

V. EXCRETION

1. The Color, Smell and Turbidity of Urine

The color of normal urine ranges from colorless through straw, yellow, amber and brown and depends on the concentration of various pigments (urochrome, uroerythrin, urobilin). The color of urine can be influenced by:

- pH: acidic urine usually darker.
- concentration: pale yellow urine is usually hypotonic, while dark urine is hypertonic (except in osmotic diuresis -e.g. in glucosuria- where the pale colored urine has high specific weight).

The color of urine might change due to different substances or under pathologic conditions. Interpretation of atypical urine color is as follows:

yellow/orange: concentrated urine, urobilinogen, bilirubin,

red/red-brown: red blood cells, hemoglobin, myoglobin, phenolphthalein (laxative), blackberries, menstrual contamination, porphyrin, amidazophen

brown-black/black: melanin, methemoglobin, homogentisic acid, (the homogentisic acid defect: homogentisic acid does not decompose, but rather is transformed into alkaptonochrome while standing) fecal contamination

blue-green: pseudomonas infection, chlorophyll, biliverdin, methylene blue,

milky/cloudy: pyuria, lipids, mucus, radiographic dye, microorganisms

The observation of turbidity or lack of it in a urine specimen is made by the examination of a well mixed, uncentrifuged sample held against a good light source.

Terms: clear, hazy, cloudy, opaque.

2. The pH of the Urine

The pH of the urine is depending on the acid-base equilibrium of the organism and may vary between pH 4 and 8. In the case of a normal mixed diet it is slightly acidic. Protein-rich diets shift the pH into the acidic range, while vegetables shift it towards a basic value.

The pH of the urine can be determined simply with indicator paper: dip the reagent strip into the urine and remove immediately. Compare the reagent strip to the color chart and record the result.

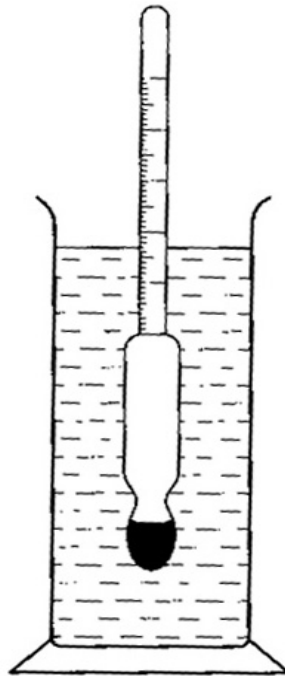
3. The Specific Gravity of the Urine

The specific weight (gravity) of urine indicates the relative proportions of dissolved solid components to the total volume of the sample. Normal value is between 1015-1025 g/l (slightly hyperosmolar). Under extreme conditions it may vary between 1001-1030 g/l. Determination of specific weight may give information about concentrating or diluting processes in the kidney.

Determination is made with an urometer (hydrometer): it is a simple pycnometer or gravimeter calibrated at room temperature (20° Celsius) and having a measuring range of 1.000-1.060. The urometer is placed into the urine sample such a way that it does not touch

the wall of the container. The value of the specific weight will be where the surface of urine touches the mark.

Correction is to be made if the temperature of the urine differs from the calibration temperature of the urometer: for every 3 °C 1 unit (0,001) should be withdrawn or added to the value seen. Glucose or protein in the urine may increase the specific gravity.



Urometer for the determination of the specific weight of the urine

4. Quantity of the Urine

The average volume of the urine excreted per day in an adult is 1000-1500 ml.

Terms: less than 500 ml/day: oliguria (in pyrexia, exsiccosis, shock)

More than 2000 ml/day: polyuria (polydipsia, diabetes insipidus, diabetes mellitus, and reduced concentrating capacity of the kidney)

Less than 100 ml/day: anuria.

Under normal circumstances urine excretion is less at night than in daytime. If the amount of daytime and nocturnal excretion equals or the amount of the latter is higher we talk about nycturia (heart insufficiency, chronic kidney disease).

