Non-protein nitrogen compounds

General Medicine

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1 Non-protein compounds of nitrogen

Blood serum contains compounds of nitrogen other than proteins and peptides. ***Urea, creatinine, uric acid, ammonia and amino acids*** are the most important of them and have implications in clinical biochemistry. These nitrogen compounds remain dissolved even after precipitating the serum proteins with deproteinizing agents. Metabolism of some nitrogen compounds is tightly connected.

Major low-molecular weight compounds of nitrogen (acc. to Burtis et al. 1994):

Low-molecular weight nitrogen compound	Source	Clinical and biochemical significance
Amino acids	Proteins	• Liver disease • Renal disease • Inborn errors of amino-acid metabolism
Ammonia	Amino acids	• Liver disease • Renal disease • Inborn errors of enzymes of the urea cycle
Urea	Ammonia	• Liver disease • Renal disease
Creatinine	Creatine	• Renal disease
Uric acid	Purine nucleotides	• Purine metabolism disorders • Excessive cell lysis

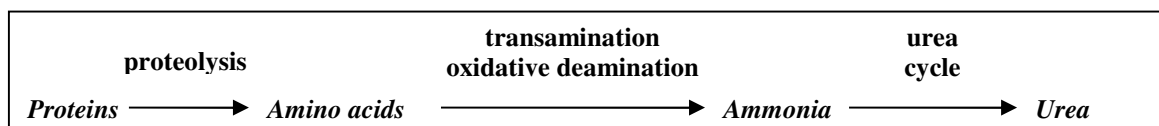|	transamination oxidative deamination		urea cycle	
proteolysis				
Proteins ⟶	*Amino acids*	⟶	*Ammonia* ⟶	*Urea*

Fig. 1: Relationships among some nitrogen compounds

2 Creatinine

Creatinine (inner anhydride of creatine) is formed in muscles by irreversible non-enzymatic dehydration and cleavage of phosphate from creatine phosphate, which serves in muscle as a source of energy for muscle contraction (Fig. 2).

Rate of creatinine production in the body is relatively constant. It reflects amount of muscle mass and under condition of physical rest and meat-free diet is stable. Creatinine is excreted in the kidney by glomerular filtration; an additional secretion by renal tubules becomes significant only at elevated serum creatinine levels.
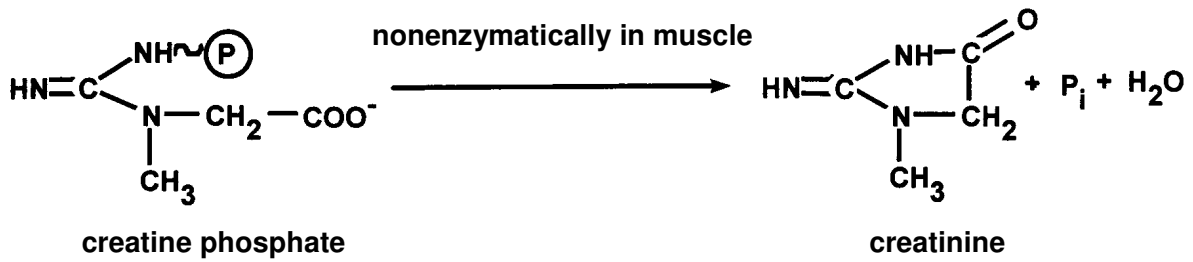
Fig.2: Origin of creatinine

2.1 Creatinine in serum

Concentration of creatinine in serum is directly proportional to the function of kidney glomerules and muscle mass of the body; for this reason it is usually somewhat higher in men than in women.

Estimation of creatinine in serum is a ***valuable indicator of glomerular filtration*** and is widely used mostly for ***monitoring of kidney diseases, including dialysed patients***. The relationship between serum creatinine and glomerular filtration is hyperbolic: creatinine excretion decreases with falling glomerular filtration, but its serum levels do not rise above reference limits until the glomerular filtration drops below 50 % (Fig. 3). It is evident from this relationship that for an early diagnosis of an initial stage of kidney damage estimation of serum creatinine is not very sensitive; rather, measurement of creatinine clearance should be used for this purpose. On the other hand, in an advanced stage of glomerular damage serum creatinine is a good measure of glomerular filtration.

