

RENAL PATHOPHYSIOLOGY

[Arno Helmborg](#)

These lecture notes accompany my lectures on pathophysiology in the study module "Kidneys and Urinary Tract" at the Medical University of Innsbruck. The English version serves two purposes: as a learning aid for international students and to encourage German-speaking students to familiarize themselves with medical English; the lectures are delivered in German. The translation from the original [German version](#) is my own; I am afraid it will occasionally sound appalling to native English speakers, but it should at least be intelligible.

Version 1.3 e ©Arno Helmborg 2016-2018

Pdf- version of <http://www.helmborg.at/renal-pathophysiology.htm>

Terms of use: <http://www.helmborg.at/terms.htm>

Why do we have kidneys? If we produce water-soluble metabolites which are hard to transport across cells and are toxic beyond a certain concentration, we need a direct pathway from the extracellular fluid compartment to the outside to get rid of them. In fact, such metabolites exist: for example, when we use amino acids to produce energy, what remains are the amino groups. In the form of either ammonium or the less toxic urea, nitrogen is hard to secrete. This problem is solved by shuttling it directly out.

Yet, directly pumping out extracellular fluid would kill us within minutes, unless we succeed in reabsorbing everything we need with utter efficiency, in a precisely regulated process, starting with water. Aside from important additional functions initiated by renin, active vitamin D and erythropoietin, that's what kidneys are about. And that helps to understand what's going wrong in kidney disease. Let's start with water.

1. WATER AND FLUID COMPARTMENTS

Water is the most abundant constituent of our body, at 50-60 % of our body mass (75% in infants). Why this large variation? The wide range is mainly due to individual differences in fat stores and muscle mass. Fat excludes water; therefore, fat cells contain very little water. Under the influence of androgens, males on average have a higher proportion of "watery" muscle to water-excluding fat, while even lean females have a lower proportion and hence a lower percentage of water, tending to 50%. Almost two thirds of body water resides inside cells, the rest is extracellular fluid.

The body of a lean woman of 70 kg thus may contain 35 liters of total body water, of which approximately 21 liters (60%) are intracellular fluid and 14 liters extracellular fluid, including interstitial fluid and blood plasma (about 3 l). In comparison, a 70 kg non-obese male may contain 42 liters of total body water, with 25 l of intracellular and 17 l of extracellular fluid. For both sexes, blood volume is the sum of three liters of plasma plus the volume of cells

contained in the blood, adding up to 5 l in females and 5.5 l in males: hematocrit, the cellular fraction of the blood, is a little higher in males.

Let's review a few necessary definitions:

Osmole is a unit of measurement expressing the number of moles of solute that contribute to the osmotic pressure of a solution

Osmotic concentration (Osmolarity) is expressed in osmol/l. In medicine, it is usually not determined precisely but approximated by calculation, as in:

Osmotic concentration of serum =

$$= 2 \text{ Na} + \text{Urea} + \text{Glucose (all in mmol/l)}$$

or, in case urea and glucose are given in mg/dl:

$$=(2 \times \text{serum sodium [mEq/l]}) + (\text{BUN [mg/dl]}/2.8) + (\text{glucose [mg/dl]}/18)$$

Osmolality, on the other hand, relates not to a volume but rather to a kg of solvent (osmoles/kg solvent). Osmolality is measured directly by either freezing-point depression of water or vapor pressure techniques.

For a given solution, osmotic concentration is slightly lower than osmolality, because the volume used in calculating osmotic concentration includes the solutes, while osmolality is based on 1 kg of solvent excluding the weight of any solute. In other words, to get from osmolarity to osmolality, you have to "top up" your solution, including more particles. In practice, with the relatively small amounts of solutes per volume or mass of solvent in our body, the difference is almost negligible.

Osmolality of intracellular volume, interstitial volume as well as blood plasma is equal at 290 mosmol/kg; otherwise water would shift until a new equilibrium is reached. The composition of solutes is very different, however. Life started in the oceans, and multicellular organisms migrating elsewhere took their "inner ocean" with them. Therefore, our extracellular compartment is rich in salt, NaCl, while within cells, our main cation is K⁺. Cell membranes constitute the border between intracellular and extracellular compartments. The main factor maintaining this asymmetry between the two compartments is Na-K-ATPase, which is pumping three Na⁺ ions out of the cell in exchange for 2 K⁺ ions. Regarding extracellular compartments, the main asymmetry between blood plasma and interstitial fluid is due to protein content. The liver constantly releases proteins into the blood, many of which are too big to pass the endothelial barrier as long as there is no inflammation.

Thus, our main fluid compartments differ as follows:

Solute	Plasma	Interstitialium	Cells
Na ⁺ (mM)	142	145	15
K ⁺ (mM)	4	4	120
Ca ²⁺ (free, mM)	1.2	1.2	10 ⁻⁴
Ca ²⁺ (total)	2.5		
Mg ²⁺ (free, mM)	0.6	0.6	1
Mg ²⁺ (total)	0.9		18
Cl ⁻ (mM)	102	116	20
HCO ₃ ⁻ (mM)	24	25	15
Phosphate (free, mM)	0.7	0.8	0.7
Proteins (g/dl)	7	1	30
Albumin (g/dl)	4.5	1	
Osmolality (mosmol/kg)	291	290	290

Albumin

Albumin is our most abundant extracellular protein. It is synthesized by the liver at a rate of 9-12 g per day. It is a 66 kDa protein of triangular shape, with a strong negative net charge of -15. Therefore, it lines up at the anode-near end of all proteins in serum electrophoresis. Although we think of albumin as a plasma protein, there is actually more interstitial albumin than plasma albumin: albumin concentration is four times higher in plasma, but interstitial volume is five times plasma volume. The rate at which albumin leaves the plasma depends on the tightness of capillaries in the respective organs. While very little albumin passes the wall of brain capillaries, capillaries in gut, muscle or fat tissue are much more permeable, not to speak of the fenestrated endothelia of liver and glomeruli. For example, albumin concentration has been reported to be 1.3 g/dl in muscle and 0.7 g/dl in fat interstitial fluid. Albumin is recycled to the blood via the lymphatics. If there were no lymphatic removal, interstitial protein concentration would eventually equal that in the plasma.

Regulation of body water

If water is the most abundant constituent of our body, how do we regulate its volume? Imagine a barrel: it's easy to determine when it's full. Yet, our body lacks the rigid structure of a barrel; it's more like a balloon: for its elasticity, it's hard to determine its "correct" volume.

In fact, our body regulates water volume in a two-step procedure.

1. First, it regulates osmolality very tightly: it adjusts water to keep osmotic pressure constant, which in practice means it adjusts water to keep extracellular Na⁺ concentration constant. That still leaves us with the balloon problem: we may put more or less isotonic saline into the balloon of our body. That is actually the case: depending on our Na⁺ intake,

our extracellular volume varies a lot more than our osmolality. Put another way: our osmoregulation is stricter than our volume regulation.

2. Yet, it is clear we also need to measure and regulate volume in a second step, if with much less precision. For this purpose, we use pressure sensors in arteries, cardiac atria and the vasa afferentia of renal glomeruli. Yet, our ability to gauge extracellular fluid volume is restricted by these measuring devices: we do not have an ability to measure excess extracellular volume in the form of interstitial edema, ascites or pleural effusion.

Note that we have a single, precise device to measure osmolality which does a perfect job and several devices to gauge effective circulatory volume, which leave much to be desired because of their inability to determine total extracellular volume.

2. REGULATION OF RENAL BLOOD FLOW

The kidneys have a combined weight of less than 0.5% of the entire body, yet receive approximately 20% of cardiac output. While resting, cardiac output may be near 5 l/min, of which 1 l/min goes to the kidneys. This is primarily necessary to be able to excrete solutes that cannot be secreted by cells. In addition, it reflects high metabolic activity: the kidneys account for 7-10 % of total O₂ consumption of our body in a resting state.

Renal plasma flow can be measured with the help of para-aminohippurate (PAH). While traces of PAH may occur in our body by metabolic processes, for the purpose of determining plasma flow, large quantities are infused intravenously. PAH is freely filtrated at the glomerulus and in addition actively secreted by the proximal tubule. Almost all of PAH reaching the kidney is eliminated in a single pass (92%), so that PAH clearance approximates renal plasma flow and, with the help of the hematocrit, can be used to estimate renal blood flow.

About 90% of the blood leaving the glomeruli perfuses the cortex; only 10%, from a subpopulation of juxtamedullary glomeruli, go to the medulla via the vasa recta. Because of the high fluid resistance of these long capillaries, very little of this blood reaches the papilla. This is important: otherwise, the high osmotic gradient in the medulla would wash out and we would not be able to concentrate urine. On the other hand, it puts cells in the medulla in a precarious position: as soon as there is a problem with blood flow, these cells are prone to suffer damage from lack of oxygen.

Regulation of glomerular filtration rate

A selective increase of afferent arteriole resistance decreases glomerular filtration rate (GFR). In contrast, increasing the resistance of the efferent arteriole has a biphasic response. At the lower end of the resistance range, an increase in resistance increases GFR as a result of increased filtration pressure. In addition, filtration fraction goes up: a higher fraction of blood entering the glomerulus is filtrated, meaning somewhat less blood is available to perfuse renal

parenchyma "behind" the vas efferens. Yet with further increases of resistance, renal plasma flow declines, causing GFR to first level off and then decline.

Two levels of regulation affect the glomerular arterioles. Autoregulation relies on feedback within an individual nephron and affects only the afferent arteriole. Systemic regulation adds to this via the autonomous nervous system, as well as via chemical mediators.

Autoregulation

Autoregulation is the result of two independent mechanisms:

1. Myogenic response: An increase in arterial pressure opens stretch-activated nonselective cation channels in smooth muscle cells of afferent arterioles, depolarizing the membrane and opening voltage-dependent Ca^{2+} channels, leading to contraction.
2. Tubuloglomerular feedback: an increase of single nephron GFR brings more NaCl to the *macula densa* in the thick ascending limb. This is sensed by the cell via its Na-K-2Cl-cotransporter. Higher uptake of Cl^- indirectly causes membrane depolarization of the macula densa cell: uptake via the Na-K-2Cl-cotransporter is electroneutral, but basolateral trickling out of Cl^- acts depolarizing. Ca^{2+} influx then leads to the release of paracrine agents, like adenosine or thromboxane, which trigger contraction of smooth muscle cells in the afferent arteriole.

Pharmacology cross reference: by blocking the Na-K-2Cl-cotransporter, furosemide not only blocks the uptake of Na^+ and Cl^- , it also inhibits tubuloglomerular feedback. GFR thus remains high in the face of sodium chloride losses, which contributes to diuresis. The opposite is true for thiazide diuretics.

A high protein diet increases glomerular capillary pressure and glomerular filtration rate. By an unknown mechanism, increased protein intake enhances reabsorption of NaCl in the thick ascending limb of the loop of Henle. With less NaCl reaching the macula densa, tubuloglomerular feedback opens the vas afferens and increases GFR. This mechanism helps to get rid of incremental nitrogen from amino groups, but increases the risk of pressure damage to the glomerulus. Thus, a high-protein diet on the margin increases the risk of renal damage.

Systemic regulation:

Sympathetic stimulation increases both afferent and efferent resistances. Intense sympathetic stimulation leads to drastic reductions in both renal blood flow and GFR. Sympathetic stimulation also increases renin release via β_1 -receptors.

Renin- angiotensin II: A decrease in arterial pressure is sensed by the afferent arteriole, which acts as a baroreceptor. It directs neighboring granular cells to release renin, to bring up arterial pressure and to maintain extracellular volume as far as possible. Angiotensin converting enzyme (ACE), anchored to the plasma membrane of glomerular endothelial cells,

locally produces angiotensin II, which thus constricts the efferent arteriole more than the afferent one, with the net effect of maintaining GFR while minimizing loss of volume.

ANP. High extracellular volume is sensed by atrial myocytes, which release ANP. ANP vasodilates afferent and efferent arterioles (afferents more) and lowers the sensitivity of tubuloglomerular feedback. Together, it increases GFR, lowers osmotic pressure in the medulla and increases diuresis.

Prostaglandins. Prostaglandins are produced by endothelial cells, vascular smooth muscle cells, mesangial cells and tubule and interstitial cells in the renal medulla. They are predominantly produced in response to angiotensin II, sympathetic activation and ADH. Thereby, prostaglandins locally limit the effects of these vasoconstrictors to maintain blood flow and GFR under stress conditions.

Pharmacology cross reference: By blocking this mechanism, frequent use of cyclooxygenase (COX-) inhibitors on the margin increases the risk of renal damage. Such damage is bound to be most pronounced in the inner medulla, where perfusion is kept at a minimum to be able to maintain the hyperosmolar milieu required for urine concentration.

We will review these regulation systems in more detail later, after considering glomerular filtration and reabsorption of sodium and water.

3. GLOMERULAR FILTRATION

The glomerular filter consists of three components:

1. The endothelial layer: endothelial cells are fenestrated, providing no barrier for solutes; still, a role of the [negatively charged glycocalyx](#) in permselectivity is being discussed.
2. The basement membrane with *lamina rara interna*, *lamina densa* and *lamina rara externa*. The basement membrane is a joint product of endothelial cells and podocytes. It is directly continuous with the glycocalyx and consists of a dense network of collagen type IV and proteoglycans. The proteoglycans carry lots of anionic charges, e. g., in the form of sialic acid. This decreases the filter's permeability for negatively charged solutes, e. g., for albumin. The basement membrane prevents any blood cell to pass the filter.
3. The foot processes of podocytes, which are connected by slit diaphragms. The slit diaphragms are a sieve of protein strands consisting of at least five proteins, including nephrin and podocin.

Congenital nephrotic syndrome of the Finnish type: this very rare syndrome is caused by mutations in the gene encoding nephrin. Massive amounts of protein are filtrated and lost via the urine. Results are kidney failure, malnutrition and infections, as antibodies are lost, too. It thus seems likely that the slit diaphragm is the structure primarily responsible for permselectivity.

Two factors contribute to the permselectivity of the glomerular filter:

1. Physical pore size: with about 8 nanometers in diameter, pore size prevents larger proteins from passing through. The defining property of a protein in this respect is its Einstein-Stokes radius, which is the radius of a hard sphere diffusing at the same velocity as the protein. Globular ("ball-like") proteins with Einstein-Stokes radii of 4 nm, equaling diameters of 8 nm, typically have masses around 70 kDa: larger proteins cannot pass the filter. For example, antibodies (150 kDa and larger) are completely retained, while isolated immunoglobulin light chains (22 kDa) or their dimers (44 kDa) are able to pass through.
2. Charge selectivity: both basement membrane and slit diaphragm are characterized by dense arrays of negative charges. As most plasma proteins are also negatively charged at the physiological pH of 7.4, electrostatic repulsion prevents them from slipping through pores that spatially would be wide enough to let them pass. To think of an analogy, you might be able to squeeze through between the bars of crowd barriers, but if they are freshly painted, you will probably refrain from trying. Human serum albumin, for example, is a 66 kDa protein. It is not globular, but rather triangular or heart-shaped, with an effective diameter of 7 nm, which would allow it to pass through the 8 mm pores. Yet, albumin is negatively charged, so that less than 1% of all albumin molecules actually pass the glomerular filter.

