Physiology Labs Protocols

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Labs aim: Explore biology in context through brain and hands

Physiology of the Kidneys, Water and Solute Balance, Polyuria, Clearance

AIMS of the lab, Objective and Output Knowledge:

- *Understanding the importance of the kidneys in maintaining balanced water and solute levels (concentrations)*
- *Understanding the mechanism by which the kidneys concentrate urine.*
- *Answering the question why a decompensated diabetic is at risk of dehydration.*
- *Understanding the principles of clearance and its evaluation (glomerular filtration, renal blood flow)*

REQUIRED KNOWLEDGE:

Blood flow through the kidney, glomerular filtration, tubular processes, threshold substances, the significance of the long loop of Henle for renal concentrating ability, obligatory and facultative reabsorption, hormonal regulation of water and solute reabsorption.

- Diuresis (volumes of urine under normal physiological conditions not confined to rest, thermoneutral environment, or unrestricted water supply)
- Polyuria, osmotic/water (consideration of various schemes of water and food ingestion)
- Concentration of urine – mechanisms of formation of definitive urine
- Renal threshold for glucose reabsorption, principles of tubular transportation mechanisms

You can watch the video: Wendy Riggs: How the Osmotic Gradient is Created in the Kidney, What Is the Counter-current Multiplication System https://www.youtube.com/watch?v=4-zXoYFdUR0

Practical preparation at home:

- *Perform a full-day measurement of fluid intake and diuresis on two separate days under the following conditions:*
    - *Maximum fluid restriction*
    - *Significantly increased fluid intake (e.g., 5 liters distributed throughout the day)*

  Bring the measurement results to the renal function lab.
- *Bring a sample of morning urine collected before the lab in a non-sterile test-tube.*
- ***For 3 healthy volunteers (without kidney disease, diabetes, or other chronic conditions), the following drinking regimen will be followed before the lab:***
    - *Consume 500 ml of fluid during the morning, with no further fluid intake after 12:00 "*

Introduction

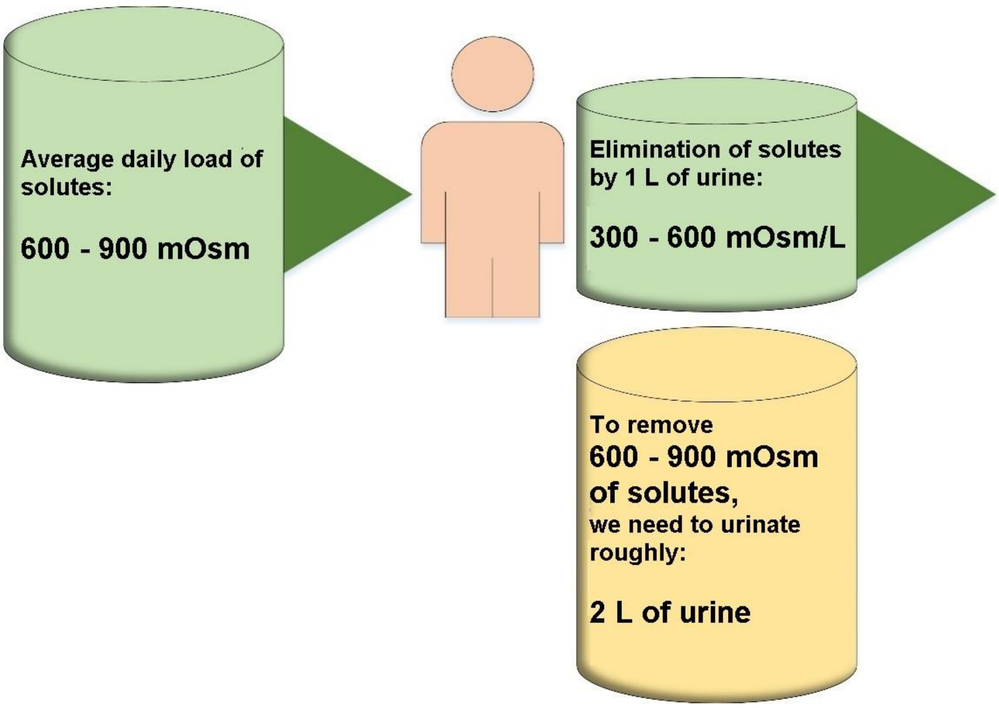
Adequate water intake and optimal diuresis

When quantitatively considering water intake and output, it is necessary to consider several facts. The most fundamental is that the intake and output of water in our body must be balanced in the long term, maintaining the body's water content at approximately constant levels. Mind, that water intake is not always determined solely by the endogenous physiological needs of the individual (for example, if the individual has a tendency to consume larger amounts of fluids - for example, beer drinkers, or in some individuals trying to maintain a “proper” drinking regime, or during the infusion therapy where larger volumes of fluid could be introduced into the patient’s circulation). On the other hand, the individual's will has much less influence on the water output eliminated by urination, since under physiological circumstances, it is more strictly governed by the physiological endogenous needs to maintain a stable water content and osmolality of extracellular fluid.