Creatininemia can be elevated also as a result of release of creatinine from muscles due to ***acute destruction of skeletal muscles*** (rhabdomyolysis).
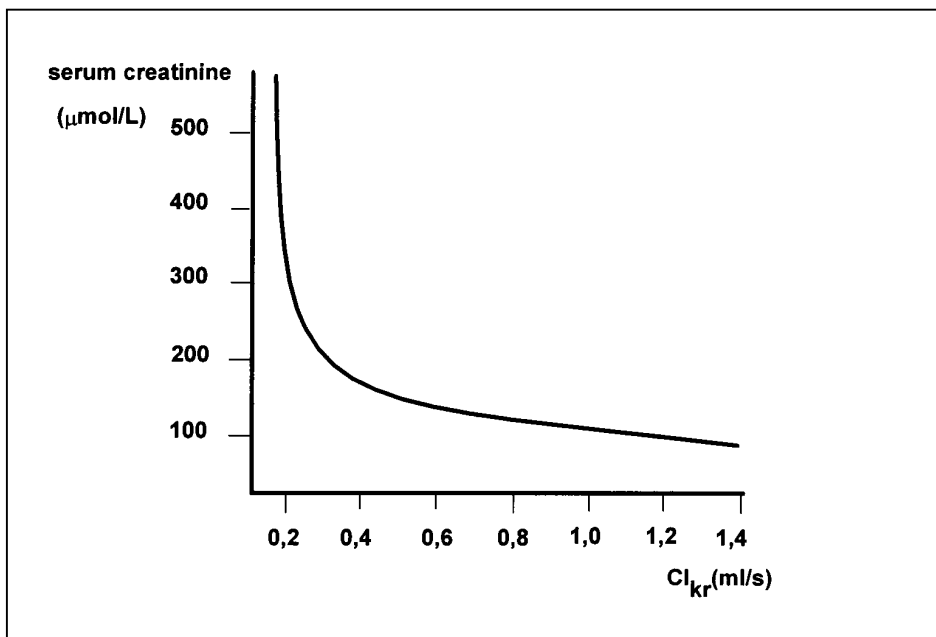

Fig.3: Relationship between serum creatinine and glomerular filtration

Reference values (S-Creatinine):
Women: **44 – 104 μmol/l**
Men: **44 – 110 μmol/l**

2.2   Creatinine in urine

When compared to other endogenous substances, creatinine excretion into urine is rather constant during the day; in persons with normal glomerular filtration it reflects amount and activity of muscles.

Concentration of creatinine in urine can be used as a ***check of 24-hour urine collection*** used for measurements of daily output of some substances into urine. Incorrect or incomplete urine collection often makes these measurements faulty; and simultaneous estimation of creatinine output provides a simple way to check it. Creatinine is estimated in a sample of urine from the 24-hour collection. The result in μmol/kg/day is compared with the reference values for urinary creatinine output in dependence on age and gender:

Age	*Creatinine Output (μmol/kg of body weight/day)*	
	Men	*Women*
20 – 29	210± 20	174± 34
30 – 39	194± 13	180± 34
40 – 49	174± 28	156± 34
50 – 59	171± 26	132± 32
60 – 69	149± 26	114± 23
70 – 79	126± 26	104± 19
80 – 89	103± 35	95± 22
90 – 99	83± 28	74± 12

If output of creatinine is 30 % or more below the tabular value, it is almost certain the collection of urine was incomplete.

Next, urinary creatinine is used for ***standardisation of urinary output of other substances*** if information on the 24-hour volume of urine is entirely missing. Concentration of the substance of interest is expressed per 1 mmol of creatinine.

Reference values:
Concentration of creatinine in urine (U-Creatinine):   3.0 – 12.0 mmol/l
Daily output of creatinine into urine (dU-Creatinine):   8.8 – 13.3 mmol/24 hours

2.3   Properties of creatinine and demonstration of creatinine in urine

Creatinine is well soluble in water. Its aqueous solution is weakly alkaline. Creatinine can be proved in a sample using the *Jaffé reaction* and the ***reaction with sodium nitroprusside***.

A. Reaction with sodium nitroprusside:

In alkaline medium, creatinine reacts with sodium nitroprusside and red color results. The test is not very specific; it is actually identical to the Legal test for acetone and acetoacetate in urine. Differentiation is possible by subsequent acidification of the mixture with concentrated acetic acid: color due to presence of creatinine disappears while the one due to presence of ketone bodies becomes even more intensive (the Legal test). If, instead, the color changes to blue or bluish-green, it indicates excretion of indolic melanogens (Thormählen test; occurs in patients with malignant melanoma).