Microalbuminuria

Most of this filtrated albumin is reabsorbed by the proximal tubule. In a healthy person, less than 30 mg of albumin per day is excreted in the urine. Interestingly, some people, especially young adults, when standing or sitting in an upright position, excrete somewhat higher amounts. This phenomenon is termed orthostatic proteinuria and is not considered pathologic. Apart from that, albumin excretion in the range between 30 and 300 mg per day is called microalbuminuria and constitutes a sensitive parameter for glomerular damage, e.g., by hypertension or *diabetes mellitus*.

Glomerular filtration rate (GFR)

In a 70 kg male young adult, the total filtrated volume is approximately 180 liters per day, or 125 ml/min. Renal filtration correlates better with body surface area than with weight. As the surface area of the standard human being is assumed to be 1.73 m², normal GFR in males is usually reported as being 125 ml/min per 1.73 m². In young females, the respective GFR is 110 ml/min per 1.73 m².

GFR varies greatly with age: until the age of two, babies have smaller GFRs due to incomplete development of kidneys. In a young adult, each kidney contains about 1 million nephrons. A typical feature of aging is the progressive loss of nephrons, which goes hand in hand with a gradual decline in GFR.

How to measure GFR? The experimental gold standard is via inulin clearance. Inulin is a polysaccharide extracted from plants. Water-soluble and non-toxic, it freely passes the glomerular filter but is afterwards neither absorbed nor secreted by tubulus cells. If the plasma level of inulin is kept constant by continuous infusion, the amount of inulin filtered over a given time equals the amount excreted in the urine over the same time:

inulin plasma concentration \times GFR = inulin urine concentration \times urine volume/sampling time

from which GFR is easily calculated as the ratio of the concentrations times the urine flow.

With normal renal function, the concentration of inulin in the urine is much higher than in plasma, as all the filtered inulin is excreted, but almost all of the filtered water is reabsorbed in the tubule and collecting duct. In other words, virtually the entire filtered volume is cleared from inulin. Thus, inulin clearance equals GFR.

More generally speaking, the **clearance** of a specific solute is the virtual volume of blood plasma that is cleared from the solute per unit time via excretion into the urine. Clearance may vary between the extremes of

- zero, e. g. for glucose, which is quantitatively reabsorbed by the tubule and normally does not appear in urine, and
- "all", about 700 ml/min, which is the total volume of plasma that reaches the kidneys, for substances that are efficiently secreted by the tubules so that all of the solute brought to the kidney ends up in the urine after a single pass. As we have seen before, this is true for infused para-aminohippurate, which thus can be used to measure renal plasma flow.

Determining inulin clearance is a precise method to measure GFR, but measuring inulin concentrations is too cumbersome to use this technique in daily routine. Easier to measure are substances that behave similarly in the kidney but are radioactively labeled: ^{125}I -Iothalamate and ^{51}Cr -EDTA.

Still, measuring GFR this way remains a pain, as you have to mess around with radioactive substances and as prerequisite reach a steady-state level by continuous infusion. It would be much more convenient to use a solute that is produced by our organism itself in a steady state way, that is filtered freely and that is neither reabsorbed nor secreted by the tubule. Well, this ideal substance doesn't exist, but creatinine comes close.

Creatinine clearance as a proxy for GFR

Creatinine is a metabolite of creatine, a liver-synthesized product that functions as an ultra-short-term energy store in skeletal muscle in the form of phosphocreatine. Creatinine is continuously released from muscle. It is freely filtrated, yet also slightly secreted by the tubule. On the other hand, the usual colorimetric assay tends to overestimate plasma creatinine concentration, so both effects tend to cancel each other out.

Usually, the patient is asked to collect urine for 24 h and to refrain from eating a lot of meat, which contains creatinine. Three measurements are required: the volume of urine, the concentration of creatinine in collected urine and the concentration of creatinine in plasma. Creatinine clearance can then be calculated as follows:

Creatinine clearance (ml/min) = urine creatinine / plasma creatinine x urine volume (ml) / duration of collection (min): usually 1440 for 24 h.

The patient's height and weight may be measured so that surface area can be calculated and creatinine clearance adjusted to the standard body surface area of 1.73m².

It is important to keep in mind that several factors may impinge on the reliability of the calculated creatinine clearance:

- the patient may have been sloppy in collecting urine, usually at day time (e. g., didn't want to be seen to go to the bathroom with a giant dark bottle)
- the patient nonetheless may have succumbed to the temptation of a steak
- the patient may have a health problem affecting skeletal muscle
- the patient may have an impairment of liver function. Synthesis of new creatine occurs mainly in the liver

Serum creatinine as a measure of renal function

The next step down the ladder of reliability is to throw out urine collection altogether, and to use steady state serum creatinine to assess renal function. In theory, a halving of GFR should lead to a doubling of serum creatinine concentration. In everyday medicine, we use a level of 1 mg/dl as a cutoff: whoever doesn't exceed it is considered to have acceptable renal function. By doing this, however, we miss a considerable percentage of patients who have low actual GFR while still managing to keep serum creatinine levels below the threshold. Typically, this is observed in patients with lower muscle mass, e. g., in delicately built females or in people of advanced age.

Estimated GFR (eGFR)

To improve on these shortcomings, formulas have been developed to calculate **estimated GFR** from serum creatinine measurements by using empirical correction factors, e. g. for female sex, for age or for African ancestry implying increased muscle mass. One of these was developed by the Modification of Diet in Renal Disease Study Group and thus termed MDRD-formula:

$$\text{eGFR (ml/min)} = 186 \times \text{serum creatinine (mg/dl)}^{-1.154} \times \text{age}^{-0.203} \times [0.742 \text{ if female}] \times [1.210 \text{ if black}]$$

Parameters like eGFR and albumin-to-creatinine ratio in urine (ACR) contribute to prediction of renal outcomes, as well as to prediction of vascular outcomes beyond traditional risk factors.

4. REGULATION OF SODIUM AND EXTRACELLULAR VOLUME

In the extracellular fluid compartment of our body, which accounts for a little more than a third of its total fluid, we carry a lot of Na^+ with us. Life on our planet originated in the sea, and in our extracellular volume, we carry a diluted form (from 3.5% to 0.9% salinity, or from about 480 mmol/l Na^+ to 140 mmol/l and from about 550 mmol/l Cl^- to 110 mmol/l) of "inner" sea with us, which we need to maintain carefully. About 65% of the total Na^+ in our body is located in the extracellular fluid. An additional 5-10% is in the ICF compartment. Together, these 70-75% of the total sodium pool of our body constitute the "exchangeable" sodium stores. The word exchangeable stems from experiments with radioactive Na^+ , which rapidly equilibrates with this part of our total body Na^+ . The rest is non-exchangeable, meaning osmotically inactive, at least in the short term, and bound in some form, mostly in skin, muscle and bone.

Non-exchangeable Na^+ has been shown in the skin, where it seems to be bound to glycosaminoglycans. There, it is not completely osmotically inactive: it seems to be "locked away" in a hyperosmotic compartment, the accessibility of which is modulated by the density of lymphatic vessels, which in turn is regulated by macrophages via VEGF-C (vascular endothelial growth factor-C). Prolonged intake of a high-salt diet may over time increase these stores, which increase with age and are higher in hypertensive than in normotensive persons. Our knowledge about the pathophysiological significance of these stores is quite inadequate; experimental data suggest that over a longer time frame, these stores may still act as an additional sodium buffer. This way, they may contribute to salt-sensitive hypertension. In tightly controlled space simulation experiments with fixed NaCl input over months, periodic oscillations in sodium storage of 200-400 mmoles were observed, with rhythms of weekly and monthly or longer periodicity. Thus, while sodium balance is by and large maintained (input equals output), this is not strictly true for specific 24-hour periods.

One gram of salt contains 17 mmol of NaCl. If we take up 140 mmol (8.2 g) of NaCl, for osmotic reasons, we expand our inner sea, our extracellular volume, by roughly 1 liter. Today, on our Western Diet, we take up a lot of salt. But that has not always been the case.

Until a few thousand years ago, maintaining our inner sea was a challenge, because Na^+ was quite scarce in human nutrition. Thus, our organism is optimized for salt conservation. For millions of years, the human organism confronted the following problem: Our "inner sea" consists mainly of water and salt; while water is a *sine qua non* and had to be readily available to fill up, salt was not. The human body needed to excrete a lot of water-soluble substances (like urea), yet retain the salt.

This problem is solved by our kidneys. Every single day, we filtrate up to 180 liters of "inner sea" containing (180x140 mmol) 25 mol salt. This is about 1.5 kg of salt, while our daily intake contains only a few grams! There is only one option: we need to retrieve 99.5% of all filtrated salt. This is achieved in steps: 67% of filtrated sodium is reabsorbed in the proximal tubule, mainly in exchange for H^+ . Twenty-five percent is reabsorbed in the thick ascending

limb of the loop of Henle by cotransport of Na-K-2Cl. In the distal convoluted tube, a further 5% are absorbed by Na-Cl-cotransport. Actual Na⁺-excretion is fine-tuned by the renin-angiotensin-aldosterone system, which controls reabsorption of the last 0-3% in the collecting duct via the epithelial Na⁺ channel (ENaC).

Let's take a look at the individual segments:

- In the proximal tubule, there is a huge gradient between the 140 mM Na⁺ in the primary filtrate and the cytoplasm of the tubule cell, which is continuously evacuated from Na⁺ by the powerful basolateral Na-K-ATPase. In addition, there is the cell-interior negative electrical potential generated by more Na⁺ leaving the cell than K⁺ entering. This powerful combined electrochemical gradient produces an inward Na⁺ cascade that supplies the energy to cotransport solutes like glucose, amino acids, phosphate, citrate etc. out of the filtrate into the cell. Reabsorption of all these valuable solutes is enabled simply by inserting the respective cotransporters ("mills") into the apical plasma membrane. From a quantitative viewpoint, however, a second mechanism is even more important: the Na⁺ cascade's energy is used to extrude H⁺ from the cell via the Na-H-exchanger (NHE), with the ultimate goal of HCO₃⁻ recovery. The speed of this exchange depends on the rate of intracellular proton generation. In the cytoplasm, H⁺ and HCO₃⁻ are generated by carbonic anhydrase II; HCO₃⁻ and Na⁺ leave the cell basolaterally to the blood. Numerous aquaporin units in the membranes of proximal tubule cells allow water to freely follow sodium, resulting in isotonic reabsorption. Apical inflow of Na⁺ into the cell and basolateral outflow of K⁺ to the blood create a lumen-negative transepithelial voltage (caution: don't confuse this with the transmembrane voltage!), causing some of the transported Na⁺ to leak back into the lumen by the paracellular pathway.

Pharmacology cross reference: blocking carboanhydrase (by acetazolamide) reduces the amount of H⁺ available to exchange against Na⁺. While at first glance that would seem to produce a huge diuretic effect, compensatory Na⁺ uptake in later nephron segments reduces it to a rather mild end result. As a diuretic, acetazolamide is obsolete, yet it continues to be used for glaucoma and other indications.

- In the thick ascending limb, Na⁺ and Cl⁻ gradients drive uptake into the cell via the Na-K-2Cl cotransporter. In this process, the limiting factor is K⁺, which is low in extracellular fluid and has to be dragged in by its partners against its concentration gradient. In order to make more of it available, K⁺ is recycled from the cell via an unusually high number of apical K⁺-channels (ROMK, renal outer medullary K⁺ channel). The resulting strong apical outflow of K⁺ leads to a lumen-positive transtubular voltage, which is the driving force for additional, paracellular Na⁺-flow. About 50% of Na⁺ reuptake in the thick ascending limb is due to this passive transport. Of course, the same mechanism also drives paracellular reuptake of other cations such as K⁺, Ca²⁺ and Mg²⁺. An important feature of the thick ascending limb is a remarkably low water permeability of its apical membrane, which is due to the selection of transmembrane proteins expressed on it (e.g., no aquaporins); tight junctions between cells are water-impermeable, too. Na⁺ is

transported out, water stays in: this leads to a diluted, hypoosmolar fluid at the end of this "dilution segment", while interstitial osmolality is built up.

Pharmacology cross reference: Loop diuretics (furosemide) block the Na-K-2Cl cotransporter by competing for the chloride site, producing a strong diuretic effect. Therefore, a lot of Na⁺ and Cl⁻ arrive at the *macula densa* at the end of this segment. Normally, that would induce a massive reduction of GFR via tubuloglomerular feedback. Yet, such reduction would be mediated by Cl⁻ uptake into *macula densa* cells via precisely the Na-K-2Cl cotransporter that is being blocked by the drug. Thus, there is **no** reduction of GFR, which contributes to the diuretic effect. Of course, this also reduces the passive, paracellular reabsorption of other cations and may cause considerable losses of Ca²⁺ and Mg²⁺. As we [noted previously](#), hypocalcemia increases the open probability of Na⁺ channels in neurons and muscle cells and may lead to cramps. Via the same mechanism, loop diuretics may induce secondary hyperparathyroidism. The other way round: loop diuretics may be used in the treatment of hypercalcemia. Obviously, downstream nephron segments try to compensate for the looming Na⁺ loss, causing increased excretion of K⁺. The mechanism of this "exchange" is explained below, in the subsection on aldosterone.

- In the distal convoluted tubule, the diluting effect has taken Na⁺ concentration down to about 70 mM. That is still more than 5 times the intracellular level. Na⁺ enters the cell via the Na-Cl cotransporter and is pumped out the back door via Na-K-ATPase.

Pharmacology cross reference: Thiazide diuretics, like hydrochlorothiazide, block the Na-Cl cotransporter. Here again, distal attempts to make up for Na⁺ losses lead to increased excretion of K⁺.

- In cortical collecting tubule and collecting duct, Na⁺ enters tubule cells via epithelial Na⁺ channels (ENaC), which are under control of aldosterone and ANP. Aldosterone increases the number of open sodium channels; ANP decreases it. Importantly, the inflow via ENaC is electrogenic, as Na⁺ enters without an anion partner. This is different from the electroneutral uptake in the previous segments and leads to an indirect exchange with K⁺, as we will see in a minute in the aldosterone subsection.

Pharmacology cross reference: Amiloride and triamterene block ENaC; spironolactone blocks the mineralocorticoid (aldosterone) receptor. The effect of these diuretics is mild, as they only affect the last 3% of Na⁺ left in the lumen. These drugs do not lead to losses of K⁺; to the contrary, they may cause K⁺ retention.

ANP

Atrial natriuretic peptide (ANP) is released from myocardial cells of the atria in response to volume expansion, which is sensed as atrial stretch. It is a 28-amino acid polypeptide binding to a receptor with guanylate cyclase activity so that its second messenger is cGMP. ANP has two effects:

1. it closes apical Na^+ channels (ENaC), reducing Na^+ reabsorption. In addition, it increases GFR by vasodilating the vas afferens more than the vas efferens and leads to washout of the osmotic gradient in the medulla. Both mechanisms contribute to increase urinary sodium and water excretion.
2. at higher concentrations, it acts as a direct vasodilator, lowering systemic blood pressure.