5. Microscopic Investigation of the Urine Sediment

First morning urine is the most suitable for this investigation since it is more concentrated than the one secreted during daytime. It is also more acidic and casts and other formed elements are more stable in acidic urine. If possible, the urine sample should not stay longer than one hour before the test.

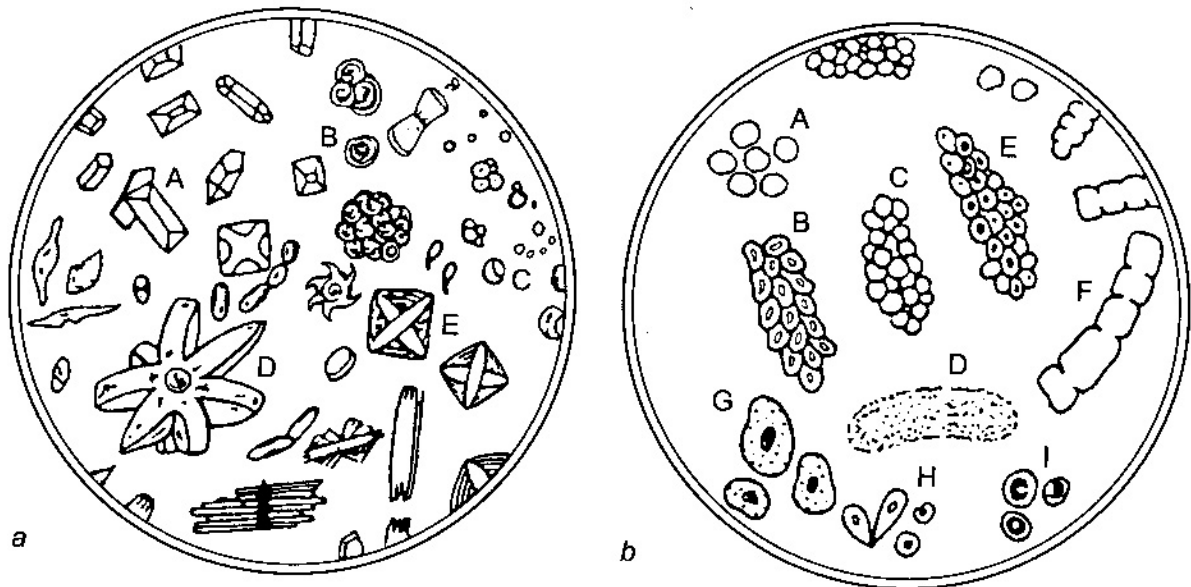
Urine is first centrifuged for 5 minutes at 1000-2000 rpm. The supernatant is discarded from the sediment, stirred and one drop is smeared on a clean glass slide. It is covered with coverslip and viewed under the microscope (condenser in the lower position, narrow diaphragm).

Urine sediment may contain a few epithelial cells and white blood cells in normal conditions.

In acidic urine the following crystals can be found:

Normal (Fig.a): A) ammonium-magnesium (triple) phosphate (coffin-shaped), calcium sulfate, B,C) ammonium urate, D) uric acid, E) calcium oxalate, abnormal: urea oxalate, tyrosine, bilirubin,

In alkaline urine: calcium phosphate, magnesium phosphate, calcium carbonate,



Urinary sediment crystals

In pathologic urine beside unorganic sediment organic ones also can be found:

a., Cells (Fig.b):

- A) red blood cells: pale, anucleate, biconcave refractive disks of 7 μm diameter, with sharp double contour. They shrink in concentrated urine and swell or burst in hypotonic urine to produce "ghost cells". One or two erythrocytes in 3-5 visual fields are considered normal.
- G, H) epithelial cells: renal tubular, bladder epithelial or squamous cells. Size and shape depends on the portion of urinary track from which they originate.
- I) leukocytes: segmented neutrophil or polymorphonuclear leukocyte is the predominant type. They are round cells with granulated surface and with a diameter of 10-12 μm . One or two per visual field is not pathologic but they are accumulated in pyuria.

b., Casts (cylindrical shaped aggregates of protein-like material) (Fig.b):

They are protein-containing casts of tubuli and collecting tubules. The slightly alkaline pH of protein containing filtrate changes to acidic in the distal tubuli; protein of sol state turns into gel state: cast are formed which correspond to the form of the tubulus. Cylinders can be classified according to their shape and to the various substances or cellular elements deposited on them. Their refractive capacity differs only slightly from their surrounding, their recognition therefore is rather hard. Using narrow diaphragm and lowered condenser, cylinders should be detected at lower magnification and then identified at higher magnification.