Another aspect influencing the amount of water intake is that optimized elimination processes require, under normal conditions, a daily urine production volume of around two liters (dependent on the composition of the diet or individual activity), i.e., a volume in which the daily load of solutes (osmotically active substances ingested and created by the body) is dissolved so that the resulting osmolality of urine does not significantly exceed the osmolality of extracellular fluid.

Thus, the osmotic load of food is one of the important factors determining the amount of urine produced, as it changes the osmolality of the internal environment and regulates the thirst and herewith the daily fluid intake. It should be noted that the osmotic load that an average individual receives daily with food and also produces through metabolism is rather constant over a longer time period, but in the short term, for example, over a few days, can fluctuate considerably.

Let's consider that the average amount of solutes excreted by our body daily (primarily Na^+, Cl^-, and K^+ ions from food, urea produced by metabolising proteins, and to a lesser extent other osmotically active substances) reaches approximately 600 – 900 mOsm/24 hours. Furthermore, let's assume that it is advantageous to produce urine with an osmolarity not greatly exceeding the osmolarity of the extracellular environment (eliminating the need to concentrate urine to higher osmolarity values) - thus, we consider a urine osmolarity of 300 – 600 mOsm/L. Then, the daily diuresis can be estimated as the ratio of excreted solutes (e.g., 600 mOsm/24 hours) and the urine osmolarity (e.g., 300 mOsm/L), resulting in 2 L/24 hours for the given values. It's interesting that this estimated diuresis corresponds to the volume of fluids commonly ingested daily in order to optimally dissolve the excreted solutes with the given osmolarity of urine (in this example 300 mOsm/L). If the values change (water or solute intake increases or decreases above their usual intake), the kidneys can physiologically produce hypotonic (up to 50 mOsm/L), or hypertonic (up to 1200 mOsm/L) urine, in quantities significantly different from those two liters[1].



It is therefore evident that water and osmotic balance cannot be described separately, since they represent systems interconnected with homeostatic mechanisms optimally regulating the food (and water) intake and the volume and osmolarity of produced urine. It is interesting to recall that the

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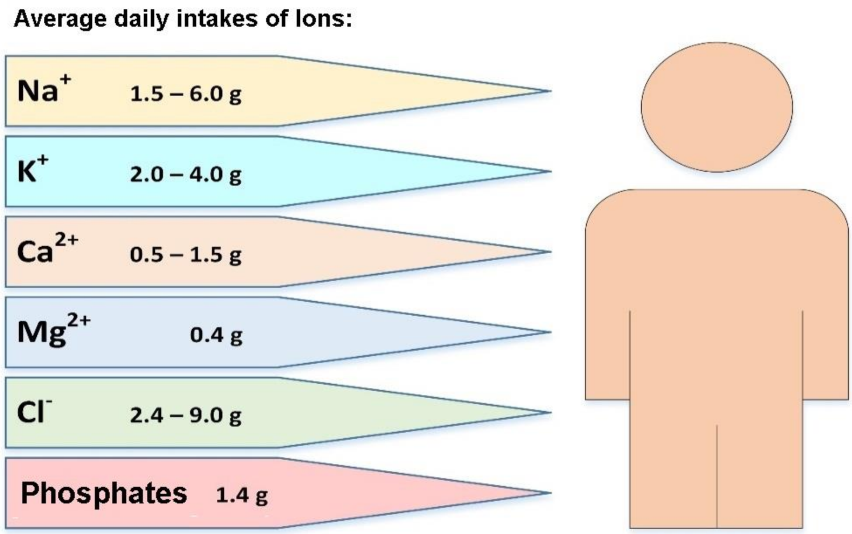
[1] It should be noted that in these outlined considerations, the impact of extrarenal losses of water and salt is not discussed. However, it is generally accepted that under normal circumstances, if a person is more or less at rest and in a thermoneutral conditions, these stated balances and the principles derived from them are not significantly affected.

daily excreted osmotic load reaches approximately 600 – 900 mOsm, values coming from the osmotic load of food, which naturally accompanies its caloric component consisting of carbohydrates and lipids (substances that create minimal osmotic load after being metabolised) and proteins (whose metabolism creates a significant osmotic load excreted to urine in the form of urea). After subtracting the daily load of eliminated urea (approx. 200 – 300 mOsm/day), which contributes to the osmotic load by about 1/3, the osmotic load from food, consisting of Na^+, Cl^-, and K^+ ions, represents approximately 600 mOsm over 24 hours[2]. This ion load needs to be excreted daily by the kidneys[3], for which, as already mentioned, an adequate amount of water must be allocated (mixed with ingested food by adequate volume of consumed beverages), allowing for the excretion of these ions from the body by appropriate formation of urine.