As the second effect limits GFR and with that, the excretion of Na^+ and water, the actual natriuretic effect of ANP remains modest, and the system is no match for the powerful RAAS. Patients with congestive heart failure have high levels of ANP, yet avidly retain Na^+ .

The effect of the Renin-Angiotensin-Aldosterone system on extra cellular volume

Renin

A decrease in effective circulating volume increases renin secretion in three ways:

1. Deactivation of stretch receptors in the afferent arteriole directly leads to renin secretion.
2. Deactivation of stretch receptors in the carotid and the aortic arch result in enhanced sympathetic signaling to the juxtaglomerular apparatus via adrenergic β_1 -receptors.
3. The mechanism mediating tubuloglomerular feedback affects renin secretion as well. As we noted before, delivery of more NaCl to the *macula densa* leads to a reduction of GFR. The other way round, if less NaCl is arriving at the macula densa, this is sensed via reduced uptake of Cl^- by the Na-K-2Cl cotransporter and somehow translated into an increase in renin release. It is not yet clear how the message travels from *macula densa* to adjacent granular cells; possibly via alterations in the rate of prostaglandin production.

However, we should keep in mind that renin release in response to increased sympathetic activation may also occur in the absence of a decrease in effective circulating volume. In this case, the result is an increase in circulatory volume, which may play an important role in the development of arterial hypertension.

Angiotensin II

Renin, a protease released into the vas afferens, excises the decapeptide angiotensin I from the α_2 plasma globulin angiotensinogen, which is continuously produced by the liver. Angiotensin I is converted to the octapeptide angiotensin II by angiotensin converting enzyme (ACE) via removal of two amino acids. ACE is expressed by endothelial cells, at high levels in lung and glomerular capillaries, and exposed on their luminal surface. The kidney receives systemic angiotensin II and produces additional angiotensin II on the endothelial surface of the glomeruli. The result is that angiotensin II concentrations at the vas efferens are higher than elsewhere, and that vasoconstriction affects the vas efferens more than the vas afferens.

The majority of angiotensin II's effects are mediated via the AT_1 receptor:

- The predominant constriction of the efferent arteriole increases the filtration fraction and diminishes blood flow in downstream peritubular capillaries. The increased filtration fraction automatically increases colloid osmotic pressure of the remaining plasma going to the medulla. In combination with a reduced washout of the osmotic gradient in the medulla, these effects enhance Na^+ and water resorption. Therefore, angiotensin II is able to increase Na^+ reabsorption on its own, regardless of aldosterone.
- Angiotensin II raises the sensitivity of the tubuloglomerular feedback mechanism. Delivery of a given amount of Cl^- to the macula densa thus leads to a more pronounced reduction in GFR.
- Angiotensin II, like norepinephrine, induces the expression of Na-H exchanger in proximal tubule and thick ascending limb, enhancing Na^+ reuptake capacity.
- Angiotensin II more generally induces hypertrophy of tubule cells. Previously, we noted a similar effect on myocardial cells.
- In the hypothalamus, angiotensin II increases the sensation of thirst and acts on receptors on neurons in the *organum vasculosum laminae terminalis* (OVLT) and subfornical organ (SFO) to promote release of ADH.
- Of course, angiotensin II stimulates the synthesis of aldosterone in the *zona glomerulosa* of the adrenal cortex.

Together, these mechanisms contribute to maintain and fill up extracellular volume.

Aldosterone

Aldosterone has two main functions: firstly, it is our Na^+ saving hormone, secondly, it is required to excrete K^+ excess. Consequently, aldosterone is activated by angiotensin II as well as by an increase in plasma K^+ concentration.

Aldosterone has a threefold effect on principal cells of the collecting tubule:

1. it induces Na-K-ATPase. An increase in activity over and above levels in other cells is necessary, as Na^+ entry via ENaC is passive and works only as long as there is a concentration gradient. In cases where urine Na^+ approaches its lowest possible value of about 3 mM, intracellular Na^+ has to be lower.
2. it increases the surface area of the basolateral membrane to accommodate more units of Na-K-ATPase
3. it induces the apical ENaC (epithelial Na^+ channel)

Indirect exchange of Na⁺ for K⁺:

Aldosterone makes more Na⁺ cross the apical membrane. Inflow of these positive charges affects membrane potential. Membrane potential is primarily a function of K⁺ trickling out. The outflow of K⁺ limits itself, as accumulation of positive charges on the outside and negative charges on the inside stop the flow electrostatically at a certain level of polarization. Incoming Na⁺ reduces this voltage, allowing more K⁺ out. The more Na⁺ is ushered in by aldosterone, the higher net secretion of K⁺. If Na⁺ reabsorption in upstream tubulus segments is inhibited by diuretics such as furosemide or thiazide, high amounts of K⁺ are excreted with the urine. In contrast, if little Na⁺ is left in the fluid reaching the collecting tubule, K⁺ secretion is low. Therefore, the ratio is also flow-dependent: at low tubular flow, a larger proportion of Na⁺ is taken up in upstream segments together with Cl⁻, and a lower proportion is exchanged for K⁺.

Glucocorticoids

Physiologically, glucocorticoids increase the GFR and decrease water permeability of the distal nephron. Thereby, they increase flow and the amount of Na⁺ reaching the collecting duct, which enhances K⁺ secretion.

Cortisol and aldosterone are quite similar. While in principle, each of them has its own receptor, both hormones bind to both receptors. In cells that ought to respond to aldosterone, this causes a problem: cortisol, with its much higher molar concentration, would bind and activate the mineralocorticoid receptor, causing an unregulated all-out response. To prevent that, these cells express the enzyme 11β-hydroxysteroid-dehydrogenase (11β-HSD), which converts cortisol to cortisone. Cortisone does not bind to the mineralocorticoid receptor (nor does it bind to the glucocorticoid receptor).

Pharmacology cross reference: When used as drugs, glucocorticoids reach far higher molar concentrations than in the physiological situation. The sheer number of glucocorticoid molecules overwhelms the capacity of 11β-HSD to protect the mineralocorticoid receptor. Thus, the higher the dose of glucocorticoids, the higher the proportion binding to the mineralocorticoid receptor and the more pronounced the aldosterone-like effects, producing, e.g., arterial hypertension. Glycyrrhizin, a constituent extracted from the liquorice root, inhibits 11β-HSD, increasing the mineralocorticoid effect of glucocorticoids. Depending on the ingested dose, it may cause Na⁺ and water retention by its own.

ADH or AVP

ADH (anti-diuretic hormone = AVP, arginine vasopressin), a nonapeptide, is synthesized in neurons of the *Nucleus supraopticus* and the *Nucleus paraventricularis* of the hypothalamus. Neurons of these nuclei are stimulated by nearby osmoreceptor-neurons directly exposed to

plasma osmolality by their proximity to two breaches in the blood-brain barrier: the *Organum vasculosum laminae terminalis* and the subfornical organ, two of the circumventricular organs. The firing rate of these osmoreceptor neurons is influenced by mechanosensitive cation channels that are able to sense osmotic swelling or shrinking.

Separate, but parallel osmoreceptor-neurons in the same organs also project to hypothalamic regions generating thirst.

On rises in plasma osmolality, ADH is released in incremental amounts from the posterior lobe of the pituitary. Normally, no ADH is secreted below 280 mOsm. However, there is an exception: in case of plasma volume depletion, ADH may be secreted even at low plasma osmolality. In this case, the stimulus for ADH release is originated by high-pressure baroreceptors in the carotid sinus and aortic arch and by low-pressure baroreceptors in the right and left atria. ADH release is much more sensitive to osmoregulation than to volume regulation. Increases of 1-2% in plasma osmolality will stimulate release of ADH, while a 10-15% reduction in blood volume or pressure is needed. However, once this threshold is reached, further response to volume depletion is exponential. As ADH is a product of the hypothalamus, under specific circumstances additional central nervous stimuli, which we do not yet understand sufficiently, are likely to influence its release. For example, pain, nausea and vomiting, IL-6 release, elevated body temperature as well as hypoglycemia have been reported to increase ADH release.

The hormone has a short half-life of about 20 minutes. Depending on initial levels, the maximum diuresis after a water load is therefore delayed for one to two hours.

Regular effects via the V2 receptor:

- At the distal nephron, ADH activates AVPR2, also known as V2-receptor, a G_s-coupled receptor signaling via cAMP. PKA-mediated protein phosphorylation causes fusion of aquaporin-2 water channel-containing vesicles with the apical membrane, increasing water permeability of the cortical collecting tubule as well as outer and inner medullary collecting ducts. Thus, in the distal nephron, osmotic resorption of water may be increased more than tenfold. Longer term stimulation by ADH induces aquaporin-2 gene expression, leading to higher numbers of protein units per cell.
- In addition, ADH increases urea permeability only at the inner medullary collecting duct by adding and phosphorylating units of UT1 (urea transporter-1). This second function is crucial for urea recycling required to build peak osmotic pressure in the antidiuresis situation. Under conditions of maximal antidiuresis, urine is isotonic with the inner medulla of the kidney. Thus, the degree of hypertonicity of the medullary interstitium determines maximum urine concentration.
- ADH increases NaCl reabsorption in the thick ascending limb by stimulating Na-K-2Cl cotransport.

Stress-related effects via the V1 receptor:

- Only at maximal secretion in acute stress situations does the molecule actually act as "vasopressin", i.e. lead to contraction of smooth muscle cells in arteriolar walls via AVPR1a (V1a receptor).
- Another renal effect of ADH via the V1 receptor is to stimulate production of prostaglandins in many cell types, including the glomerular mesangium and the tubulus. The prostaglandins promote vasodilation and in all likelihood are important to maintain GFR and renal perfusion in the face of elevated ADH levels and antidiuresis.

Pharmacology cross reference: the widespread use of NSAIDS has the unwanted side effect of suspending this safeguard mechanism.

- V1a receptors are also expressed by hepatocytes and platelets, promoting gluconeogenesis and clotting readiness.
- Via AVPR1b (V1b) receptors on corticotrophs in the pituitary, ADH/AVP promotes the release of ACTH.

Pharmacology cross reference:

- Stimulants of ADH release may be nicotine and drugs like morphine or barbiturates.
- Inhibitors of ADH release are alcohol and opiate-antagonists.

Diabetes insipidus

Diabetes insipidus ("unquenchable flow-through") is the result of failing ADH function, which may be caused either by lack of ADH itself (central *diabetes insipidus*) or by a failure of the kidney to respond (nephrogenic *diabetes insipidus*). As the latter parts of the nephron remain impermeable to water, the patient produces large amounts of diluted urine (polyuria). The loss of water tends to result in hypovolemia, making patients thirsty and inducing them to drink large amounts (polydipsia). If patients do not drink enough, they quickly develop hypotension, marked hypernatremia and shock. Central *diabetes insipidus* may be treated by nasal administration of the synthetic ADH analog desmopressin or DDAVP (1 Desamino-8-D-Arginine-Vasopressin).

Pharmacology cross reference: Nephrogenic *diabetes insipidus* may be an unwanted response to certain drugs, e. g., colchicine and Li^+ . Lithium therapy is used to treat bipolar disorder. Li^+ -ions decrease cAMP, the second messenger of ADH, by inhibiting adenylyl cyclase.

SIADH

The syndrome of inappropriate ADH secretion (SIADH) is the opposite of diabetes insipidus, with inappropriately high levels of ADH in relation to osmolality. Full-fledged water resorption leads to volume expansion with ANP release. Water retention combined with

excretion of concentrated urine containing relatively high amounts of Na^+ may result in critical hyponatremia.

Causes of SIADH:

- Certain malignant tumors may produce ADH or ADH-like peptides, e. g., small cell lung carcinomas.
- CNS disorders, including traumatic brain injury, meningitis or encephalitis.
- Pulmonary diseases such as pneumonia, tuberculosis.
- SIADH is sometimes seen in the course of surgical procedures, probably via pain afferents. Most likely, this is abetted by genetic factors, although we don't yet know the responsible genes and alleles.
- SIADH is likely to be part of the problem in exercise-associated hyponatremia, which is caused by overdrinking during endurance sports. Normally, overdrinking should just result in production of more urine. Yet, most probably again on the basis of genetic factors, some individuals retain the fluid, develop hyponatremia and, in extreme cases, exercise-associated hyponatremic encephalopathy, a form of cerebral edema. As in the case of surgical procedures, ADH secretion is due to non-osmotic stimuli. Concomitant use of NSAIDs to alleviate aches and pain may compound the problem by reducing GFR and renal perfusion. At the end of the 2002 Boston Marathon, 13% of surveyed participants were reported to be hyponatremic.
- **Pharmacology cross reference:** Likewise, a long list of drugs may increase production of ADH (central SIADH) or sensitize renal tubules to ADH (renal SIADH). SIADH may occur in patients receiving opioids, tricyclic antidepressants or chemotherapy containing cyclophosphamide or vincristine.

Pregnancy: During pregnancy, ADH release and thirst are stimulated already at lower osmolalities to satisfy the need for increased blood volume. Thus, pregnancy is often associated with an 8 to 10 mosmol decrease in plasma osmolality. A similar but smaller change may occur in the progesterone-dominated late phase of the menstrual cycle, resulting in slightly increased body weight.

Pharmacology cross reference: The effect of ADH may be blocked by V2 receptor antagonists like Tolvaptan.

Hyponatremia

Causes. In theory, hyponatremia may result either from loss of sodium or from retention of water. While we may lose considerable amounts of sodium by vomiting, diarrhea or sweating, Na^+ concentration in these fluids is almost always lower than in plasma. Thus, we lose more water than Na^+ which tends to increase plasma Na^+ concentration. Consequently, hyponatremia is only seen in cases of water retention leading to an excess of water in relation to Na^+ . As long as everything works according to plan, we have an enormous capacity to excrete water by completely shutting down ADH. In the absence of ADH, urine osmolality

can fall to 40 mosmol/kg, about one-seventh of the value in plasma (290 mosmol/kg). Therefore, water retention resulting in hyponatremia occurs only when there is an inability to suppress the secretion of ADH. An exception to this rule occurs in patients who drink excessive amounts due to mental diseases.

Persistent ADH release in the face of reduced osmolality is seen in:

1. Effective circulatory volume depletion
 - [Congestive heart failure](#)
 - Hepatic cirrhosis
 - Use of thiazide diuretics, in combination with the next point:
 - electrolyte-containing fluid (renal, gastrointestinal, skin) losses partially replaced by water
2. SIADH, as discussed above (paraneoplastic, CNS disease, tuberculosis, surgery, overdrinking in endurance sports)
3. Cortisol deficiency (adrenal insufficiency)
4. Hypothyroidism

Some patients with asymptomatic mild hyponatremia have a reset osmostat. In these patients, the threshold for ADH release is reduced below the normal osmolality of 280 mosmol/kg, so that a lower level of plasma Na^+ is maintained. In these patients, hyponatremia cannot and should not be treated, as these patients would only increase ADH release and develop thirst on attempts to elevate their plasma sodium concentration.