- hyaline casts have a diameter of 10-15 μm , are transparent, have cylindrical shape. Few might occur in normal sediment, but generally they cannot be observed in routine tests.
- D) shape and size of granulated cylinders is similar to that of hyaline but their surface is granulated due to the sedimentated amorphous granules.
- F) waxy casts are pale yellow, more corpulent forms, sometime retractions can be observed on the surface.
- E) leukocyte casts: easy to recognize because of the presence of white blood cells attached to the surface of the hyaline matrix
- C) red blood cell casts: may be readily recognized because of the red blood cells on the surface of the casts. In time the red cells will undergo hemolysis and give a golden-brown color of the cast.
- B) epithelial casts: these casts are made up of a hyaline cast matrix with the epithelial cells embedded in the casts. The cells have a large spherical central nucleus.

c., Microorganisms: bacteria, protozoa may also show up in the urine sediment.

6. Detection of UBG in Urine with Ehrlich's Aldehyde test.

Conjugated bilirubin secreted by the liver is reduced in the intestinal tract. These reduced products (mesobilirubin, stercobilinogen, urobilinogen) are partly excreted, partly reabsorbed and secreted again by the liver (enterohepatic recirculation).

Normally there is 0.5-2.5 mg UBG in the urine collected for 24 hours. UBG is secreted in higher amounts in pathologic cases, like in hemolytical and hepatocellular diseases. If bile flow into the intestine is obstructed neither UBG nor urobilin will be formed and they are not present in the urine.

UBG is always detected in fresh, cool urine (it oxidizes to urobiline if left standing in the air and urobiline cannot be detected with Ehrlich's reagent).

Few drops of Ehrlich's reagent (p-dimethyl-aminobenzaldehyde dissolved in 20% HCl) is added to 3 ml urine and the sample is inspected after 15-20 s:

- Normal UBG concentration: no significant change in color, urine is light pink if seen from above and colorless if held against the light but becomes red upon boiling.
- Slightly increased UBG: light red if held against the light.
- Markedly increased UBG: expressed red color if held against light.
- Decreased UBG: red color does not appear even upon boiling.

The reaction is not very specific, it can be induced by porphobilinogen and various medicines (e.g. p-amino salicylic acid).

7. Detection of Calcium According to Sulkowitsch

Equal amounts of Sulkowitsch's reagent (ammonium oxalate, oxalate, acetate) and urine are put in a test tube. Acetic acid, as an acidic medium accelerates the reaction and in the presence of Ca-oxalate insoluble precipitates will be formed. This test is a semi-quantitative test and serves only for gross information.

Ca content of the urine might be:

normal: light turbidity (haze) develops

increased: immediate heavy turbidity (opaque) develops

decreased: a light opalescence develops after a while.

8. Detection of Sugar According to Nylander and Fehling:

Usually the urine sample is checked for glucose but sometimes the detection of fructose, galactose, lactose and pentoses might be necessary as well. Glucosuria physiologically does not exceed 130 mg / day which cannot be detected with routine tests. Glucose amounts in the urine exceeding this value are called glucosuria. It's most frequently cause is diabetes mellitus. It may also occur in healthy humans after excessive carbohydrate consumption or when the diet is containing mainly carbohydrates: this is called alimentary glucosuria.

Determination of glucose in the urine is based on the reducing capacity of glucose: reducing sugars in an alkaline medium are reducing Cu, Bi, Hg, Fe, Ag-ions from metal hydroxides.

a., Nylander 's test

Reagent: 100 g NaOH, 20 g bismuth-nitrate 40 g Seignette-salt (K-Na-tartrate) in 1000 ml distilled water).