Reaction with nitroprusside	*Color*	
	Alkaline pH	*After acidification*
Creatinine	red	disappears
Acetone and acetoacetate	red	deepens to red-violet
Indolic melanogens	red	blue to bluish-green

B. Jaffé reaction (see below).

2.4   Estimation of creatinine by Jaffé reaction

A simple, albeit not quite specific *Jaffé reaction* is based on the ***reaction of creatinine with salts of picric acid in alkaline medium***. Electrophilic oxogroup of creatinine allows dissociation of proton from a methylene group. Creatinine anion is then conjugated with positively polarized carbon of picrate ion and a red-orange complex is formed (Fig. 4). The test is used for estimation of creatinine both in serum and urine. In addition to creatinine, other compounds of body fluids, such as pyruvate, acetate, oxaloacetate, glucose, ascorbic acid, and acetone react with picrate as well – so called *Jaffé-positive chromogens*. The values of "true" creatinine in serum are normally 9 – 18 μmol/l lower than those determined by this method.
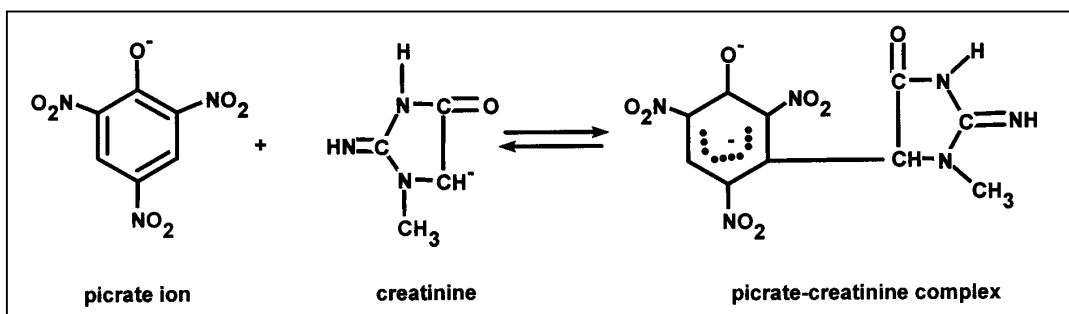

Fig.4: The Jaffé reaction

2.5 Clearance of endogenous creatinine

The term clearance in general applies to clearing of the internal environment by all means of excretion (kidney, liver etc.).

For excretion of low-molecular substances that pass freely the kidney filtration membrane this relationship applies:

$$\mathbf{GF \times P \; = \; U \times V}$$

U: concentration of substance in urine
V: volume of urine
GF: volume of glomerular filtrate
P: concentration of substance in plasma

If a substance is excreted into urine only by glomerular filtration, amount of the substance passing glomerular membrane in unit of time must be equal to the amount excreted into urine in the same time period. And if the amount of substance excreted into urine is U × V per second, then there must be a certain (theoretical) volume of blood plasma that has been "cleared" from the substance. This volume of plasma is called clearance.

Renal clearance of a certain compound is the volume of blood plasma that is completely cleared of the compound in kidneys within unit of time.

Measurement of clearance for various substances can provide information on various renal functions. If a substance is chosen that is excreted only by glomerular filtration, its clearance tells us value of the glomerular filtration. If a substance in use is both filtered and excreted by tubular secretion (e.g. p-aminohippuric acid), its clearance can be used for estimation of renal blood flow.

Compounds eliminated only by filtration through glomerular membrane can be employed to measure glomerular filtration. *Inulin* is an example of substance that passes the glomerular membrane, and then in tubules is neither secreted nor absorbed; measurement of *clearance of inulin* provides the exact value of glomerular filtration. The procedure for determination of inulin clearance, however, is demanding since a constant plasma level of inulin must be kept by a continuous intravenous infusion. Therefore, usage of this examination is limited to research purpose, while in clinical practice *clearance of endogenous creatinine* is used for the assessment of glomerular filtration. Creatinine is excreted largely (about 90 %) by glomerular filtration, and its plasma concentration is fairly stable. In comparison to clearance of inulin, the value of creatinine clearance is higher. The examination of creatinine clearance is useful especially in patients with less severe kidney damage, in which *glomerular filtration is lowered to 50 – 80 % of the normal*, and serum creatinine is still below reference limit.

On the other hand, at higher values of serum creatinine (above 180 μmol/l) the fraction of creatinine excreted by tubular excretion increases and validity of the examination of creatinine clearance becomes rather limited. In these cases estimation of serum creatinine is more useful.