Symptoms. Hyponatremia is critically more dangerous if it develops acutely than if it develops chronically. With acute hyponatremia, plasma osmolality falls, and water starts to move inside cells. Cells start to swell by osmotically taking up water. For most cells, this is no big problem, yet it is for brain cells, which are encased in a rigid skull. The brain can accommodate a maximum increase of 7-8% in volume by shifting fluid from ventricles out of the skull, but then starts to herniate through the *Foramen magnum* at the base of the skull. The patient may develop headaches, nausea, vomiting, confusion and eventually, seizures and coma. In the worst case, brain stem compression may lead to respiratory arrest and death.

If hyponatremia develops slowly and chronically, on the other hand, brain cells adapt by releasing osmotically active substances. These comprise K^+ and Na^+ , but also organic solutes, e. g., myoinositol, choline compounds, glutamine and glutamate. Therefore, chronic hyponatremia typically produces no symptoms for a long time.

When diagnosing and correcting hyponatremia, it is essential to differentiate between the two states:

- Hyponatremia in an overhydrated marathon runner may be safely and rapidly corrected by administration of hypertonic saline. The SIADH component requires that Na^+ concentration of the infusion be higher than the Na^+ concentration in the excreted urine.
- Were the same treatment administered to a patient with asymptomatic chronic hyponatremia, the rapid increase in extracellular osmolality would induce a massive shift

of water out of the already adapted brain cells and produce an osmotic demyelination syndrome with irreversible neurologic damage (central pontine myelinolysis).

Hypernatremia

Causes. Any increase in osmolality beyond normal levels induces a strong sensation of thirst. Hypernatremia is thus usually seen in situations involving unreplaced water losses where thirst is either impaired or cannot be quenched.

Water losses may be due to:

1. Sweating and insensible losses via skin and respiration: people in the desert or on boats at sea; patients with fever and infections
2. Urinary losses due to diabetes insipidus or osmotic diuresis due to hyperglycemia
3. Gastrointestinal losses

Symptoms. As with hyponatremia, symptoms are predominantly seen when the condition develops rapidly and include lethargy, seizures and ultimately, coma. In chronic hypernatremia, elevated levels of Na^+ may be reached in the absence of symptoms other than thirst.

Hypernatremia is commonly seen in elderly patients in nursing homes suffering from an infection, especially if their mobility or mental status had already been reduced to begin with. Alternatively, hypernatremia may develop in infants and toddlers yet too small to help themselves or unable to express themselves. Think of children left in car seats in the heat.

Hypo- and hypernatremia may also be seen in patients suffering from *diabetes mellitus*. Hyperglycemia causes osmolality to increase, leading to osmotic water movement out of cells. In addition, increased osmolality leads to the sensation of thirst, causing the patient to drink more water. Both effects contribute to lowering plasma Na^+ by dilution. The longer this state of hyperglycemia is maintained, the more water is lost due to osmotic diuresis. Yet, Na^+ concentration in the urine is well below plasma concentration: proportionally, more water is lost than Na^+ . Treatment with insulin will correct hyponatremia by causing water (with glucose and potassium!) to shift back into cells, unmasking the previous relative accumulation of Na^+ . Plasma Na^+ may now climb through normal values and temporarily reach levels of hypernatremia.

The ability to concentrate urine is inextricably linked with conditions that pose a danger to the medulla

Like a penguin is able to regulably separate its warmth (40°C!) from the coldness in its feet by a counter-current procedure, our kidneys are able to regulably build a steep osmotic gradient by an osmolality counter-current procedure. This gradient is the basis of our kidneys' ability to concentrate urine. Like the system allows the penguin in the cold to lose little

warmth to the environment, it allows us to lose little water to the environment if water is scarce.

The kidney's ability to concentrate urine depends on its handling of NaCl and of urea. Of these two, urea is more variable. Urea is produced by the liver when metabolizing amino acids. The higher the dietary protein content, the greater the kidney's concentrating ability. In the deepest portion of the medulla next to the papilla, interstitial urea contributes about half of total osmolality. Additional solutes that sometimes have to be excreted at high concentrations are integrated into the gradient.

The kidneys keep our organism's osmolality constant by adjusting excretion of osmotically active solute particles. They are able to produce a wide range of urine concentrations, from very concentrated to extremely dilute. A given urine volume may be thought of consisting of two parts: part 1 is the volume required to dissolve all the excreted solutes at the same osmolality as in plasma (290 mosmol/l). Part two is the volume of pure or solute-free water that has to be added or subtracted to arrive at the given urine volume. In case urine osmolality is lower than that of plasma, this free water volume is positive; if urine osmolality is higher, free water volume is negative. Excreted volume of free water per unit time is called (solute-) free water clearance.

With a normal diet, our daily production of excreted solutes amounts to about 600 mosmol. Dissolved in an average urine volume of 1.5 l, they result in urine osmolality of 400 mosmol/kg, which is a little more concentrated than the 290 mosmol/kg of plasma. Free water clearance that day would be negative at about -0.5 l, because isoosmotic excretion of 600 mosmol would require a little more than 2 liters.

A patient with *diabetes insipidus* produces extremely diluted urine around 40 mosmol/kg. At that dilution, the patient excretes $600 : 40 = 15$ liters of urine. This is the amount he also has to drink to prevent hypovolemia. Free water clearance of that day would be +13 liters.

If we get lost in the desert, we need to concentrate the 600 mosmol in the smallest volume possible. Our young kidney is able to push up osmolality to 1300 mosmol/kg. That way, the 600 mosmol can be excreted in a little less than half a liter of urine. Free water clearance per day is -1.6 liters.

Urine specific gravity is easily measured, allowing a rough estimate about its concentration and osmolality:

1010 g/l: corresponds to the 290 mosmol/ kg of our body

1015-1022 g/l: a frequent range, with slightly concentrated urine

1001 g/l: extremely dilute, around 40 mosmol/kg

1040 g/l: extremely concentrated, about 1300 mosmol/kg

If extremely concentrated urine at osmolalities up to 1300 mosmol/kg is excreted, of course, all solutes contribute to this enormous osmolality according to their individual concentrations: Na^+ , Cl^- , K^+ , urea, NH_4^+ , phosphate, etc.

Why is it impossible to prevent dying from thirst by drinking sea water?

In young healthy adults, the people with "optimal kidneys", maximum measured concentration ability for Na^+ in urine is about 270 mmol/l, with individual maxima varying between 240 and 295 mmol/l. Na^+ concentration of sea water is about 480 mmol/l. Thus, drinking sea water would only worsen the situation. Here, we assess the problem using the more familiar numbers for Na^+ , yet the result is even more valid for Cl^- .

Papillary necrosis

To provide a zone with extreme osmolality in the kidney, it is necessary that this zone be perfused as sparingly as possible to prevent the otherwise inevitable washout. Of course, this entails the danger of hypoxia, and with prolonged hypoxia, the danger of necrosis. The papilla is therefore the most endangered region of the kidney once additional cell-stressing factors coincide. These additional stress factors may be remembered by the mnemonic POSTCARDS:

- Pyelonephritis
- Obstruction of the urogenital tract
- Sickle cell disease
- Tuberculosis
- Chronic liver disease
- Analgesia or alcohol abuse
- Renal transplant rejection
- Diabetes mellitus
- Systemic vasculitis

How to protect one's kidneys? Drinking sufficiently helps! If it's not necessary to concentrate urine, perfusion of renal medulla is higher and oxygen supply is better.

Edema formation

Starling's law states that net filtration is proportional to the permeability of the capillary wall, the surface area available for filtration, and the differential between hydraulic pressures in capillary and interstitium minus the differential in oncotic pressures. The differential in oncotic pressures is codetermined by the reflection coefficient of proteins across the capillary wall, σ . How much of this filtrated volume remains in the interstitium is determined by the rate of fluid removal via the lymphatics.

Thus, edema may result from:

1. Increased capillary pressure
2. Decreased oncotic pressure differential
3. Increased capillary permeability
4. Lymphatic obstruction

Each of these conditions causes a reduction in plasma volume, which is corrected by the kidneys by retaining Na^+ and water until plasma volume is normalized. In other words, in case of edema, renal retention of Na^+ and water is an appropriate compensation to normalize perfusion of all organs of the patient's body. We may feel that we would like to remove the underlying edema by treating the patient with a diuretic, yet we have to keep in mind that this may diminish tissue perfusion in parts of her organism.

Edema does not become apparent until the interstitial volume has increased by at least 2.5 l. The only way to recognize it earlier on is by weighing the patient on a daily basis.

Pharmacology cross reference: Classes of drugs known to sometimes promote sodium and water retention include glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs), Ca^{2+} -antagonists and thiazolidinediones. For glucocorticoids, the effect is due to activation of the mineralocorticoid receptor. Prostaglandins not only maintain perfusion and GFR under stress, they also have a slight tubular effect promoting excretion of Na^+ and water; NSAIDs counter this effect. Ca^{2+} -antagonists increase capillary filtration pressure, because they dilate arterioles more than venules; in the kidney, they tend to promote diuresis. For Thiazolidinediones (glitazones), which have been used to treat patients with *diabetes mellitus* type 2, the exact mechanism is not known. It has been shown that steady state levels of several proteins involved in renal sodium and water transport are increased. Note that thiazolidinediones are PPAR γ agonists. Activated by endogenous lipids like fatty acids, PPAR γ is a nuclear receptor regulating expression of a large number of genes. Obviously, some of these gene products affect handling of Na^+ and water.

5. DISORDERS OF POTASSIUM HOMEOSTASIS

Disorders of potassium balance may occur by losses, retention or excessive administration of potassium or, rarely, by a very low-potassium diet over a long time. In addition, imbalances may occur by potassium shifts between intra- and extracellular volume.

While exact regulation of Na^+ is required for its importance in determining extracellular volume, exact regulation of K^+ is essential for its role in membrane excitability. Membrane potential is foremost a K^+ -potential, because at equilibrium, membrane permeability is highest for K^+ , which trickles out of the cell following its concentration gradient. High intracellular K^+ concentration is maintained by Na-K-ATPase, which is not simply chugging along, but rather modified in its activity by external factors. Insulin, epinephrine via β_2 receptors and aldosterone make it pump harder. Acidosis reduces the pump's activity. Pondering this, we

realize that we have to expect shifts in potassium once we use drugs like insulin, beta-blockers, β_2 -adrenergic agonists or aldosterone antagonists.

With a potassium-rich meal, we take up a high load of K^+ , while K^+ concentration in blood is low and needs to be kept strictly within a narrow range. How do we manage to do that? Short-term, the K^+ taken up is shoveled into cells. This is strongly assisted by insulin, which increases following a meal. Insulin is secreted into portal blood, reaches the liver at high concentrations and helps to move a large part of the K^+ load into hepatocytes. Plasma K^+ increases a little, yet after an hour, most of it has vanished into cells. Longer term, the kidney is able to smooth over the strong ups and downs of potassium intake with food. This is done via the collecting tubule, which is able to secrete large amounts of potassium via the ROMK channel of principal cells, or to reabsorb almost all K^+ by α -intercalated cells. A healthy kidney has an enormous K^+ -excreting capacity.

In response to prolonged nutritional K^+ deprivation, the apical membrane of intercalated cells, which contains H^+ - K^+ -ATPase pumping in K^+ from the lumen in exchange for protons, gets amplified much like in parietal cells of the stomach.

Plasma K^+ concentration may also be perturbed by intense exercise. In a fast sprint, we use almost all of our muscles. At depolarization, Na^+ streams into muscle cells, at repolarization, K^+ pours out. Concurrent sympathetic activation guards against an excessive increase in plasma K by prompting other cells, e.g., hepatocytes or cells of inactive muscles, to take up more of it. Once the sprint is over, epinephrine still remains active for a while. In summary, K^+ tends to increase during strenuous exercise, but tends to decrease following cessation.

Regarding the effects of changes in extracellular K^+ , we may reflect as follows:

Extracellular hypokalemia should expedite the flow of K^+ out of cells, at least, if this change occurs within a short time frame. This increases membrane polarization. With membrane potential farther from the threshold, it is harder to trigger an action potential.

Extracellular hyperkalemia should impede K outflow and reduce membrane polarization. That should facilitate triggering action potentials in neurons and muscle cells. Unfortunately, it's not as simple as that. [As we have seen in cardiac pacemaker cells](#), at low polarization, Na^+ channels sometimes fail to regenerate after an action potential, reducing the probability of further action potentials.

At this point in our considerations, we give up and content ourselves with the general assessment that deviations of plasma K concentration in both directions may cause arrhythmia and weakness in skeletal muscle.

Acidosis leads to hyperkalemia. Low pH inhibits Na-K-ATPase, leaving more K outside in extracellular space. Inside cells, increased protons displace K ions from proteins so that more K^+ exits from cells. Likely, these effects are most prominent in the large volume of muscle tissue. In addition, acidosis reduces renal excretion of K^+ in spite of increased plasma

concentrations: acidosis reduces the open-probability of the apical K^+ -channel of principal cells.

Alkalosis leads to hypokalemia. This is mainly due to uptake of K^+ into cells, but in addition, more of this intracellular K^+ is secreted into the tubulus by cells of the collecting tubule.

Hyperkalemia

Causes: A single high K^+ reading in routine labs is no reason to panic. Frequently, this is caused by artificial hemolysis due to improper blood drawing or sample handling, resulting in release of K^+ from red blood cells.

Yet, K^+ released from lysing cells may cause actual hyperkalemia *in vivo*. Examples are:

- tumor lysis syndrome in the initial phase of chemotherapy
- burns
- crush injuries
- rhabdomyolysis

A classical cause for hyperkalemia due to a shift of K^+ out of cells is ketoacidosis in a patient with diabetes mellitus type 1. In this situation, both lack of insulin and acidosis contribute to the inhibition of Na-K-ATPase.

Pharmacology cross reference: some drugs have a tendency to induce hyperkalemia:

- Beta blockers
- Digitalis cardiac glycosides, which directly inhibit Na-K-ATPase, leaving K^+ outside
- potassium-sparing diuretics: aldosterone antagonists, triamterene or amiloride
- Trimethoprim blocks ENaC, as well
- to a lower extent, ACE-blockers and AT_1 -antagonists

Due to the kidneys' enormous capacity to excrete K^+ , persistent hyperkalemia is only possible if this function is impaired. Conversely, as our kidneys usually secrete K^+ , hyperkalemia is typical in end stage renal disease.

Symptoms of hyperkalemia are fairly unspecific, including malaise, muscle weakness, arrhythmia. Sudden cardiac arrest or ventricular fibrillation is possible. ECG changes start with a "tented", tall and peaked T wave, then the p wave flattens or disappears and the QRS complex widens to a pattern reminiscent of a bundle branch block.