Proteins, if there are any, should be eliminated from the urine sample before.

Few drops of Nylander reagent are added to 2 ml of urine in a test tube and boiled for 3 minutes. In the presence of glucose black precipitate develops: the precipitate is bismuth in metallic form. The test is fairly sensitive, positive results can be observed at the presence of as much as 0.08% glucose. Results should be read immediately after cooling, because after prolonged standing the boiled urine becomes black anyway even if glucose is not present. Compared to other reducing tests the advantage of the Nylander test is that in case of increased uric acid or creatinine content of the urine (they also have reducing capacity) it does not give misleading positive reaction.

b. Fehling's test:

Reagent: Fehling I = 3.5% CuSO_4 solution

Fehling II = 17% Seignette salt dissolved in 5% NaOH.

Equal volumes of the two reagent solutions are mixed. Boil it and add few drops of urine to the hot solution. The solution having azure blue color (resulting from mixing Fehling I and II solutions), turns to red (copper (I) oxide) when urine having glucose is added. The background of the reaction is that Cu^{++} ions bound in complex are reduced to Cu^+ ions by glucose.

Both tests give positive results not only in the presence of glucose but also in the presence of other kinds of sugar (pentose, fructose, lactose, galactose) present in the urine.

9. Detection of Proteins

Protein content of the urine is 2-8 mg/100 ml under physiological conditions; upper limit of the daily protein excretion is 100-150 mg, on average 40-80 mg. Excretion higher than that is called proteinuria. The urine may contain serum albumin, globulins, paraproteins, or protein degradation products. Albuminuria is the most frequent one. Generally materials of small molecular weight have higher clearance thus they are present in higher amounts in the urine.

Protein in the urine can be detected with precipitation tests. Since these protein tests might give positive result with the cellular elements in the urine it is suggested to make the tests with a centrifuged urine sample.

a., Sulphosalicylic acid test:

It is a fairly sensitive test; few drops of sulphosalicylic acid (20%) is added to about 3 ml of urine. In the presence of protein opalescence or precipitate can be observed (alkalic urine should be acidified previously). Content of test tube should be compared to reagent-free urine.

Evaluation:

- negative: no change can be observed
- protein in traces: just a slight, smoke-like turbidity can be observed against a black background.
Protein content: 0.1-0.5 g/l.
- slightly positive: moderate turbidity
+ = 0.5-1 g/l
- positive: turbidity without precipitate
++ = 1-2 g/l
- positive: turbidity with fine precipitate
+++ = 2-5 g/l
- positive: increased precipitate
++++ = above 5 g/l

b., Boiling test:

Urine proteins coagulate and form a precipitate upon boiling in slightly acidic medium. A few drops of acetic acid (1%) is added to 5 ml urine and the sample is boiled. In the presence of protein precipitate is formed. If precipitate cannot be dissolved with acetic acid, this indicates the presence of protein, if the precipitate dissolves, it indicates the presence of phosphates and carbonates.

Evaluation:

- opalescence (formation of sediment after few minutes): < 10 mg/100 ml urine
- turbidity: about 10 mg/100 ml
- precipitate: > 10 mg/100 ml

c., Heller's test:

The principle of the reaction is that proteins are denaturated by strong acids. HNO_3 is layered under urine in the test tube. (Caution! Urine has to be put first into the test tube, than put HNO_3 with a Pasteur pipette to the bottom of the tube.) A marked white disk develops at the interface of nitric acid and urine the thickness of which indicates the approximate amount of proteins.

10. Detection of Blood and its Decomposition Products with the Benzidine Test

Hemoglobin in the urine can be found usually in intact RBCs, which is called hematuria. If hemoglobin is released from the RBCs in the blood vessels it will show up in a dissolved state in urine: this is called hemoglobinuria (the hemoglobin binding plasma-haptoglobin is saturated due to the marked intravascular hemolysis, and free hemoglobin is filtered into the urine).