Dietary Aspects of Na^+, Chloride Cl^-, Potassium K^+, and Other Ion Handling and Their Renal Balance

Ions (primarily Na^+, Cl^-, and K^+) are regularly ingested in excess from our diet, with daily intake ranging from 1.5 g to 6 g of Na^+, 2.4 g to 9 g of Cl^-, and 2 g to 4 g of K^+ depending on the composition of food and the assumption of average caloric intake. Approximately 90% of these ions need to be excreted in urine (an intake of these ions in case they are not be properly excreted by kidneys due to the renal insufficiency could be lowered below to 1 g Na^+, 1 g K^+, 1 g Cl^- [4]).



It is important to note that all mentioned ions are present in blood plasma, and they are inevitably filtrated into the ultrafiltrate. If we calculate the amount of leaked ions into the ultrafiltrate, we arrive at quite interesting figures. For instance, for Na^+, it is 180 L × 150 mmol/L = 27000 mmol (the

[2] This corresponds to approximately the osmotic equivalent of 18 g NaCl, i.e., a dose that osmotically correlates in our region with the common daily intake of 15 g NaCl plus 3 g K^+

[3] Common extrarenal losses of salts (especially Na^+) are, unless Na^+ is significantly lost through increased sweating, approximately an order of magnitude lower (around one to two grams per day), and these losses indirectly suggest that if we would not excrete these ions by the kidneys, one gram of Na^+ per day could be sufficient to cover its losses.

[4] Approximately 10% of K^+ is excreted in feces, but if it is ingested in food in large excess, fecal excretion can reach up to 30%. Potassium intake, especially its balance, must be carefully monitored in cases where it is released by the kidneys to a greater extent (side effect of some diuretics) or, conversely, inadequately removed from the body by the kidneys (renal function disorders).

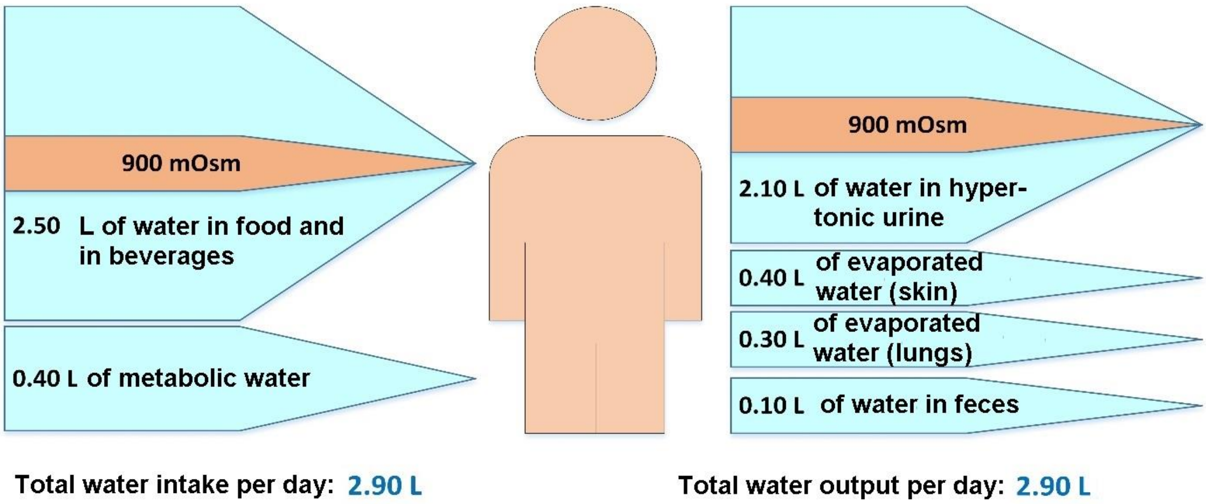
amount of ultrafiltrate filtered per day multiplied by the concentration of the soluble ion fraction in plasma), which equals 1173 g of Na^+, more than one kilogram, exceeding its daily intake from the food by more than two orders of magnitude (approximately 200 times). For other ions like Cl^-, K^+, Ca^{2+}, and Mg^{2+}, the corresponding calculation shows smaller amounts (650 g for Cl^-, 35 g for K^+, 10 g for Ca^{2+}, and 4 g for Mg^{2+}) being filtered into the ultrafiltrate, yet these also exceed their daily intake (and, of course, their final excretion) by at least one to two orders of magnitude.
It is therefore essential that more than 90% of these ions are reabsorbed back from the ultrafiltrate into the blood to maintain their overall balance. From the aforementioned quantitative analysis, it naturally follows that the initial setting and regulation of the resorption mechanisms must be very precisely adjusted according to the current state of the body, so that even minimal changes in the amount of produced ultrafiltrate cannot lead to significant ion imbalance, which could result in life-threatening conditions or even organism death[5].