Therapeutic options include fast and longer-term measures. The fastest way to stabilize membrane potential is careful injection of Ca^{2+} . Ca^{2+} ions impede Na^+ channels, making it harder to trigger action potentials. Administration of insulin, together with glucose to prevent hypoglycemia, moves extracellular K^+ into cells. In case hyperkalemia is combined with metabolic acidosis, it may be corrected by bicarbonate: infusion of HCO_3^- helps to move K^+ into cells in exchange for H^+ . Furosemide, perhaps concomitantly with an infusion of NaCl, will increase excretion of K^+ provided the kidney is still in a state to do that. Nonabsorbable

cation exchange resins, introduced orally or by enema into the gastrointestinal tract, may bind K^+ and remove it from the body. In end stage renal disease, dialysis is the only way to keep K^+ values down.

Hypokalemia

Causes: Hypokalemia may result from losses of potassium from the body, or from moving extracellular K^+ into the cells.

Losses may occur in the following situations:

- diarrhea or repeated vomiting, as gastrointestinal secretions contain a considerable amount of K^+
- hyperaldosteronism, in the majority of events secondary to loop diuretics or thiazides or by high-dose glucocorticoid therapy with overwhelming of 11β -HSD

Intense exercise on a warm, humid day may cause hypokalemia by a combination of K^+ -losses by sweating and uptake of K^+ into cells by adrenergic stimulation.

Shifting of K^+ into cells may be caused by administration of insulin or β_2 -sympathomimetic drugs. For example, using solely insulin to treat a diabetic patient with high blood glucose may result in hypokalemia. Instead, insulin should be accompanied by a K^+ -containing infusion, which has the additional benefit of filling up volume.

Strong sympathetic activation by itself may lead to hypokalemia as well, e.g. by the combination of a drop in blood pressure, pain and fear in myocardial infarction.

Symptoms: muscle weakness and cardiac arrhythmia, e. g., *torsades de points*. In ECG, a flattened T wave merges in a prominent U wave.

For **therapy**, it is important to figure out what caused hypokalemia in this particular patient. In case hypokalemia has been the result of a shift of potassium into cells, it has to be expected to get out again at some point in time. Filling up K^+ too forcefully risks hyperkalemia later on. This is of little concern in hypokalemia by chronic losses, where intracellular K levels are on the low side, either.

Therapy at first sight looks easy: can't we just fill up the K^+ deficit? Two things have to be kept in mind when trying to do this by infusion:

- K^+ has to be properly diluted and the infusion has to run slowly. Blood from the veins reaches the heart very quickly, and locally increased K^+ concentration there would wreak havoc with cardiac excitability.
- In addition, extracellular K^+ is one of the main pain-generating agents. Consequently, an infusion running too fast may cause pain or discomfort along the patient's vein.

Therefore, if critical hypokalemia needs to be treated quickly, it is advisable to do this by central venous access in combination with heart monitoring. The infused solution should not

contain components like glucose or HCO_3^- that might shift additional K^+ into cells. Rapid administration should proceed only until plasma K^+ approaches the lower end of the normal range (about 3 mmol/l); then there is enough time to complete the task slowly.

The safer route is to fill up by oral administration. This is best done by having the patient swallow a solution containing KCl, although this treatment is not popular, as the solution tastes quite awful.

6. GLUCOSE AND DIABETES

Glucose is freely filtered at the glomerulus. At physiological concentrations of around 100 mg/dl (5.5 mM), that is a lot of valuable energy-containing fuel: 1 g for every single one of the 180 liters of filtrate we produce in a day. Still, under normal circumstances, we excrete no glucose at all. Virtually the entire load is reabsorbed in the proximal tubulus. In the early part of the proximal tubulus, this is an easy process. Low affinity/ high capacity Na^+ -Glucose transporter 2 (SGLT2, SLC5A2) shovels most of it into the cells, from where it passes the basolateral membrane via GLUT2-mediated facilitated diffusion. What glucose remains in the lumen is taken up in the downstream part of the proximal tubule by the high-affinity/ low capacity SGLT1 transporter, which uses the energy of two Na^+ -ions to push in the glucose unit. Here, the basolateral back door is GLUT1.

Being a transporter-mediated process, glucose uptake is saturable. Once plasma glucose concentration exceeds 250 mg/dl (14 mM) in diabetics, the two cotransporters work at capacity, and incremental glucose is excreted, causing osmotic diuresis. This has given the disease its name: *diabetes mellitus* is Greek for "honey-sweet flow-through".

Pharmacology cross reference: In patients suffering from diabetes mellitus type 2, one treatment option is to inhibit SGLT2 by specific blockers such as Dapagliflozin or Empagliflozin. Although backup transporter SGLT1 works like crazy, it is not able to fully compensate for the loss of SGLT2 activity. Lots of glucose is excreted via the urine, taking water with it. This lowers glucose levels by decreasing the threshold for excretion to values well below the normal 250 mg/dl. Compared to other treatments, the advantage is that excreted glucose cannot any more be metabolized to fat. In many cases, weight loss is an added benefit. The disadvantages: loss of glucose may lead to tiredness, excessive weight loss, dehydration and ketoacidosis. In addition, bacteria in the urinary tract are well fed by all this glucose, increasing the probability of urinary tract infections.

Don't have patients trouble taking up glucose from the intestines? Glucose uptake in the small intestine is not affected by SGLT2-blockers, as it is mediated by SGLT1. Although SGLT1 and -2 are closely related, it would take a thousandfold concentration of Dapagliflozin to inhibit SGLT1.

7. PROTEINS AND PEPTIDES

Filtrated proteins and peptides are 100% reabsorbed in the proximal tubule. We need to realize that "reabsorption" means something different for proteins than for electrolytes or glucose. Once "outside" by filtration, proteins cannot reenter as such, they have to be broken down and digested to amino acids. Still, amino acids are valuable enough to be retrieved.

Most proteins and peptides are bound and endocytosed by a receptor complex combining the three transmembrane proteins megalin-cubilin-amnionless. For proteins and peptides small enough to be filtered, renal elimination is a determining factor for their plasma levels. Via this mechanism, end-stage renal disease can lead to elevated levels of glucagon, gastrin and ANP and may further increase secondary hyperparathyroidism.

8. ACID-BASE HOMEOSTASIS

The pH of our blood, one of the most closely regulated parameters in our body, is about 7.4. In most other fluid compartments, it is very near that value. It is essential that pH remain near 7.4, because the conformations of our proteins and thus, the activities of many enzymes, are very sensitive to changes in pH.

In diluted solutions like our fluid compartments, pH approximates the negative of the logarithm to base 10 of the concentration of hydrogen ions in mol/l. In other words, the hydrogen ion concentration in our blood plasma is $10^{-7.4}$ mol/l, that is 0.000 000 04 mol/l or 40 nmol/l. This 40 nM proton concentration is a dynamic equilibrium, resulting from an ever ongoing flourishing trade in protons by thousands of different donors and acceptors. At every point in time, a tiny fraction of protons belongs to nobody and is hurry-scurrying about: only this fraction determines pH. The situation is complicated by the fact that this trade is conducted in compartments separated by biological membranes. Some of the traders, like CO_2 , can pass these membranes effortlessly, while others, like phosphate, cannot. Furthermore, proton trade across membranes can't go on for long unless it satisfies the requirement for electroneutrality. Unfortunately, our mind is very ill-equipped to handle this complex trade involving thousands of interdependent dynamic equilibria in an open system. Even our mathematical models are inadequate. Our "knowledge" stems from experiments where we try to vary the concentration of the biggest traders while keeping everything else as constant as possible.

The biggest trading house in our body's proton market is CO_2 & HCO_3^- . Luckily for us, the acidic one of its two partners has the habit of vanishing into thin air, and if he didn't, the trade would go sour very soon. Each day, we eliminate 15,000 mmol of CO_2 , a potential acid, via the lungs.

Recovery of HCO_3^-

On the other hand, the buffering partner HCO_3^- , our main defense against acidification, is in danger of vanishing via the kidney back door. Each liter of primary glomerular filtrate contains 24 mmol HCO_3^- . At 180 liters per day, that is $180 \times 24 \text{ mM} = 4320 \text{ mmol}$, or in the range of 4-5 moles. Loss of any appreciable part of this would result in catastrophic metabolic acidosis: complete reabsorption is therefore essential. This is accomplished by secreting H^+ into the tubule, converting HCO_3^- to CO_2 , which is easily reabsorbed. This process is aided by carbonic anhydrase IV, which is GPI-anchored to the outside of the apical membrane, and carbonic anhydrase II inside the cell, which catalyzes the opposite reaction. At the basolateral membrane, HCO_3^- is exchanged for Cl^- or, following its gradient, cotransported out together with Na^+ . This is accomplished via the electrogenic Na-HCO_3^- cotransporter NBCe1 (N for Na^+ , B for bicarbonate, C for co-transporter, e for electrogenic, SLC4A4). The carrier is able to transport in both directions. The amount of HCO_3^- generated in the proximal tubule cell is such that three HCO_3^- push a reluctant Na^+ out of the cell. Eighty to ninety percent of HCO_3^- is recovered in the proximal tubule, the rest by α -intercalated cells in the distal tubule and collecting ducts.

H^+ is extruded into the lumen via three mechanisms. The first two of them are active in both the proximal and the distal tubule, while the third one is restricted to distal tubule and collecting ducts:

1. The Na-H exchanger (NHE) is driven by the steep lumen-to-cell Na^+ gradient in the proximal parts of the nephron. It mediates the majority of H^+ transport but cannot build a strong proton gradient, i.e., a lower pH in the lumen than in the cell.
2. An electrogenic H^+ pump uses ATP to build a lumen-positive proton gradient. This pump is expressed in all tubule segments, but highest expression levels are found in α -intercalated cells of the cortical collecting tubule and the medullary collecting duct.
3. An electroneutral $\text{H}^+\text{-K}^+$ -pump in the distal tubule uses ATP to exchange luminal K^+ for H^+ . This system may contribute to the generation of hyperkalemia in metabolic acidosis.

Thus, the majority of H^+ is secreted in the proximal tubule, but if a proton gradient is required, this is built up in the distal tubule by α -intercalated cells. With all these mechanisms working at capacity, urine pH may reach a value as low as 4.4; that is a thousand times the H^+ concentration in blood (40 $\mu\text{mol/l}$ compared to 40 nanomol/l in blood). That sounds and is impressive, yet quantitatively, these 40 $\mu\text{mol/l}$ sink into insignificance compared to the 4-5 moles of recovered HCO_3^- and, as we will appreciate in a minute, compared to the up to 300 mmol of acid equivalents eliminated in the form of NH_4 per day. The kidney is thus able to excrete acid equivalents over and above the recovery of HCO_3^- . If there is a problem with acid excretion in an otherwise functioning kidney, we speak of **renal tubular acidosis**, which may result from many genetic as well as acquired causes.

However, the kidney is also able to cope with an alkaline challenge, e. g., loss of acid by vomiting. In this case, it simply reduces H^+ secretion so that a corrective amount of HCO_3^- is excreted with the urine.

Pharmacology cross reference: Acetazolamide blocks carbonic anhydrase and strongly inhibits HCO_3^- reabsorption, leading to the excretion of alkaline urine.

Excretion of NH_4^+

On a typical Western diet, our metabolism produces a surplus of about 70 mmol H^+ as nonvolatile acids per day, which we have to eliminate to prevent acidosis. This is mainly the result of a diet rich in proteins. The more we rely on proteins to satisfy our energy requirements, the more nitrogen we have to dispose of and the more acidity we produce. In the liver, gluconeogenesis produces waste from clipped-off amino groups, which can be disposed of in two ways.

1. Usually, and depending on acid-base status, more than 90% are detoxified still in the liver by integration into urea. While far more complicated in detail, the process boils down to fusing two proton donors in the form of NH_4^+ and one proton acceptor in the form of HCO_3^- into one electroneutral molecule of urea, leaving one acid equivalent on the table. So, this process consumes HCO_3^- .
2. The liver's second option to dispose of nitrogen is by packaging toxic NH_4^+ in non-toxic glutamine for transport. The kidney retrieves the NH_4^+ . The proximal tubule cell cleaves two ammonium ions from glutamine, leaving α -ketoglutarate. The NH_4^+ is secreted. Two units of α -ketoglutarate and 4 H^+ from 4 $CO_2 + 4 H_2O$ are combined to glucose, leaving 4 HCO_3^- . Therefore, for each NH_4^+ secreted into the tubule lumen, one HCO_3^- leaves the cell at the basolateral membrane. Gluconeogenesis with integrated acid secretion, in fact energy extraction coupled with waste disposal: really genial! Whether HCO_3^- is actually gained in the kidney, or rather previously in the liver, by obviating the need to consume it in urea synthesis is a matter of perspective. In summary, secretion of acid equivalents in form of NH_4^+ saves HCO_3^- that would otherwise be consumed in urea synthesis.

Because the pK_a of NH_4^+/NH_3 is 9.2, almost all of it is in the ionized form at pH 7.4 (At pH 7.2, 1% would be NH_3). While NH_3 readily passes most cell membranes, NH_4^+ does not. As the luminal fluid usually becomes more acidic in the later sections of the tubule, it becomes progressively more difficult for NH_4^+ to slip back through the membrane during the ultra-short time frames it adopts the form of NH_3 ; NH_4^+ thus becomes trapped and can be excreted. The lower the urine pH, the higher is the ammonium excretion.

The kidney builds a considerable ammonium gradient in the medulla, which helps to excrete ammonium and, thereby, acidity. The plasma membrane of the thick ascending limb is relatively impermeable for NH_3 . Here, NH_4^+ is reabsorbed by taking the slot of K^+ in the Na-K-2Cl-cotransporter and in K^+ -channels. At the higher intracellular pH, it is easier for

ammonium to leave the cell at the basolateral side in the form of NH_3 , leading to accumulation of NH_4^+ in the interstitium of the renal medulla: the deeper into the medulla, the higher the concentration of NH_4^+ . This gradient leads to net secretion into the descending parts of the nephron and a bypass of the cortical portions of the distal nephron.

This way, it is possible to excrete higher percentages of the relatively toxic ammonium. In addition, with every molecule of NH_4^+ , an acid equivalent leaves the body.

Excretion of titratable acids

Some of the protons extruded into the lumen are buffered by acceptors like HPO_4^{2-} (pKa of H_2PO_4^- : 7.2), urate (pKa 5.75) and creatinine (pKa 5.0). They are called titratable acids as the amount of buffered protons in acidic urine may be determined by measuring the amount of sodium hydroxide required to bring pH back up to 7. NH_4^+ is not considered a titratable acid, because with a pKa of 9.2, it does not release its proton in this maneuver.

If increased amounts of protons need to be excreted, titrated acids are of little help. Phosphate buffering, the most important, is limited by the amount of phosphate present in plasma. Only in case of ketoacidosis, ketoanions like β -hydroxybutyrate may contribute to a considerable increase in titratable acids.

Thus, in acidosis, the predominant way to dispose of additional acid equivalents is by increasing excretion of ammonium. Ammonium excretion may increase up to 300 mmol per day.

"Acid ash" diet does not lead to osteoporosis.