2.5.1 Measurement of clearance of endogenous creatinine

In order to measure clearance of creatinine, its concentration in serum and in urine, as well as the volume of urine per given period of time, must be found. Patient usually collects urine for 24 hours. Errors in urine collection may be less likely if the collection period is shortened to 6 or 12 hours. The volume of collected urine is measured in a cylinder with precision to 10 ml, and after mixing a sample is taken, in which concentration of creatinine is estimated.

At the end of the collection period a blood sample for estimation of serum creatinine is taken as well. Information on the patient's body height and weight accompanies the samples to the laboratory.

Formula for calculation of clearance of endogenous creatinine:

$$Cl_{Cr} \text{ (ml/s)} = \frac{U \times V}{P}$$

U: concentration of creatinine in urine (mmol/l)
V: volume of urine in ml/s
P: concentration of creatinine in serum (mmol/l)

Values for clearance obtained in this way are hardly comparable between different people even if reference values are determined – clearance depends on the surface area of glomerular membrane. This surface area is different in every person; it is however assumed that the filtration area is proportional to the body surface. Hence, the value of clearance is **corrected** to the so-called ideal body surface, which is 1.73 m^2. The body surface of the examined patient is obtained from patient's body height and weight; it is either found in tables, or calculated according to formula:

$$A = 0.167 \times \sqrt{m \times l}$$

0.167: empirical factor
m: patient's body weight in kg
l: patient's body height in meters

Calculation of the **corrected clearance of creatinine** is following:

$$Cl_{Cr} \text{ corr. (ml/s)} = Cl_{kr} \times \frac{1.73}{\text{Patient's body surface in } m^2}$$

2.5.2 Estimation of creatinine clearance from serum creatinine

Clearance of endogenous creatinine can also be estimated from serum concentration of creatinine without any collection of urine using the **Cockroft & Gault formula**, which takes into account some factors known to affect glomerular filtration, such as age, gender, and body weight (related to muscle mass).

Estimation of Cl$_{Cr}$ for men:

$$Cl_{Cr} \text{ (ml/s)} = \frac{(140 - \text{Age [years]}) \times \text{Weight [kg]}}{44.5 \times \text{Serum creatinine (µmol/l)}}$$

Estimation of Cl$_{Cr}$ for women:

$$Cl_{Cr} \text{ (ml/s)} = 0.85 \times \frac{(140 - \text{Age [years]}) \times \text{Weight [kg]}}{44.5 \times \text{Serum creatinine (µmol/l)}}$$

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Recently, this estimation has been replaced by a more reliable calculation, called **MDRD formula**. It was suggested by Levey et al. in 1999. MDRD is an empirical formula that was derived from data of a large, multicentric study on the effect of diet in renal disease (Modification of Diet in Renal Disease – MDRD).

General formula is

GF = 2.83 × (0.0113 × serum creatinine)$^{-0.999}$ × age$^{-0.176}$ × (2.8 × serum urea)$^{-0.17}$ × (0.1 × serum albumin)$^{0.318}$

For women, the result is to be multiplied by 0.762.

Results of this estimation are in good agreement with measured values especially in patients with decreased glomerular filtration. None of these calculations is suitable for people with normal or just moderately decreased renal function.

Reference values for Cl$_{Cr}$ (ml/s):

The glomerular filtration decreases with age:

Age (years):	13 – 49	50 – 59	60 – 69	70 and more
Women:	1.58 – 2.67	1.0 – 2.1	0.90 – 1.80	0.8 – 1.3
Men:	1.63 – 2.60	1.2 – 2.4	1.05 – 1.95	0.7 – 1.0

The ideal clearance of creatinine with respect to the age can be also found using the equation: $Cl = -0.00946 \times age \text{ [years]} + 2.118$. The patient's value should be within ± 30 %.

2.6 Estimation of glomerular filtration using serum concentration of cystatin C

Cystatin C is a protein composed of 120 amino acids. Many tissues produce cystatin C at various rates. This protein is one of the most important inhibitors of extracellular cystein proteases. Rate of its synthesis is almost constant and unaffected by inflammation, catabolism or diet. Cystatin C is due to its relatively low molecular weight (approx. 13,000) freely filtered through glomerular membrane. Then, it is fully reabsorbed and degraded in proximal tubules. Thus, *plasma concentration of cystatin C* is a *good measure of glomerular filtration,* while its *urinary concentration correlates with impairment of tubular function*. Concentration of cystatin C can be measured by immunochemical techniques. Reference values differ according to the analytical method used; however, introduction of international standardized calibration is expected. Estimation of cystatin C provides several considerable advantages: it is sensitive for early stage glomerular defect; there is no need for 24-hour urine collection that is frequently faulty; and its measurement is not influenced by non-specific reactions as for creatinine. Although this estimation is rather expensive and still reserved for research it is believed that cystatin C will broaden the repertoire of commonly used examinations of renal functions in the near future.