It should be noted that the energetically demanding tubular reabsorption of these ions from the ultrafiltrate is the corresponding price for the kidney's cleansing functions enabled by high volumes of glomerular filtration (180 L/day), effectively eliminating unnecessary, potentially harmful, or toxic substances that automatically pass through filtration into the ultrafiltrate without needing to be recognized by the body through specific selective mechanisms.

Water management and associated balance considerations

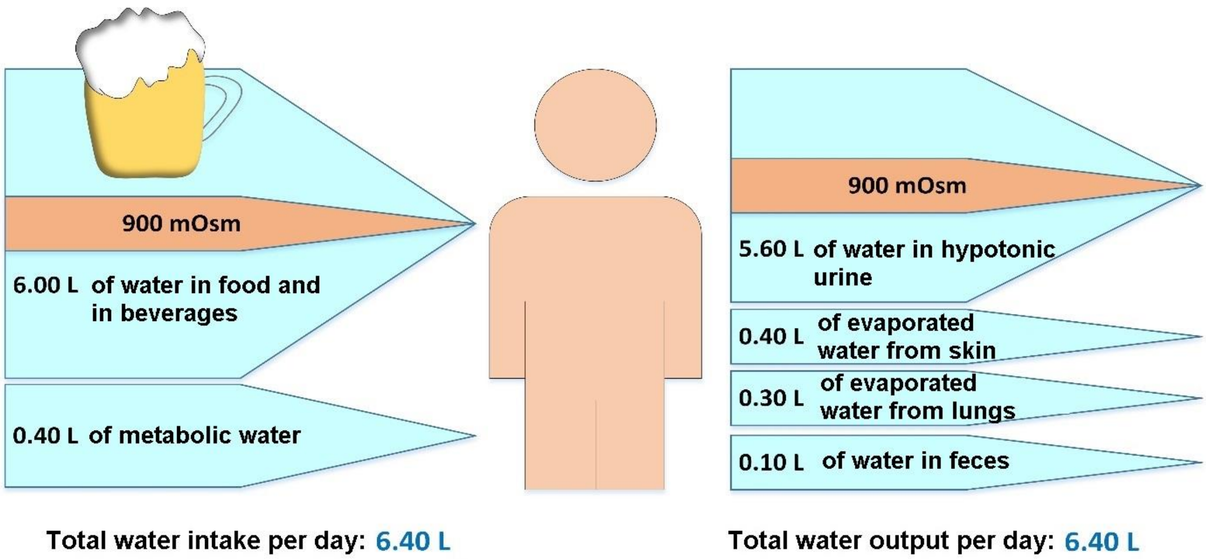
Water intake by the body is influenced by many factors and must, in the long term, match its output. If we temporarily omit extrarenal water losses through sweating and respiration, which are described in detail in the chapters on thermoregulation and respiration and which, under resting and thermoneutral conditions, do not exceed 600–800 ml/day (though they are roughly compensated for by the production of metabolic water, i.e., approximately 300–500 ml/day), we are left primarily with water intake from the food and its excretion by the kidneys in the balance equation (water losses through feces can be neglected in this consideration, as they typically amount to only about 100 ml/day). However, let us recall what is quite crucial in terms of water intake regulation (and not immediately visible at first glance) - namely, the amount of solutes ingested with food (600–900 mOsm/24 hours), which need to be excreted in urine with an acceptable osmolarity (300–600 mOsm/L). We must be aware that diuresis with higher osmolarity, while reducing the amount of water needed as a solvent for a given amount of solutes, does so at the expense of increased demands on the kidneys to produce such hypertonic urine. Optimization of solute excretion essentially determines reasonable, or optimal, water intake (typically in the range of approximately 1.5–2.5 L/day), consisting of water in food (approximately 500–1000 ml - usually water is an obligatory component here) and in beverages (approximately 1000–1500 ml - the intake of this water is modulated by thirst to ensure that the osmotic daily load of 600–900 mOsm/day is eliminated by roughly 2 L of urine with the osmolarity of 300–600 mOsm/L, as already outlined in previous paragraphs, requiring a total water intake of 2 liters). Let's us focus on few examples:

[5] In theory, if for some reason the reabsorption of these ions in the tubular system were to cease, while the production of ultrafiltrate remained unchanged, within a few hours there would be a complete disruption of the body's ion balance.