There is a common and often-repeated misperception that, via generation of acid equivalents, a Western diet contributes to [osteoporosis](#). There is no scientific basis for this assertion. While it is true that osteoclasts break down bone substance with the help of acidification, as we will [discuss](#) when dealing with [bone metabolism](#), osteoclasts are careful to limit this acidification to their very small operating range. And while there are good reasons to be critical of our Western diet, the production of acid equivalents is not one of them. Healthy kidneys are perfectly able to dispose of the 70 mmol per day of non-volatile acidity resulting from a Western diet; in fact, acid elimination could be ramped up to a multiple of that. Of course, blood and bone marrow pH remain the same, irrespective whether people are stuffing themselves with sausages or prefer a vegan diet. Sausage stuffers have higher blood pressure, more atherosclerosis and more myocardial infarctions, but not more osteoporosis.

The situation is very different once there is a serious problem with renal acid elimination. Renal tubular acidosis, which may be due to genetic causes or may be secondary to, e.g., autoimmune diseases or sickle cell anemia, leads to osteomalacia or to rickets in children. In this case, acid equivalents are in fact buffered by phosphate from [hydroxyapatite](#), which is thereby dissolved.

pH-dependent excretion of pharmaceuticals: weak acids and bases

Excretion of weak acids and bases depends on pH, as their non-ionic forms pass the membranes of tubulus cells a lot more easily than their charged forms. Consider acetylsalicylic acid as an example. At low urine pH, most of it is protonated and diffuses back into the blood. At high urine pH, it is ionized as acetylsalicylate, stays in the tubulus lumen and is excreted. The opposite is true for a weak base like quinine. As many pharmaceuticals are weak acids or bases, urine pH has a profound influence on their excretion. For example, an overdose of acetylsalicylic acid may be treated by alkalinizing the urine by an infusion of HCO_3^- .

Elimination of organic anions and cations

The late proximal tubule secretes several organic anions and cations. Among the anions are oxalate, α -ketoglutarate, bile salts and liver-manufactured glucuronate and sulfate conjugates. The yellowish color of normal urine is mainly due to bilirubin-derived metabolites such as urobilin. Among secreted cations are dopamine, epinephrine and norepinephrine. Therefore, the rate of production of catecholamines may be determined from their concentration in 24-hour urine.

Correction of acidosis and alkalosis

Respiratory acidosis:

An increase in arterial P_{CO_2} is compensated by increased renal H^+ secretion, which translates to production of new HCO_3^- via excretion of NH_4^+ . In chronic respiratory acidosis, the capacity of this mechanism is ramped up by induction of the apical Na-H-exchanger and the basolateral Na- HCO_3^- cotransporter. This adjustment leads to a new equilibrium at an increased HCO_3^- concentration.

Respiratory alkalosis:

In response to reduced P_{CO_2} , the kidneys reduce H^+ secretion, leading to secretion of a corrective amount of HCO_3^- with the urine.

Metabolic acidosis:

If, for some reason (e.g., lack of oxygen), tissues are incapable of metabolizing glucose or fatty acids to CO_2 and water with the help of mitochondria, they have to resort to anaerobic glycolysis; the result is lactic acidosis. Prolongued absence of insulin action makes the body switch into hunger mode: the result is ketoacidosis. Whatever the cause, increased acid equivalents are first buffered by HCO_3^- , generating CO_2 and H_2O . The first response is increased ventilation to reduce P_{CO_2} . In many cases, metabolic acidosis is the result of impaired renal function; of course, this precludes renal compensation. Yet, in chronic

diarrhea, lactic acidosis or diabetic ketoacidosis, metabolic acidosis is caused by extrarenal factors. In these cases, the kidneys react via the same lines as in respiratory acidosis: H^+ secretion is increased via induction of Na-H-exchanger and electrogenic H^+ pump, combined with induction of the basolateral $Na-HCO_3^-$ cotransporter. In addition, NH_4^+ production is increased by activation and induction of the enzymes involved in liver and kidney, enabling elimination of more acid equivalents which translates to generation of more HCO_3^- .

Base excess

Base excess allows estimating the magnitude of metabolic deviations from acid-base equilibrium. Base excess is defined as the amount of acid that must be added to each liter of blood to return the pH to 7.40 at standard respiratory conditions (100% oxygenation and P_{CO_2} 40 mm Hg at 37°C). In the absence of any metabolic deviation, there is nothing to titrate; reference range is 0 ± 2 mEq/l. Somewhat incongruous, the "excess" may be positive or negative. Positive values > 2 mEq/l indicate metabolic alkalosis, negative values < -2 mEq/l metabolic acidosis.

For all practical purposes, base excess is not assessed by separate titration. Rather, it is calculated in an arterial blood gas test, which is performed anyway in case of acid-base problems.

[Just to illustrate that: base excess is calculated with pH and P_{CO_2} as input, from a combination of Siggaard-Anderson and Hendersen-Hasselbalch equations:

$$base\ excess = 0.02786 \times P_{CO_2} \times 10^{(pH - 6.1)} + 13.77 \times pH - 124.58]$$

The best treatment of acidosis is correction of the underlying cause. Sometimes, however, metabolic acidosis needs urgent correction. In this case, base excess helps to assess the required amount of buffer. What is the fluid volume we need to take into consideration? Of course, it is not possible to correct only "the blood". A better approximation would be extracellular fluid volume: in our 70 kg female, that would amount to 14 liters and in the respective 70 kg male, to 17 liters. Yet, part of our buffer, sodium bicarbonate, equilibrates with intracellular volume which is also affected by acidosis. Therefore, 30% of body weight is assumed as target volume, or 21 liters in our 70 kg standard persons. As blood is only part of that volume, the calculation of base excess is slightly modified. Hemoglobin acts as a buffer itself. *Standard base excess* refers to a hemoglobin concentration of 5 g/dl, down from 12-18 g/dl in blood, to better reflect the properties of the target volume. If metabolic acidosis with a standard base excess of -10 mEq/l needs to be corrected in our 70 kg person, the amount of $NaHCO_3$ required is approximately $70 \times 0,3 \times 10 = 210$ mmoles. Caution! This type of correction is a critical procedure reserved for specialists, as it entails considerable risks (rapid increase in P_{CO_2} , risk of overcorrection, K^+ -shifts, osmotic effects like hypernatremia. Typically, half the dose is administered very slowly, followed by reassessment of the situation).

Anion gap

The size of the metabolic deviation is important, yet it is of little help in determining the cause of metabolic acidosis in the individual patient. The next useful step in this regard is determining the anion gap. Plasma electroneutrality implies that the number of anions equals the number of cations. The majority of these ions are "visible" in routine labs: Na^+ , K^+ , Cl^- , and HCO_3^- . A small remainder is invisible. In the patient with acidosis, could an unusual amount of acids hide in this invisible corner? Lactate, for example, or maybe ketone bodies acetoacetate or β -hydroxybutyrate? Well, it is possible to estimate the amount of these anions by comparing anion and cation concentrations. In doing this, we are lazy, ignoring K^+ : its concentration is small anyway, and we are not going to fuss about a constant error of 3%. There we go:

$$\text{Anion gap} = [\text{Na}^+] - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

Normally, this value equals 12 ± 2 , reflecting...Um, what again? Anions, of course, for example phosphate, but that's small,...Oops, almost forgot the proteins! Proteins bear positive and negative charges, yet in total, more negative ones. Think of albumin's 15 negative net charges. In fact, albumin constitutes no less than 60% of plasma protein. Accordingly, the normal anion gap of 12 is mainly albumin. But, albumin may be lost if there is a problem with the glomeruli, albumin synthesis may decline in liver disease: we will have to take it into account if albumin values are in any way unusual.

Acidosis with increased anion gap: if albumin is normal, yet the anion gap is increased, the plasma obviously contains an unusual amount of acids. We have to think about lactic acidosis, ketoacidosis, uremia and a number of alternative causes.

Acidosis with normal anion gap: in this case, HCO_3^- has been reduced without concomitant addition of acids. Possible causes include HCO_3^- losses by chronic diarrhea or renal tubular acidosis.

Metabolic alkalosis:

Among typical causes are persistent vomiting (as in bulimia) or treatment with diuretics. Furosemide or thiazides activate aldosterone, which exchanges not only K^+ , but also H^+ for Na^+ in the collecting duct. Interstitial HCO_3^- at the basolateral side of proximal tubule cells is increased: more HCO_3^- leaks back into the tubule lumen via the paracellular route. In addition, the increase depresses the transport rate of the Na-HCO_3^- cotransporter, causing HCO_3^- to back up in the cytosol. So, intracellular carbonic dehydratase, too, has to push against the backlog of its product, generating lower amounts of H^+ for secretion into the tubule lumen. Apart from this effect in the proximal tubule, an additional adaptation is found in the cortical collecting tubule: a shift in the ratio of α to β intercalated cells, increasing the proportion of β cells. While α cells pump H^+ into the lumen and release HCO_3^- into the blood, β intercalated cells have the opposite orientation, secreting HCO_3^- . Thus, in metabolic alkalosis, the cortical collecting duct may switch from acid secretion to HCO_3^- secretion.

Volume contraction stimulates H⁺ secretion

Volume contraction activates the RAAS and sympathetic systems. Both Angiotensin II and norepinephrine stimulate Na-H exchange in the proximal tubule, and aldosterone enhances H⁺ secretion in the collecting duct. By these mechanisms, volume depletion not only lowers urine volume; it lowers urine pH, too, which may contribute to stone formation. Calcium oxalate stones and [uric acid](#) stones form especially at low urine pH (in contrast, calcium phosphate stones form at increased pH).

Hypokalemia stimulates H⁺ secretion

In the proximal tubule, K⁺ depletion activates Na-H exchange and electrogenic Na-HCO₃⁻ cotransport. In addition, K⁺ depletion activates K-H exchange by α -intercalated cells in the collecting tubule. Hypokalemia may thus lead to metabolic alkalosis.

9. DISEASE MECHANISMS AFFECTING GLOMERULAR FUNCTION

In a young adult, every kidney contains about one million glomeruli. Age is the invincible enemy of our glomeruli: the older we become, the more glomeruli we lose. These microfilters can only function if they

- constantly receive an adequate flow of blood and
- remain structurally intact

Problems with blood flow may be due to atherosclerotic lesions, to hypovolemia, to hypertension or to other causes, which we deal with elsewhere.

Structural alterations have many causes as well. For example, arterial hypertension or diabetic metabolic state over time results in structural damage and loss of glomeruli. Many of the mechanisms leading to damage of the glomerular structure are immune in nature. As we have seen when dealing with our defense system against microbial invaders, our immune system commands a range of sharp weapons. The primary route of delivery of complement components, neutrophils, monocytes, antibodies and T cells is via the blood. Accordingly, plenty of these tools reach the glomeruli and in addition, there is ample opportunity for them to get stuck in the filter. Depending on where exactly the primary damage occurs, clinical symptoms may vary widely.

Damage to podocytes: mainly nephrotic glomerular diseases

Membranous glomerulopathy

Recall that the slit diaphragm is the part of the filter with the tightest pores. Antibodies may thus reach the capillary-facing side of the podocytes, even when slit diaphragms are intact. If any of these antibodies cross-react with structures on the podocyte surface, complement may be activated and podocytes may react to the ensuing damage by retracting their foot processes. Consequently, the slit diaphragm disappears, while the basement membrane part of the filter

remains largely unaffected. Proteins are filtrated in great quantities, but blood cells are unable to pass. Now, antibodies reach the lumen-facing side of podocytes as well.

Normally, with complement activation, small cleavage products like C3a, C4a, and C5a diffuse into the surrounding tissue and attract leukocytes. Here, in the midst of a rushing plasma cascade, any chemoattractant is washed away. Accordingly, no inflammatory infiltration is seen in light microscopy.

Yet, antibody and complement deposits sit on the basement membrane-facing side of the podocytes, and podocytes react by producing more basement membrane material. Basement membranes consist of proteins like collagen type IV and laminin, as well as polysaccharides. The polysaccharide component can be visualized with periodic acid Schiff (PAS)-staining. Basement membranes appear markedly thickened, and analogous changes may be seen in electron microscopy. In fluorescence microscopy for IgG or complement, a grainy structure of staining follows the glomerular basement membrane.

Emphasized membranes in the absence of leukocyte infiltration have led to the morphological designation membranous glomerulopathy. The term membranous glomerulonephritis is used frequently, too, but is somewhat misleading as there is no inflammatory infiltration.

Which podocyte structures are targeted by antibodies? In majority of cases, it is either a phospholipase-A₂ receptor or neutral endopeptidase (aka CD10 or CALLA). In children, antibodies against nutritional bovine serum albumin from cow milk or beef have also been shown to play a role. Having passed the intestinal barrier, maybe via M cells, the protein is modified to a cationic state. In the cationic state, it becomes implanted in the capillary wall near the surface of podocytes, which is studded with numerous negative charges. There, it is attacked by antibodies against the foreign protein.

Nephrotic syndrome:

With membranous glomerulopathy, the patient usually loses large amounts of proteins of all sizes (thus including IgG) in the urine, but no erythrocytes or other cells. Note that nephrotic syndrome also may be caused by marked, isolated loss of albumin. Nephrotic syndrome is characterized by loss of plasma protein combined with Na⁺ retention and edema. What is cause and what is effect? This is debated and may vary from case to case:

Underfill hypothesis: Loss of protein reduces intravascular oncotic pressure, so that fluid is lost to the interstitium. Vascular underfill activates the renin-angiotensin-aldosterone system causing compensatory Na⁺-retention.

Overflow hypothesis: Increased glomerular filtration of proteins causes damage to the nephron resulting in primary Na⁺ and fluid retention, potentially via ANP resistance. Increased intravascular volume leads to overflow and secondary edema formation. Several clinical and experimental observations seem to support this view. In many cases with edema formation, oncotic pressures of plasma and interstitium go down in parallel, leaving

the differential constant. If patients are treated with glucocorticoids, frequently edema retreats before plasma albumin increases.

In nephrotic syndrome, GFR usually remains normal, but may be reduced in some cases.

To interfere with proliferation of and antibody production by B cells, treatment of membranous glomerulopathy includes intensive immunosuppression.

Damage to podocytes resulting in nephrotic syndrome may come in two additional variants, "light" and "heavy":

- light functional damage: minimal change disease
- massive destruction: primary focal segmental glomerulosclerosis (may also develop without causing nephrotic syndrome if glomerular destruction stops filtration of proteins)

In both cases, antibodies are usually not detected; therefore, the causative damage must be of a different nature. Severity, rather than quality of the damaging mechanism may determine which of the two forms develops.

Minimal change disease

By light microscopy, the glomeruli look normal, neither antibodies nor complement are detected by fluorescence microscopy. It is speculated that podocytes react to soluble cytokines by retracting foot processes, a change seen only in electron microscopy. Why cytokines? Usually, the patients enter remission with glucocorticoid therapy and as we have learned, glucocorticoids inhibit expression of many cytokines. Frequently, glucocorticoid withdrawal is followed by relapse.