In both cases tests for detection of Hb are positive. In case of hemoglobinuria, however, the characteristic color of the urine does not change even after centrifugation, while in hematuria RBCs sediment during centrifugation and the supernatant of the centrifuged urine remains clear.

Hb present in the urine releases atomic oxygen from H_2O_2 in the reagent solution which oxidizes the chromogen in the reagent (benzidine, o-toluidine, guayacol-resin) into a colored end product.

The reagent is usually fresh-made. A knife-tip amount of benzidine is dissolved in 2-3 ml acetic acid; 3% H₂O₂ solution is prepared separately, the reagent is the mixture of the two solutions. Add 3 ml of reagents to the same amount of urine. In the presence of Hb a blue-green color will appear.

11. Detection of Acetone

Ketone bodies (acetic acid, beta-hydroxy-butyric acid, acetone) are present in the urine in very small amounts only. They are produced in higher amounts even in healthy humans, if carbohydrates are missing from the diet. In this case the antiketogenic effect of carbohydrates is missing, fats are not burned completely to CO₂ and H₂O. Diabetes mellitus is the most frequent cause of ketonuria; it's presence indicates the marked disturbance of carbohydrate and fat metabolism which may lead to serious ketoacidosis. In addition to diabetes mellitus ketonuria might occur after repeated vomiting and during prolonged starvation.

a., Rothera's test

For the detection of ketones a color reaction given by sodium nitroprusside in alkalic medium is suitable. A small amount ("knife-tip") of the reagent powder is put on filter paper and few drops of urine is added. In the presence of ketones a violet color will appear which might become darker (purple) with time.

Preparation of the reagent (modified Rothera mixture): 10g of powdered sodium nitroprusside is added first, then 200 g of ammoniumsulphate and 200 g dehydrated sodium-carbonate are added, then mixed and stored in a glass stoppered flask.

b., Legal's test:

Principle: Urine containing acetone turns the mixture of sodium-hydroxide and sodium-nitroprusside solution to burgundy red; this color changes to purple upon adding concentrated acetic acid.

To 5 ml urine in a test tube about 5-10 drops of sodium nitroprusside solution (dissolved in water) is added, this is alkalized with NaOH until the color turns red (that means positive reaction). Then acetic acid is added to the tube and observed; if the reaction is due to creatinine the color will fade and turn into a light blue, but if the red color originates from acetone there is no change in the color observed or the red tint becomes darker.

12. Detection of Bile Pigment

Normal urine does not contain bilirubin. The kidney threshold is about 30 µmol/l. Bilirubinuria may be observed at serum levels higher than that. The color of the excreted urine is characteristic: from saffron yellow to dark brown (the foam of the urine is also yellow). The principle of detection is that bilirubin turns into biliverdin having green color when treated with strong oxidizers.

a., Gmelin's test:

Proteins are previously precipitated and HNO₃ is layered under the urine. First colored rings will be formed at the interface, finally the whole solution becomes yellow. The different steps are as follows:

biliverdin (green) ⇒ bilicyanin (blue) ⇒ biliprasin (violet) ⇒ choletelin (yellow)

b., Rosin's test

1% alcoholic iodine solution (iodine tincture) is layered on about 3 ml of urine. In the presence of bilirubin a green ring develops at the interface. (With bovine bile used on the practical demonstrations a red-brownish reaction can be observed).

c., Rosenbach's test:

The urine to be tested is filtered through filter paper several times and a few drops of HNO_3 is dropped on the filter paper. The filter paper absorbs bilirubin and colored rings can be seen around the spot where the HNO_3 dropped which corresponds to oxidized products of bilirubin.

13. Detection of pus with Donne's Test (principle)

Pus in the urine (dead leukocytes, bacteria, tissue debris) might origin from kidney, bladder or urethra (pyelonephritis, pyelitis, urocystitis, urethritis). In women vaginal discharge (vaginal fluor) might be misleading.