Example 1. *The excreted load of 900 mOsm dissolved in urine with an osmolarity of 428 mOsm/L gives us 900 mOsm / 428 mOsm/L = 2.10 L of final urine. Then, considering the intake of 2.5 L of water in food (totaling 2.9 L due to the production of an additional 0.4 L of metabolic water), there is a shortfall of 0.76 L - the volume corresponding to extrarenal losses through the skin (200 – 400 ml Insensible perspiration, also known as transepidermal water loss), and losses through the respiration (200 - 300 ml at 25°C and 40% relative humidity), and feces (approximately 100 ml on a normal diet).*

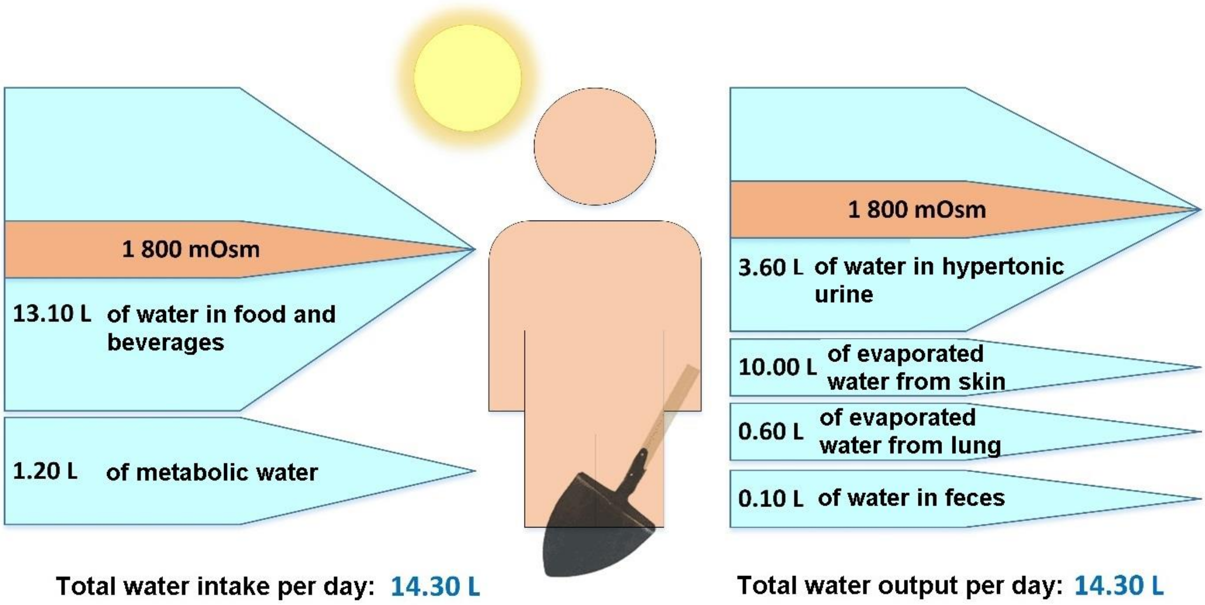
However, this balance example is only a model illustration explaining the basic typical water balance and providing a template for more complex considerations (as well as more complex balance calculations with various parameters of water and osmotic balance) for individuals who consume more (or less) fluids and whose daily osmotic load differs from average values.



Example 2. *Another illustrative and not so uncommon example might be, for instance, the excessive consumption of beer, where, with a normal diet determining the daily osmotic load, the urine osmolarity decreases proportionally with the amount of fluids consumed, for example, with a total daily water intake of 6 L, the amount of urine will be approximately 6 L - 0.8 L + 0.4 L (0.8 L being extrarenal losses, 0.4 L metabolic water) and its* ***osmolarity 151 mOsm****/L (850 mOsm / 5.6 L), corresponding to hypotonic urine.*

On the contrary, in cases of restricted water intake, such as limiting it to 1 liter per day (for example, due to insufficient fluid intake for various reasons), urine must be concentrated to much higher osmolarity values in order to eliminate the daily osmotic load (for instance, 700 mOsm/day: 700 mOsm/day / (1 L + 0.4 L - 0.8 L) = 1166 mOsm/L, which approaches the maximum concentration capacity of the kidneys being 1200 mOsm/L, and suggests that with a lower intake of fluids, it is no longer possible to maintain the osmotic balance with a given daily osmotic load). It is worth noting that diuresis with maximal urine osmolarity (reaching 1200 mOsm/L), under which it is no longer possible to excrete the normal daily osmotic load, is called oliguria, and it corresponds to urine output less than 0.5 L/day.