Primary focal segmental glomerulosclerosis

This disease is characterized by necrosis and loss of podocytes, which cannot be replaced. The result is massive flow through the basement membrane. Proteins too big to pass through the pores of the basement membrane get stuck and accumulate until the filter is completely clogged. "Glomerulosclerosis" indicates collapse or obsolescence of glomerular capillaries. "Segmental" indicates this involves part of the tuft of an individual glomerulus; "focal" means less than 50% of glomeruli are affected as judged by light microscopy.

Damage to the entire capillary wall: mainly nephritic symptoms

(Diffuse) proliferative Glomerulonephritis

Any filter is prone to collect particles; if soluble immune complexes circulate in the blood, lots of them are bound to end up in the glomerular basement membrane. As there is no basement membrane separating capillary and mesangial space, immune complexes also enter the mesangium. Alternatively, immune complexes may assemble locally in the basement membrane if antibodies bind any of its components.

Typical examples for the entrapment of circulating immune complexes are poststreptococcal glomerulonephritis and lupus nephritis. Complement is activated and C3a and C5a are generated in the immediate vicinity of endothelial cells and leukocytes. Endothelial activation

causes neutrophils and monocytes to attach and to wriggle their way between endothelial cells to phagocytize the complexes. Some of these leukocytes accumulate in the mesangium. In light microscopy, increased numbers of cells or nuclei are visible, which enticed pathologists of yore to describe the process as "proliferative". Although this has proved incorrect, as it is infiltration rather than proliferation, the name has stuck. Activated macrophages secrete IL-1 β , TNF α , IL-6 and release their protease-containing granules. Locally, the basement membrane is damaged or broken down, causing erythrocytes to pass the filter.

Nephritic symptoms:

The damage to the basement membrane causes hematuria; erythrocytes and casts are found in the urine sediment. Proteinuria is present but in many cases remains less pronounced than in nephrotic syndrome, because not all glomeruli are damaged: edema may or may not form. Depending on the extent of damage to the glomeruli, GFR may range from normal to markedly reduced. RAAS activation may cause hypertension.

This diffuse proliferative glomerulonephritis ("diffuse" means most glomeruli are affected, as opposed to "focal") is typically seen in postinfectious glomerulonephritis and frequently resolves within a few weeks, after the invading pathogen has been eliminated. However, if formation of immune complexes does not stop, another type of morphology develops:

Membranoproliferative glomerulonephritis

If infections become chronic, as in viral hepatitis, or in case of chronic autoimmune processes like systemic lupus erythematosus, the load of immune complex deposits increases over time despite the effort of immigrating macrophages to clear the stuff out. Endothelial cells attempt to compensate by synthesizing a new basement membrane over the piles of immune complex debris. In the microscope, the thickened walls of capillaries seem like bands of amorphous material. In PAS stains, typical "tram track" double contours may be seen: two PAS-positive basement membranes are separated by a PAS-negative layer of immune complex debris. More visible membranes **and** more cells: membranoproliferative glomerulonephritis. Clinically, hematuria is likely to persist, GFR to come down; prognosis is poor.

A similar picture develops in individuals who, due to genetic factors, suffer from an especially trigger-happy complement system. As we have seen previously, complement is constantly being activated via the alternative pathway, yet reined in on our cell membranes by inhibiting factors like H, I or MCP (CD46). Homozygous defects in any of these regulatory factors result in variant forms of membranoproliferative glomerulonephritis called either **dense deposit disease** or **glomerulonephritis C3**. Erythrocyte lysis may complicate the situation, completing the picture of **atypical hemolytic-uremic syndrome** (aHUS).

Pharmacology cross reference: Eculizumab inhibits complement activation at C5 and may be used to treat aHUS.

IgA nephritis and Henoch-Schönlein purpura

Immune complexes containing IgA1 are the common denominator of these two related diseases. Usually, within only one or two days after the mucosal immune system has been

reactivated by a respiratory or gastrointestinal infection with an agent "seen" previously by the immune system, IgA₁-containing protein complexes cause vasculitis with multiple disseminated small bleeding episodes. If the process is limited to the kidney, hematuria may be the only symptom. If more widely disseminated, the classical Henoch-Schönlein-trias develops with purpura visible on legs and buttocks, arthritis and abdominal pain, with or without hematuria.

The IgA₁ deposited in immune complexes is unusual in that it lacks galactose units normally present near its hinge region. Why glycosylation is deficient in affected individuals is poorly understood. It has been proposed that these galactose-deficient IgA₁-units act as an autoantigen and are bound by naturally occurring anti-glycan IgA₁ or IgG antibodies. These antibodies thus act like rheumatoid factors, resulting in immune complexes consisting of two antibodies. The complexes seem to activate complement indirectly via the lectin or alternative pathway and are recognized by receptors expressed on mesangial cells. Mesangial cells start to proliferate, secrete cytokines and overproduce extracellular matrix components. Prognosis is good in children; in adults, the process tends to become chronic and complications are more frequent.

Goodpasture syndrome: Antibodies directed against the basement membrane

While the diseases mentioned above are [type III hypersensitivity reactions](#) caused by immune complexes, glomerular disease may also be caused by a [type II](#) direct antibody attack. The antibodies are directed against an epitope of the $\alpha 3$ -chain of type IV collagen, which is the main component of basement membranes. Unsurprisingly, many patients develop not only renal but also respiratory symptoms, with shortness of breath, cough, coughing up blood and chest pain. To Yours Truly, it remains unclear why basement membranes of other organs are unaffected.

Glomerular damage is usually severe, with focal necrosis and crescent formation and characteristic linear, not granular deposition of IgG along basement membranes. Accordingly, kidney failure may develop rapidly. For therapy, it is necessary to remove as much antibody as possible by plasmapheresis and to suppress formation of new antibody by aggressive immunosuppression. Regardless, in many patients kidneys are destroyed within months.

Secondary focal segmental glomerulosclerosis with hyalinosis ("renal aging"):

By light microscopy, the disease is often indistinguishable from primary focal segmental glomerulosclerosis due to direct podocyte damage. It is a sign of slowly progressing kidney damage, after nephron loss by any cause has exceeded a certain limit.

The secondary variant reflects the growing strain on remaining glomeruli, which hypertrophy and increase their single nephron glomerular filtration rate. Higher single nephron filtration is reached by opening of the vas afferens, combined with increase in systemic pressure, while the efferent arteriole is constricted. With higher pressure hammering relentlessly at the glomerular capillaries, the capillaries are distended and elongated, increasing the volume of the glomerular tuft and the surface available for filtration. Podocytes are terminally

differentiated cells unable to divide; they only increase in size. Once they are stretched to the maximum, they no longer manage to keep intact all interdigitating foot processes and slit diaphragms, leaving "tears" in the sheet below the basement membrane. At these tears, the larger pores of the remaining basement membrane allow far more protein to rush through. Because of reduced hydraulic resistance, also more plasma volume rushes through. The tubule is overwhelmed by the amount of filtrated protein. The patient develops microalbuminuria, later full-fledged proteinuria. Big proteins like IgM or fibrin are too large to pass even through the bigger pores of the basement membrane; they get stuck, over time clogging the capillary wall with a hyaline deposit.

Increased shear stress and tension occasionally ruptures the weaker part of the glomerular capillary wall towards the mesangium, exposing mesangial matrix components to platelets. Thrombosis, inflammation and organizing reaction follow, leaving behind collapsed capillaries representing an area of segmental glomerulosclerosis in the microscope.

Even before the entire glomerulus collapses, tubules suffer, too. The fall in GFR causes phosphate retention (see FGF23 below). The tears in slit membranes cause filtration of transferrin, which increases iron load on proximal tubule cells; iron promotes generation of reactive oxygen species.

The end is marked by the complete collapse of the glomerulus. Once a glomerulus obliterates, the lack of blood flowing from its efferent arteriole will damage not only that nephron's tubule, which would not matter anymore, but also tubule segments of nephrons in close proximity.

A vicious cycle develops: the more nephrons die, the higher the strain on the remaining ones. Clinically, the process manifests with gradual elevation in creatinine concentration, slowly increasing proteinuria and hypertension. Over time, it leads to end stage renal disease.

10. TUBULOINTERSTITIAL DISEASES

All of the tubules' blood supply stems from blood exiting glomeruli via vasa efferentia. If one glomerulus is lost due to one of the disease mechanisms mentioned above, the loss of the respective vessel loops negatively affects the tubules of several neighboring nephrons.

Apart from problems with blood supply, tubules may be damaged by infections, autoimmune phenomena, toxic effects or inherited conditions.

Pyelonephritis

Infections of renal parenchyma are usually caused by bacteria that cause pyelonephritis via ascending infection from the bladder. In children and adults, this occurs much more frequently in females, due to

- the short female urethra, which allows passage of bacteria, especially during sexual intercourse
- the presence of the vaginal microbiota
- the lack of antibacterial prostatic fluid

Ascending infections require a number of bacterial and host factors to occur, including properties allowing bacteria to adhere to glycolipids of the urothelium, vesicoureteral and intrarenal reflux. Intrarenal reflux is promoted in compound ("twin –peak") papillae, where collecting duct openings are less likely to be closed by the hydrostatic pressure in the pylon. Pyelonephritic scars are therefore typically found at the renal poles, where compound papillae occur.

Pyelonephritis is critical because infection and the inevitable damage from infiltrating neutrophils and macrophages affects a tissue that receives little blood supply and operates under harsh conditions in the best of times. Increases in interstitial pressure by increased vascular permeability further reduce oxygen supply. With severe infection or delayed antibiotic therapy, entire papillae vanish as lost tubules cannot be replaced. What remains is a thin layer of renal cortex filled with useless glomeruli over an empty calyx.

Drug-induced renal impairment and injury

Drugs may interfere with kidney function by vascular effects and by effects on tubule cells. Due to their concentrating effects, renal tubules are especially likely to suffer unwanted injury from drugs. Recall once more that cells in the loop of Henle and in the medullar collecting ducts, while metabolically highly active, reside in a hyperosmotic, hypoxic microenvironment. In addition, many drugs are metabolized in the proximal tubule by [cytochrome p450](#) and other systems, exposing downstream tubule segments to unusual concentrations of metabolites.

Conversely, the kidneys' central role in drug elimination implies that in pharmacotherapy, renal function has to be taken into account. In many cases, loading and maintenance doses need to be calculated based on eGFR, and concomitant volume expansion may be required to limit concentrating effects. Let's have a look at a few examples.

Drugs with primarily vascular side effects

Non-steroidal anti-inflammatory drugs (NSAIDs) probably are the most frequently used drugs worldwide. Inhibition of cyclooxygenase interferes with prostaglandins' role in keeping the vas afferens open in a broad range of physiologic and pathologic situations. Of course, this effect is magnified in the presence of hypovolemia. Use of NSAIDs in endurance sports enhances the risk of hyponatremia in case of overdrinking.

Angiotensin-converting enzyme (ACE) inhibitors and **AT₁ receptor blockers** inhibit the effect of angiotensin II, which is important to maintain GFR in the face of decreased systemic

pressure. Accordingly, use of these drugs may compromise renal function, more so when they are combined with NSAIDs, cyclosporine or tacrolimus, especially in patients with congestive heart failure or in situations of hypovolemia.

Calcineurin inhibitors, cyclosporine and tacrolimus, interfere with the autocrine IL-2 feedback loop necessary for T cell proliferation and are used for immunosuppression, e. g., following renal transplantation. They have a very narrow therapeutic index and may damage the kidney via several mechanisms, of which the most important one is a constriction of the afferent arteriole.

Iodinated radiocontrast agents are one of the most common causes of acute kidney injury in the hospital. Intravenous administration is followed by an intense constriction of the vas afferens, enhancing hypoxia in the medulla. In addition, the radiocontrast agents damage tubule cells by an osmotic effect. Typically, a rise in serum creatinine is seen within 24-48 hours. Previous administration of volume reduces the frequency of complications.

Drugs that may cause tubular injury

Aminoglycosides have a cationic structure and are freely filtrated and then reabsorbed, leading to accumulation in proximal tubule cells. This may interfere with transport of cations like K^+ , Mg^{2+} and Ca^{2+} , leading to hypokalemia, hypomagnesemia and hypocalcemia. More pronounced accumulation may result in cell death. Even at normal therapeutic concentrations, nephrotoxic effects have been reported in 10-25%.

Sulfamethoxazole-trimethoprim may cause hyperkalemia, as trimethoprim inhibits ENaC in the distal tubule, acting like the K^+ -sparing diuretic amiloride.

Amphotericin B is used to treat systemic fungal infections. Its name originates from its amphoteric properties: it places itself in lipid membranes, next to ergosterol, a sterol lipid present in fungal, but not in human membranes. Its presence interferes with normal membrane function, e. g., it makes K^+ leak out. It also has some, albeit lower, affinity to cholesterol, the sterol present in our cell membranes, which explains its toxicity. Renal toxicity starts with tubular acidosis, concentration defects and electrolyte imbalances and may end in acute tubular necrosis. As therapeutic use is limited by nephrotoxicity, a liposomal formulation has been developed that has an improved renal safety profile.

Acyclovir, administered via venous infusion to treat serious infections by herpes viridae, may induce tubular injury by precipitation. Slow infusion and volume expansion to increase diuresis are essential.

Lithium therapy is used to treat bipolar disorder. Li^+ -ions inhibit adenylyl cyclase, thus decreasing cAMP, the second messenger of ADH. This reduces the number of aquaporins brought to the membrane of the collecting duct and may cause renal diabetes insipidus.

HMG CoA reductase inhibitors or statins block the rate-limiting step in cholesterol synthesis and are among the most widely used drugs. A common side effect of this class of pharmaceuticals is myopathy, which may range from very frequent myalgia to very rare rhabdomyolysis. In rhabdomyolysis, the contents of muscle cells leak out into the bloodstream. Myoglobin, a 17 kDa protein with a heme group to bind oxygen, is released and filtrated in large amounts. Along with other muscle proteins, myoglobin is taken up via megalin/cubilin-mediated endocytosis and thus concentrated in proximal tubule cells. Recall that heme groups with their central iron are dangerous compounds that enable redox reactions in carefully regulated systems like cytochrome P450 enzymes or in the mitochondrial respiratory chain. Here, uncontrolled reactions produce radicals of reactive oxygen species that over time may result in cell death. So, rhabdomyolysis in response to statins is at least as much a kidney problem as a muscle problem.

Cisplatin is, of course, an extremely toxic molecule to begin with, intended to kill tumor cells by crosslinking DNA. This toxicity is enhanced by accumulation in proximal tubule cells.

Methotrexate blocks dihydrofolate reductase and is intended to deprive tumor cells of purines and thymidine, the building blocks for DNA synthesis. Nephrotoxicity is strongly enhanced by crystallization in the tubulus, which is favored at the acidic pH that is the typical result of our Western diet high in protein.

Polycystic kidney disease

Polycystic kidney disease is an umbrella term for genetic diseases leading to tubule dysfunction and cyst formation. There are indications that compromised function of the primary cilium may be a common denominator of various forms of the disease. Almost all cell types have a single immotile structure protruding from the cell. This primary cilium carries many receptors and might be seen as a kind of antenna that concentrates signal reception.