First of all, 2 ml of 20% KOH is added to 5 ml of urine in a test tube and shaken (just once). In the presence of pus air bubbles in the fluid are floating or ascending very slowly. Explanation: Mucus passed into the leukocytes in the urine upon the effect of reagent increases the viscosity of urine which decreases the speed with which air bubbles rise to the surface (+ = is when the ascent of the small bubbles is slow, ++++ = bubbles of a larger size do not move). Since the test might be positive in proteinuria too, it is important to perform a urine sediment test.

14. Rapid Strip Tests

Different reagent papers and pills might give instant, semiquantitative information concerning urine. Sticking to instructions found on most reagents' container is of high importance! Fresh, well mixed but not centrifuged urine of room temperature should be used. Test paper impregnated with the buffer and the special indicator is immersed into the urine for a short time (30 to 60 seconds) and evaluated by comparing the color developed to a color scale usually found on the container.

Do not touch the reagent area on the stripes!

a., Detection of protein

Bromphenol-blue indicator (yellow if adjusted to pH 3 with buffer) turns to greenish-blue depending on the kind and concentration of the protein present. The test is based on the so-called protein-error of some indicators: that is, the color change of the indicator is proportional to the concentration of the protein. The indicator is most sensitive to albumin. The cause of an occasionally false positive result can be due to the following: urine has high alkalic buffer capacity and the test paper cannot provide the acidic pH needed and bromphenol-blue becomes blue even in the absence of proteins. In this case few drops of 3% acetic acid is added to the urine and the test is repeated.

b., Detection of glucose

The test paper is impregnated with buffered glucose-oxidase, peroxidase and a chromogen indicator. Glucose is formed to gluconolactone by glucose-oxidase at the oxygen of the air, while H_2O_2 is released. This product is decomposed by the peroxidase present and the

releasing atomic oxygen oxidizes the indicator to a colored compound. The intensity of violet color developed is approximately proportional to the concentration of glucose.

c., Detection of Ketonbodies

The test is based on the nitroprusside reaction of ketones (test paper is impregnated with sodium nitroprusside). In the case of a positive reaction a violet color can be observed, color intensity is approximately proportional to the ketone concentration. The reagent is more sensitive to acetacetic-acid than to acetone.

d., Detection of Urobilinogen

The test is based on Erlich's aldehyde reaction. The test paper becomes yellowish-brown or brownish in the presence of UBG; color intensity is proportional to the concentration.

e., Detection of Hemoglobin or Blood

Its principle is similar to that of benzidine test but the chromogene used is o-toluidine. The test is positive if the paper becomes blue. It is more sensitive to hemoglobin and myoglobin than to the intact RBCs.

15. The Effect of ADH on Rats

15 ml/kg of water is given to four rats through a gastric tube (with a syringe). Two groups of two animals are formed. 0.2 ml vasopressin (Adiuretin SD) is administered to one group ip. and urine is collected for a 2 hour period. Compare the volume of urine in the two groups. Rats treated with ADH will excrete less urine than the controls.

16. Dilution and Concentration Tests in Humans (principle)

a., Concentration capacity is tested by inducing thirst; the person tested receives dry and salty supper and is asked not to consume any fluid afterwards. While collecting urine every second hour starting from next morning till noon (or, alternatively, until the patient is able to bear thirst), the volume and specific weight of urine samples is determined. If any of the specific weight values measured reaches 1025 g/l within 4 hours the test might be stopped since this value indicates a good tubular function. If cellular functions of the distal tubuli are impaired but they are still able to some adjustments of the urine concentration: this is called hyposthenuria. Asthenuria means that there is no controlling function (kidney insufficiency) and the specific weight of urine equals with that of plasma-ultrafiltrate, that is 1010 g/l. No precise results can be obtained if the patient is edematous, drinks water secretly, or consumed a lot of water right before the test.

b., Diluting capacity of the kidney is investigated by giving 1.5 liters of water to the patient with empty stomach (it should be consumed during a 15 minute period) after the first urination in the morning. Urine is collected during the next 4 hours in 30 minute intervals and the quantity and specific weight of the urine fractions are measured. In case of good diluting capacity the 1.5 l of water consumed is excreted within 4 hours, and the specific weight of at least one of the fractions is around 1003. The test cannot be evaluated right after thirst induction, or if the patient is edematous. Dilution test is not used any more in diagnosis of kidney diseases; in kidney or tubulus diseases concentrating capacity impairs sooner than diluting capacity: the concentration test is more sensitive to impaired tubulus function than the dilution test.