2.7 Fractional excretion (FE)

The amount of a compound excreted to definitive urine depends on glomerular filtration (i.e. on the amount of the compound in primitive urine) and on tubular secretion and reabsorption. To make the explanation simpler, we will assume only compounds that are not eliminated by tubular secretion (or with negligible tubular secretion) in the following text.

Portion of the compound from primitive urine that is finally excreted to definitive urine is called *fractional excretion (FE)*. FE of certain compound can be between 0 and 1 (between 0 and 100 %). FE value equal to zero means that the compound is fully reabsorbed in tubuli; on the other hand, FE 100% means that all substance filtered across glomerular membrane passes to definitive urine. *Tubular reabsorption (TR)* is a "mirror" value to FE – it is the portion of substance that is reabsorbed by tubular cells. Assuming that tubular secretion is negligible it goes that

$$FE + TR = 100\%$$

General formula for fractional excretion is a ratio of clearance of studied compound and glomerular filtration

$$FE_x = \frac{U_x \times V}{P_x \times GF}$$

The glomerular filtration GF can be estimated as clearance of endogenous creatinine. The volume of urine in the formula is then cancelled out; thus, only concentration of substance in plasma and urine and concentration of creatinine in plasma and urine is required for calculation. Collecting urine for a prolonged time period (that is frequently prone to substantial errors) is not necessary.

$$FE_x = \frac{U_x \times P_{creatinine}}{P_x \times U_{creatinine}}$$

x   studied compound
U   concentration in urine
P   concentration in blood plasma or serum
Concentrations in both plasma and urine must be in the same units.

For assessment of the renal function fractional excretions of Na^+, K^+, Cl^-, phosphates and water are useful.

Fractional excretion of water is calculated as

$$FE_{H2O} = \frac{V}{GF}$$

After replacing GF with formula for creatinine clearance and cancelling we get simply:

$$FE_{H2O} = \frac{P_{creatinine}}{U_{creatinine}}$$

Reference values for FE_{H2O}: 0.01 – 0.02, i.e. 1 – 2 %.

Elevated values are found in:
- diabetes insipidus
- excessive intake of fluids
- damage to tubular kidney cells

2.8   Tubular reabsorption of water

Knowing the clearance of endogenous creatinine and amount of urine per one second, the value of ***tubular reabsorption (TR) of water*** can be obtained. The difference between glomerular filtration and volume of definitive urine per time unit (second) corresponds with the volume of water reabsorbed in renal tubuli per second.

$$TR_{H2O} = \frac{Cl_{Cr} - V}{Cl_{Cr}}$$

V   volume of definitive urine excreted per 1 s

Normal value of TR_{H2O}: 0.988 – 0.998

Decreased values indicate disorder of tubular reabsorption of water, e.g. in diabetes insipidus.

3   Urea

In quantitative terms, urea is the most significant degradation product of protein and amino acids in the body. It is produced in the liver from ammonia released by deamination reactions from amino acids. Urea diffuses freely through cell membranes and so its concentrations in plasma and intracellular fluid are equal.

Urea is excreted from the body mainly in the kidney by a combination of glomerular filtration and tubular reabsorption. The latter is variable: lower at higher diuresis while increasing when diuresis is low.

Concentration of urea in the blood depends on amount of protein in the diet, excretion by the kidney, and metabolic function of the liver.

For instance, serum urea can increase due to **high protein intake in the food**. One gram of protein (dietary or endogenous) can give rise to 5.74 mmol (0.34 g) of urea. Increased concentration of serum urea without changes in other non-protein nitrogen compounds (esp. creatinine) is a hallmark of an ***intense protein catabolism***, which occurs e.g. in starvation, febrile state or malignancy. Children have lower catabolism of protein, and show demonstrably lower serum urea levels.

Serum urea increases in ***diseases of the kidney that lead to marked restriction of glomerular filtration*** (below 30 %); simultaneously, high levels of creatinine are found. Unlike creatinine, estimation of urea is not suitable for an early detection of decrease in glomerular filtration. Sensitivity of the urea estimation for renal insufficience is low: it starts to exceed the reference values when more than 75 % of glomerular filtration is lost. On the other hand, urea is a sensitive parameter of renal hypoperfusion – in this case not only glomerular filtration is lowered but also tubular reabsorption of urea is increased. Serum concentration of urea therefore rises much more quickly than that of creatinine. In renal failure of the pre-renal type (e.g. in hypoperfusion, most frequently in dehydration) the ratio of serum concentrations of urea and creatinine (in µmol/l) is above 160.