The outlined examples discussed the balance under resting and thermoneutral conditions. If these conditions cease to apply, more complex but perhaps even more interesting situations arise from a quantitative perspective. Below is one not entirely uncommon case:



Example 3. *Let's consider, for instance, a heavily working individual in a warm environment, who has an average daily metabolism three times higher than usual, and loses 10 liters through sweating during work. From the perspective of water balance, it becomes an interesting question how much water they should drink per day, and in addressing this question, we must again consider not only water balance but also ion and calorie balance. Let's continue with the assumption that this individual consumes a normal diet, but in triple the usual amount, thus resulting in the necessity to excrete 3 x 900 mOsm of solutes daily lessened by the loss of* Na^+ *through sweating (10 L x 2.5 g* Na^+*/L = approximately 900 mOsm). Assuming a* ***reasonable osmolarity of urine reaching 500 mOsm/L****, we need to excrete 1800 mOsm / 500 mOsm/L = 3.6 L of urine + 10 L for sweating, minus the gain of metabolic water, which at such an increased metabolism will be around 3 x 0.4 L + losses of water through respiration, which will also be higher than usual (3 x 0.2 L), minus 3 L of obligatory water in triple the amount of food, giving us 10 L of water supplied through beverages with a total optimal water intake in food reaching 13.1 L.*

Renal Clearance

Any volume, e.g., the volume of plasma, blood, ultrafiltrate, or a volume of other fluids (in principle, it can be any volume, even a theoretically conceived one), containing a substance that is completely cleared or removed by a given clearing or elimination process (such as filtration, secretion, or a combination of multiple processes) could be understood as clearance of a given elimination mechanism.

Since in this lab we are dealing with the clearing functions of the kidneys, the amount of plasma that is completely cleared of a given substance is called a **renal clearance**. If the elimination of a given substance from the plasma takes place fully using the process of glomerular filtration, then the clearance of such substance correlates with the value of glomerular filtration (i.e., with the volume of ultrafiltrate formed over a defined period of time - often expressed per minute or over 24 hours). This is understandable since the concentrations of such substance in the plasma and the ultrafiltrate are equal and thus the volume of plasma completely cleared of such substance, is, in fact, the volume of produced ultrafiltrate in which the substance is fully urinated, say eliminated eventually.

If the substance is eliminated by the glomerular filtration, and in addition to that, it is **also secreted actively** into the tubular fluid, the clearance of that substance will be bigger than the glomerular filtration rate (GFR) – this is naturally expected because the volume of the plasma completely cleared of that substance must be bigger than the volume of plasma that is only partially cleared of a given substance, say passing into ultrafiltrate by partial filtration. And conversely, if the substance that is filtered into ultrafiltrate is subsequently resorbed back into the blood (partially or completely), its clearance must be smaller than the value of GFR.

It should be emphasized that nominal clearance values of substances by means of which a certain kidney function is analyzed are known for a given size and age of the patient, and the testing itself provides a value of clearance of the corresponding renal function of the examined patient. If the tested substance is **creatinine**, which is mainly filtered only (it is not significantly secreted into, or resorbed from tubular fluid, however, when renal function declines, creatinine is increasingly secreted in the tubules after filtration), the estimated value of its clearance will correspond roughly to the patient's actual value of GFR.

To estimate the clearance of *creatinine*, we need to know

1) the volume of urine produced over 24 hours

2) the concentration of creatinine in the urine, and

3) the concentration of creatinine in the blood.

Since the creatinine is not reabsorbed from tubular fluid or significantly secreted as already outlined, the amount of creatinine in urine over 24 hours ($\mathbf{V_{urine} \times C_{creatinine\_in\_urine}}$) must be the same as the amount of creatinine that entered the ultrafiltrate ($\mathbf{GFR \times C_{creatinine\_in\_plasma}}$). Mind, that creatinine is very well filtered into the ultrafiltrate, so its concentration in the ultrafiltrate would be almost identical to its concentration in the plasma (in fact in the blood). This can be expressed simply by the following equation:

$$GFR \times C_{creatinine\_in\_plasma} = V_{urine} \times C_{creatinine\_in\_urine},$$

from which it follows that:

$$GFR = V_{urine} \times C_{creatinine\_in\_urine} / C_{creatinine\_in\_plasma}$$

In case the urine is collected over a sufficiently long time period, say over 24hours, then the amount of creatinine in the urine, divided by the plasma creatinine concentration, corresponds roughly to the GFR over 24 hours.