17. Counting Cellular Elements in Urine According to Addis's method

The Addis's count expresses the number of cellular elements (RBCs, leukocytes, cylinders) excreted with urine in one minute. The procedure of cell number determination is cumbersome and gives just questionable qualitative information, though it is rarely applied in practice.

Urine collected for definite time is mixed and its quantity measured. After tenfold dilution of a small sample of collected urine the number of cellular elements are counted in Bürker's chamber. Knowing the time of urine collection and the volume of urine the number of excreted cells over one minute can be determined. Normally is less than 2000 erythrocyte/min or 4000 leukocyte/min.

18. Determination of Clearance

Clearance is concerns particular substances excreted by the kidney; it is the virtual amount of plasma cleared from a given substance in the kidney in a minute. Clearance of a particular substance can be calculated with the following equation:

$$C = U \times V / P$$

U = urine concentration of the substance

V = urine output/min

P = plasma concentration of the substance

Each endogenous and exogenous substance has a particular clearance value, different substances thereby can be classified in the following manner:

- Zero is the clearance of substances which are not excreted under normal conditions. Their characteristics is that they are either not filtrated (plasma proteins), or if filtrated, they are actively reabsorbed (glucose).

- GFR is equal to C for those substances which are freely filtrated, but the amount once filtrated does not change in the tubuli, there is neither secretion or reabsorption. Examples include creatinine, which is a physiological component of plasma, or inulin which is an exogenous substance.

- The clearance is equal to the RPF of those substances which are filtrated and are secreted into the tubulus-lumen by an active transport; e.g. PAH.

- The clearance is between these two theoretically extreme values for those substances which are freely filtrated and undergo both reabsorption and secretion in the tubuli (e.g. potassium ion).

There are substances, the clearance values of which give information on kidney function, therefore, they are routinely checked in everyday clinical practice.

Clearance techniques:

A substance providing a relatively constant plasma concentration would be optimal for this investigation; it should be freely filtrated in the kidney and should not be toxic.

The great advantage of the clearance test of endogenous substances is that their plasma level is practically constant; opposed to exogenous substances the plasma level of which can be kept constant with continuous infusion only. Accurate collection of urine is important and the bladder should be completely emptied before the test. In testing the clearance of exogenous substances relatively frequent urine collection might be necessary. It can be achieved with a bladder catheter, which might cause some urethral infection, therefore catheterization should be performed under sterile conditions. In determination of clearance of endogenous substances a simple 24-hour-long urine collection is sufficient, thus the complications of catheterization can be avoided.

In clinical practice the clearance of endogenous creatinine is the most frequently used for the measurement of GFR.

Performance of the test:

- 24 hour urine collection
- taking blood sample from the cubital vein
- determination of creatinine concentration from urine and blood sample
- urine output/min is calculated: quantity of 24 hour collected urine is divided by 1440
- Clearance value is calculated by the equation: $U \times V / P$.

Normal value is 120 ± 25 in men, and 110 ± 25 ml/min in women.

In clinical practice the value of "**osmotic clearance**" is also frequently used. The term is identical with the well known definition of the clearance: $C_{\text{osm}} = U_{\text{osm}} \times V / P_{\text{osm}}$ and means that amount of plasma which will be cleaned from its osmotically active particles in a minute.

The "**free-water clearance**" means a difference between minute diuresis and osmotic clearance.

$$C_{\text{H}_2\text{O}} = V - C_{\text{osm}} = V - U_{\text{osm}} \times V / P_{\text{osm}} = V \times (1 - U_{\text{osm}} / P_{\text{osm}})$$