Autosomal dominant polycystic kidney disease is the most common form with an incidence of 1:500. Affected genes PKD1 and PKD2 have roles in cellular calcium transport. Already in utero, cysts start to form in a small percentage of nephrons. Over time, these cysts grow and compress surrounding parenchyma. An early sign may be a reduction in the kidney's ability to concentrate urine. The condition may eventually lead to end-stage renal disease in late adulthood.

Autosomal recessive forms are less common, but tend to be more severe.

In polycystic kidney disease, it is especially important to treat arterial hypertension. As blood pressure contributes to "inflating" the cysts, it is essential to bring it back to a normal range. In addition, affected tubule cells are usually sensitive to ADH/AVP, which may cause enhanced proliferation. Therefore, the -very expensive- V2 antagonist tolvaptan may slow progression in part of the patients.

11. RENAL HANDLING OF CALCIUM AND PHOSPHATE

We will come back to the regulation of calcium and phosphate when we study [bone metabolism](#). For now, we will concentrate on the renal aspects. Calcium and phosphate stores of our body are regulated according to different principles:

- For calcium, the main variable regulated to maintain long term stores is uptake from the intestines. This is accomplished by "switching on" vitamin D by hydroxylation. This mechanism is too slow to regulate plasma Ca^{2+} , which needs to be kept in a very narrow range. This is achieved via a short-term mechanism: if necessary, parathyroid hormone releases Ca^{2+} from bone. Even this "fast" mechanism requires a few hours to react; in the meantime, fluctuations in Ca^{2+} levels are buffered physically by the bone reservoir. Increased requirement for parathyroid hormone switches on vitamin D to fill up bone stores.
- Phosphate is treated as a precious resource: we take up all we can get and excrete only the surplus via the kidneys. The main tool to do this is fibroblast growth factor 23, which goes up in response to an intestinal phosphate load and stimulates phosphate excretion.

Let's have a look at the system in more detail:

Parathyroid hormone

Parathyroid hormone (PTH) is released by the four parathyroid glands behind the thyroid. An increase in concentration of free Ca^{2+} activates the *calcium-sensing receptor* (CaSR) located at the membrane of their chief cells, throttling PTH production.

Pharmacology cross-reference: Cinacalcet is a small molecule binding to another site of the calcium-sensing receptor, allosterically sensitizing the receptor to free Ca^{2+} . Its main use is in treating secondary hyperparathyroidism in patients with chronic renal failure

PTH increases Ca^{2+} concentration via two main mechanisms: by mobilizing it from bone and by adjusting renal function. [In bone](#), osteoclasts release Ca^{2+} and phosphate by local acidification. Not much would be gained by just taking Ca^{2+} and phosphate from bone: due to their low solubility product, they would quickly reprecipitate somewhere else in the body.

Therefore, PTH has three effects in the kidney:

1. It simultaneously lowers plasma phosphate levels by inhibiting renal reabsorption. By activating its receptor, PTH stimulates two G proteins. G_{α_s} stimulates adenylyl cyclase and, via cAMP, protein kinase A (PKA). G_{α_q} activates phospholipase C and, via diacylglycerol and Ca^{2+} release, protein kinase C (PKC). Phosphorylations by PKA and PKC promote removal of the Na-Pi cotransporter from the apical membrane into a vesicle compartment below the membrane, thus strongly reducing phosphate reabsorption in the proximal tubule.

2. It minimizes Ca^{2+} losses.

At the same time, in the thick ascending limb and the distal collecting tubule, PTH increases the open probability of the apical Ca^{2+} channel and induces a Ca^{2+} -buffering protein in the cytosol, thereby increasing Ca^{2+} reabsorption.

3. It switches on vitamin D.

In proximal tubule cells, PTH induces CYP27B1, the enzyme hydroxylating 25-hydroxy-vitamin D at position 1 to produce the biologically active 1,25-dihydroxy-vitamin D. Active vitamin D then proceeds to fill up calcium pools from outside.

Vitamin D

Lipid-soluble vitamin D₃ may be taken up from food, especially fatty fish, and may be produced in our own skin from 7-dehydrocholesterol with the help of UV-B from sunlight.

Vitamin D, which already contains one hydroxyl group, is activated by two successive hydroxylations resulting in 1,25-dihydroxy-vitamin D or calcitriol. The first hydroxylation is performed at position 25, the end of the side chain, in the liver. The second, at position 1, is performed in the proximal tubule and is carefully regulated. PTH stimulates hydroxylation, while the end product calcitriol as well as increased levels of phosphate, via FGF23, act inhibitory. Calcitriol activates the vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which is expressed by most cells in our body. As a ligand-dependent transcription factor, one of its functions is the induction of genes that are necessary to maintain Ca^{2+} reserves.

Its main target of filling up calcium pools is achieved by enhancing uptake of Ca^{2+} from food in the duodenum. In the kidney, the action of vitamin D parallels that of PTH by increasing reabsorption of Ca^{2+} in the distal tubule, although its effect is much weaker. Contrary to PTH, vitamin D also enhances reabsorption of phosphate.

Fibroblast growth factor-23 (FGF23)

FGF23, a member of the large fibroblast growth factor family of extracellular signaling molecules, primarily regulates serum phosphate levels. It is produced by osteocytes and osteoblasts in response to dietary phosphate loading and to 1,25-dihydroxyvitamin D. How bone cells get the message of intestinal phosphate loading is not yet clear. FGF23 increases renal phosphate excretion by reducing the number of Na-Pi cotransporters in the apical membrane of the proximal tubule. In this function, it acts similar to PTH. Yet, it counters PTH by inhibiting 1α -hydroxylation of vitamin D; FGF23 downregulates CYP27B1, the enzyme performing 1-hydroxylation, but upregulates an enzyme which degrades vitamin D. FGF23 is eliminated if it is fully able to do its job, although we are not yet sure about the precise mechanism. If it is not entirely successful, FGF23 levels rise.

What happens in chronic kidney disease?

With progressive loss of nephrons, fewer nephrons have to work harder to get the job done. Nephrons are admirable workers: they are able to redouble their efforts without a hitch. But of course, there is a limit: once only about a quarter of nephrons are left, they start having trouble to secrete the necessary amounts of H^+ , K^+ and phosphate. While dietary phosphate loading continues, FGF23 increases, but to no avail: the remaining nephrons excrete all the phosphate filtered, but that is not enough. Phosphate slowly creeps up. The low solubility product forces precipitation of calcium phosphate somewhere in the body, for example in the media of large arteries, leading to *Mönckeberg's* calcific sclerosis. This reduces aortic windkessel function and leads to left ventricular hypertrophy and to deterioration of coronary blood flow.

So, phosphate is on the high side, FGF23 is high, keeping active vitamin D down. Too little Ca^{2+} is taken up from outside. How do we keep up Ca^{2+} ? The only way to maintain Ca^{2+} near required levels is to crank up PTH. This is what is being done, leading to strong secondary hyperparathyroidism. This is not really a good solution: continuous nibbling on bone leads to renal osteodystrophy or CKD-MBD (chronic kidney disease- mineral and bone disorder).

12. ERYTHROPOIETIN (EPO)

How would you go about designing a system to make sure there is always just the right number of erythrocytes? Maybe we would try to count and replace the ones we break down or lose. Even if we succeeded in doing that, it would not do the trick. Once we try to climb a mountain somewhere up high, say, in the Himalayas, gaspingly, we would soon find out that we need more erythrocytes than at home. So, the system would have to be able to respond to changes in ambient pressure, better still, it should as well respond to longer-term changes in oxygen consumption. To implement such a system, there would be no way around measuring oxygen concentration somewhere in the body. Where in the human body would you place an oxygen sensor? Not in the skin, of course, because the skin receives lots of oxygen from outside; it would tell you nothing about oxygen transport capacity. Not in the gastrointestinal system, because blood supply there varies greatly: think of a fright-or flight reaction. We would have to look for a place deep inside the body with a high, reliable, constant blood supply. Notwithstanding that, at the same time it should be a place where oxygen concentration may approach its critical lower boundary.

Nature placed our oxygen sensor in the renal medulla. In an elegant feedback mechanism, a reduction in medullar partial oxygen pressure (P_{O_2}) stimulates production of new erythrocytes in the bone marrow. Like most cells, peritubular cells in the renal medulla constantly produce a transcription factor, hypoxia inducible factor (HIF-1), which at normal P_{O_2} is broken down fast and efficiently. Breakdown via the ubiquitin-proteasome pathway is initiated by an oxygen-dependent enzyme, a prolyl-hydroxylase. This enzyme just barely works as long as there is enough oxygen in the cell. As soon as P_{O_2} sinks below a certain threshold, the enzyme

ceases to function. HIF-1 stops to be hydroxylated, is not broken down any more yet remains to be constantly produced: so it starts to accumulate. In the nucleus, accumulating transcription factor HIF-1 activates its target genes, including the gene encoding erythropoietin (EPO). In summary, this sensing mechanism translates a fall in renal medullar P_{O_2} into a secretion of EPO.

EPO increases the rate of proliferation in erythroid progenitors in the bone marrow (colony forming units, CFU_E and burst forming units, BFU_E). The net effect is an increase in erythrocyte output, eventually increasing hematocrit or, in other words, the blood's oxygen-transport capacity. In time, this leads to normalization of P_{O_2} in the kidney, reactivating the oxygen-dependent hydroxylase. As soon as HIF-1 is degraded again, the negative feedback loop is closed.

Tubulo-interstitial disease interferes with the renal medulla's ability to produce EPO. Thus, chronic kidney disease is typically associated with anemia. Previously, dialysis patients had to receive blood transfusions at regular intervals, over time resulting in iron overload, infections and transfusion complications.

A big improvement was made in 1989, when the FDA approved recombinant EPO, developed by the then-small company Amgen with late-stage help by Johnson&Johnson (J&J). EPO is a 30 kDa glycoprotein of 165 amino acids, with three N-linked and 1 O-linked carbohydrate chains of variable structure providing about 40% of its mass. For patients on dialysis, it was usually administered concomitantly with dialysis two times per week.

To bring recombinant EPO to market, Amgen had entered into a contractual obligation with J&J that reserved the dialysis market in the US for Amgen, while all other areas and uses remained the realm of J&J. EPO became a huge worldwide commercial success, as not only the numbers of patients on dialysis increased greatly, EPO was also used in cancer patients on chemotherapy to minimize the duration of anemia.

Soon, recombinant EPO was used for doping in professional endurance sports like cycling and cross-country skiing, as it was an undetectable way to increase oxygen transport capacity. Eventually, it was found that recombinant EPO differed slightly from endogenous EPO in the number of sialic acid (=N-acetylneuraminic acid/ NANA) residues at the end of the carbohydrate chains. That way, doping could be identified by isoelectric focusing of EPO variants from athletes' urine. Post-hoc analysis of stored urine samples revealed numerous athletes had used the good stuff.

If differences between recombinant and endogenous EPO exist, don't they lead to antibody formation? There was indeed a scare around the year 2002. In Europe at that time, there was a drive to replace human serum albumin, which until then had been used as stabilizer of the injectable EPO solution in prefilled syringes. Human serum albumin is not produced recombinantly (too big, too expensive), yet purified from pooled blood plasma from donors. The recent cases of variant Creutzfeldt-Jakob disease had made authorities aware of the potential that prions transferred from any pooled donor material might cause problems later

on. With the best of intentions, in Europe, the stabilizer was therefore reformulated to avoid pooled donor material: albumin was replaced by the detergent polysorbate 80 plus glycine. Shortly thereafter, very low, but increasing numbers of patients developed pure red cell aplasia (PRCA) because of neutralizing antibodies, which inactivated not only recombinant, but also remaining endogenous erythropoietin. It was proposed that organic compounds leached from uncoated rubber stoppers in prefilled syringes containing polysorbate 80 might have an adjuvant effect, breaking B cell tolerance. Alternatively, it was proposed that increased formation of EPO aggregates might play a role. As a hypothetical model, EPO aggregates or EPO-including micelles formed by polysorbate 80 might cross-bridge several B cell receptors, while that may not have happened with the old formulation. To address the problem quickly, several changes were made simultaneously: subcutaneous administration was stopped, rubber stoppers were Teflon-coated and the cooling chain from production to patient was improved. With these measures, the problem soon abated; however, it was not possible to discriminate cause and effect of a single action. An undisputable lesson seems to be: there is a fine line between tolerability and antigenicity, and that line is quite unpredictable.

Over time, Amgen developed a molecule they termed novel erythropoiesis stimulating protein (NESP), which differed from EPO in that it contained two additional N-linked glycosylation sites. To reach that goal, five amino acids had to be replaced. Surprisingly, this considerable change does not lead to more frequent antibody formation. The additional glycosylation increased serum half-life, allowing less-frequent dosing. A dispute arose between J&J and Amgen whether NESP was also covered by the original contract. By arbitration, the dispute was settled in favor of Amgen, which from then on was able to sell its product in Europe under the generic name darbepoetin alfa (trade name: Aranesp).

At the peak in the year 2006, worldwide sales of EPO-related drugs exceeded \$10 billion. Then, several studies indicated that there is no added benefit, even a disadvantage resulting from thrombosis and hypertension, in trying to raise hemoglobin concentrations to levels seen in healthy controls. A target range of hemoglobin of 10 g/dl to 12g/dl was stipulated by FDA and EMEA in 2007 and 2008, which brought down sales in the following years. In the meantime, the upper limit has been further reduced to 11 g/dl. As patents have expired and the market remains attractive nonetheless, a range of biosimilars has meanwhile become available in Europe.

13. CHRONIC RENAL FAILURE

After having studied all these renal functions, let's assemble a picture of a person whose kidneys do not function adequately over a prolonged period; e.g. in end-stage renal disease (ESRD). We expect to find symptoms in the following areas:

- **Uremia** is the word we use for the combined toxic effect of all those substances that are normally eliminated via the kidney. One of those substances is ammonium, NH_4^+ . Apart

from ammonium, we measure blood urea nitrogen and creatinine as surrogate markers, but these two are not toxic at the concentrations reached. Importantly, we know very little about which other toxins are responsible for the symptoms encountered. As a complex form of intoxication, uremia affects many systems. The central nervous system is one of the first to be affected, leading to symptoms including weakness, fatigue, problems with memory and concentration, confusion, anorexia, somnolence during daytime, restlessness at nighttime. Without intervention, the condition will progress to stupor, coma and death. The peripheral nervous system is affected with polyneuropathy, the gastrointestinal system with nausea and vomiting, and the heart with pericarditis.

- **Retention of sodium and water** with edema and hypertension, as long as the person adheres to the usual Western diet. Fluid retention may be alleviated by cutting back on Na^+ intake. In principle, however, at normal water and very low Na^+ intake, hyponatremia may occur, too.
- **Acidosis**, again, at the relatively high protein intake typical of our usual diet
- **Hyperkalemia**, contributing to weakness, arrhythmia and congestive heart failure
- **Hyperphosphatemia** leading to itching and contributing to bone damage
- **Hypocalcemia**, contributing to muscle weakness, cramps and secondary hyperparathyroidism with chronic kidney disease- mineral and bone disorder
- **Anemia**, resulting in massive weakness and increased strain on the heart