It is important to stress, that the clearance of creatine reflects the functional ability of kidneys to get rid of substances by glomerular filtration, and early detection of that clearance decrease can inform in advance of incipient kidney disease affecting the glomerular filtration, resulting in a significant reduction in renal functions.

It is important to note that functional tests based on creatinine (more precisely endogenous creatinine) gives us only approximate values of renal glomerular filtration (creatinine concentration in plasma may not be completely stable due to physical activity and food intake over 24 hours). More accurate GFR values are obtained by using non-endogenous substances such as inulin. In general, these substances should neither be reabsorbed nor secreted, but rather filtered very efficiently. Inulin is typically administered via infusion to maintain stable levels in the plasma, and its quantity in the urine is then determined. However, this procedure is quite demanding and is primarily reserved for research purposes.

It is intriguing to consider the maximum possible clearance of any substance that undergoes complete filtration in the glomeruli and additional secretion from peritubular capillaries into the tubular fluid. Based on the principles outlined in the preceding paragraphs, one might assume that the clearance cannot exceed the renal blood flow, regardless of the substance being cleared. This is logical, as the kidneys (and other organs like the liver) cannot clear or eliminate more substance than what flows into them. Therefore, if the kidneys clear all the blood passing through them, the theoretical renal clearance of a substance eliminated in this manner would equal the plasma flow through the kidneys, approximately five times greater than the glomerular filtration rate (GFR) of roughly 125ml/min, or about 625 ml/min. This clearance closely resembles that of **para-aminohippuric acid (PAH)**, a substance maximally excreted by the kidneys through filtration and subsequent tubular secretion, almost completely removed from the renal blood in a single pass through the kidneys. Measuring the renal clearance by PAH (via infusion into the blood and measuring its concentration there, as well as in collected urine) is termed effective renal plasma flow (ERPF), which correlates well with the actual plasma flow through the kidneys, abbreviated as RPF:

$ERPF=V_{urine}\times C_{PAH\_in\_urine}/C_{PAH\_in\_plasma}$, then the real estimate of RPF is by approx. 10% bigger, namely= ERPF/0.9.

Practical task

Task 1A: conducted on volunteers:

1. **At the beginning of the lab,** collect a sample of freshly urinated urine, label it, and store it for later measurement of urine specific gravity (empty the rest of the bladder freely into a bowl - do not measure volume of left urine).
   During the next 20 minutes, ingest:
   - Person A: Control, consumes nothing.
   - Person B: 15 ml/kg of weak tea/water.
   - Person C: 15 ml/kg of water + 2g/kg of sucrose: apple/orange juice (10% sucrose solution, 1.5g of carbohydrates/kg) + cookies (0.5g/kg, 1 piece weighing 20g contains 12g of carbohydrates).
2. At specified intervals, collect urine, measure its volume, and determine its specific gravity refractometrically.
3. Additionally, Person C tests urine for the presence of glucose using a glucophan test strip.

A: 0 Fluids			B: H_2O 15ml/kg		C: Θ sucrose 15ml/kg water + 2g sucrose/kg		
Time(min)	V(ml)	S.G.(kg/m^3)	V(ml)	S.G.(kg/m^3)	V(ml)	S.G.(kg/m^3)	GLC +/-
0							
60							
90							
120							

S.G. morning urine:	S.G. morning urine:	S.G. morning urine:
Last fluid intake before lab: when: Volume: what kind of drink: Regimen during the previous day choose: Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑	Last fluid intake before lab: when: Volume: what kind of drink: Regimen during the previous day choose: Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑	Last fluid intake before lab: when: Volume: what kind of drink: Regimen during the previous day choose: Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑

Task 1B: Activities (tasks) of other participants in the lab:

Refractometric determination of the density of morning urine

Variance of urine density within the group, examples		
Highest achieved value	Average value	Lowest value
Previous day's regimen (select) Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑	Previous day's regimen (select) Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑	Previous day's regimen (select) Fluid intake: ↓ moderate ↑ Physical activity: ↓ moderate ↑

Assessment of 24-hour urine collection results under conditions of **restricted fluid** intake, and **fluid excess**, a home task

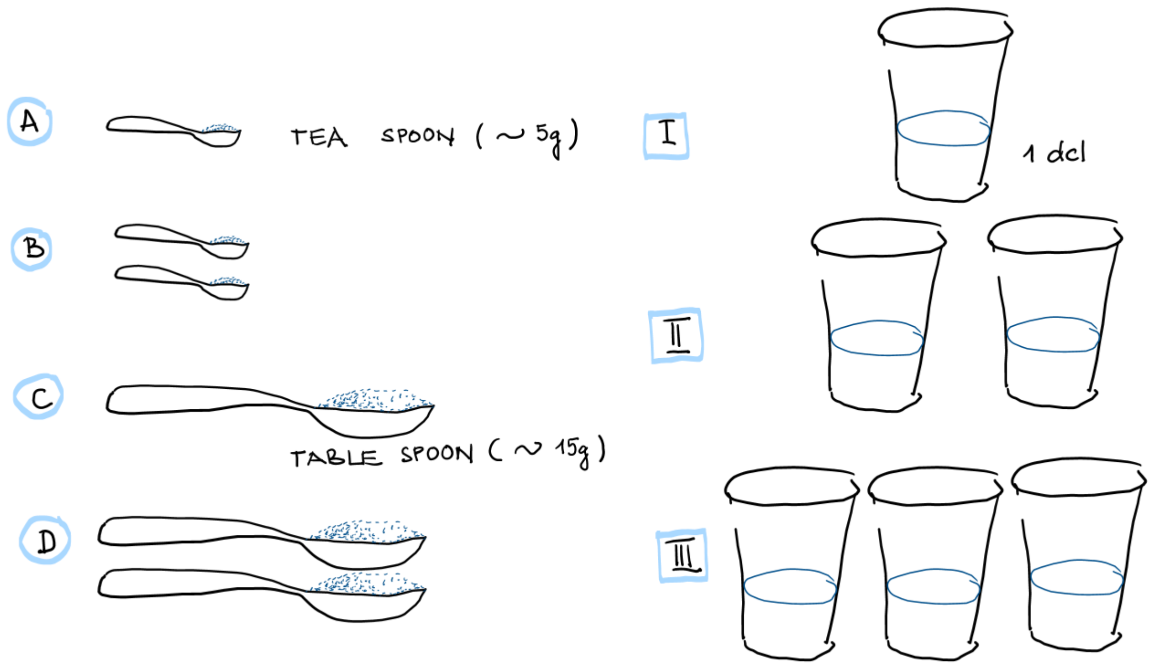
	Volume of received fluids /24 h	**Diuresis /24 h**	**Colour of urine**
Restricted fluid intake			
Distribution over the day	9-13/13-18/18-23/23-9	9-13/13-18/18-23/23-9	
Excess of fluid intake			
Distribution over the day	9-13/13-18/18-23/23-9	9-13/13-18/18-23/23-9	

Summarisation:

Examples for practical exercises in renal function

Task 2: Polyuria in a diabetic

A. The urine of a poorly managed diabetic can typically contain 10% glucose. Assign the amount of glucose (A/B/C/D) and urine volume (I/II/III) that illustrate this situation:



B. How much glucose must a diabetic patient have in urine to cause osmotic polyuria if the baseline osmotic load (ions, urea) is 600 mOsml.

- What is the kidney's maximum concentration limit?
- How does this limit change with a large increase in osmotically active substances in tubular fluid?
- What diuresis do you consider as polyuria?
- What glycaemic profile do you expect in a diabetic during the day?
- How much osmotic load will the kidneys excrete in 3 liters of urine with a urine concentration of 600 mOsml/l?
- Divide this value into glucose and other substances.
- How many grams of Glucose is that?

C. What glycaemic levels does such glycosuria/polyuria likely relate to?

Example C.1:
Imagine that glycemia fluctuates around 15 mmol/l throughout the day (so the average glycemia over the day could be considered 15 mmol/L).

- What will be the glucose concentration in 1 liter of primary filtrate (ultrafiltrate)?
- How many mmol of glucose will remain in tubular fluid behind the proximal tubule (behind the glucose reabsorption segment) for the entire day?
- Consider other osmotic load besides glucose that is excreted during the day.

- What will be the diuresis with a kidney concentration capacity of 600 mOsml/l?
- What will be the waste of glucose in urine?, calculate in grams/day.

Example C.2:

Now imagine a patient with a blood glucose level of 40 mmol/L for 2 hours.
How will the production of his urine and waste of glucose look like during this time?

- How much primary filtrate will be created in 2 hours?
- How much glucose will remain in the final urine?
- How much of other solutes needs to be typically excreted in 2 hours (assuming even distribution)?
- How much urine would this person excrete during these 2 hours if we do not consider any other substances that are also excreted by the kidneys and may be present in a diabetic (e.g., ketones)?
- What will be the waste of glucose in urine?
- What color of urine do you expect during this diuresis?
- What density do you expect?

Example C.3:

How will the amount of urine of a diabetic change if he consumes an extra 6 g of salt?

- Will it increase or decrease?
- Why?
- By how much?