In ***liver function failure***, synthesis of urea falls down, and so its concentration in the serum.

Concentration of urea in serum and urine can also be used for calculation of ***nitrogen balance***.

Some causes of changes in serum urea concentration:

Serum urea increased:	*Serum urea decreased:*
Impaired kidney function	Low-protein diet
High-protein diet	Impaired liver function
High catabolism of protein	Late pregnancy (growth of foetus demands protein)
Dehydration	

Reference values (fS-Urea):	Women:	**2.0 – 6.7 mmol/l**
	Men:	**2.8 – 8.0 mmol/l**

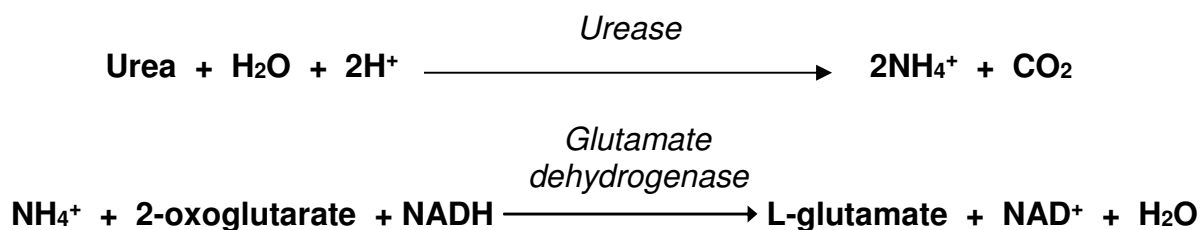**Output (loss) of urea in urine (dU-urea):**

An adult excretes daily 167-583 mmol/ 24 hours, depending on protein uptake in food and protein catabolism. Urinary urea output is calculated according to the formula:

dU-urea(mmol/24 hours) = U-urea (mmol/l) x volume of urine per 24 hours (l)

Principle of urea estimation in serum and urine:

In general, urea in biological fluids can be estimated either directly, or indirectly as ammonia. In the indirect assay, first an enzyme ***urease*** cleaves urea to carbon dioxide and ammonia that in aqueous medium exists as ammonium ion. Next, the amount of ammonium is estimated by the ***Berthelot's reaction***: ammonium ion with sodium hypochlorite and phenol or salicylate form a colored product. The reaction is catalyzed by sodium nitroprusside.

Nowadays, the recommended routine method for measurement of the ammonium ions generated by the urease reaction utilizes conversion of α-ketoglutarate to glutamate. The reaction is catalyzed by ***glutamate dehydrogenase***, and coupled to oxidation of NADH to NAD^+ (Warburg's optical test).

$$Urea + H_2O + 2H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + 2\text{-oxoglutarate} + NADH \xrightarrow{\text{Glutamate dehydrogenase}} L\text{-glutamate} + NAD^+ + H_2O$$

4 Uric acid

Uric acid is the ***end product of purine metabolism*** in humans (Fig. 5). Purine nucleotides are building blocks of the nucleic acids and other compounds with important roles in metabolism (e.g. ATP, NAD^+). Most cells are capable to synthesize purine nucleotides *de novo*. An important intermediate in purine synthesis is 5-phosphoribosyl-1-diphosphate (PRPP) that is formed in reaction catalyzed by ***PRPP-synthetase***. In the course of the subsequent reactions, the purine core is formed and principal purine nucleotide, inosine monophosphate (IMP) is produced. IMP is then converted to other purine nucleotides, adenosine monophosphate (AMP) and guanosine monophosphate (GMP).

Nucleic acids from own cell nuclei as well as from nutrition are catabolized to nucleotides, nucleosides and bases. Purine bases are finally metabolized by ***xanthine oxidase*** to uric acid. Degradation of purines is terminated at this point in humans and primates. Other mammals can further convert uric acid by uricase to allantoin which is better soluble in water. A part of purines is used for re-synthesis of nucleotides by ***hypoxanthine-guanine phosphoribosyltransferase (HPRT)*** and ***adenine phosphoribosyltransferase (APRT)*** ("salvage pathway", Fig. 6).

Lactim form
(Enol-form)
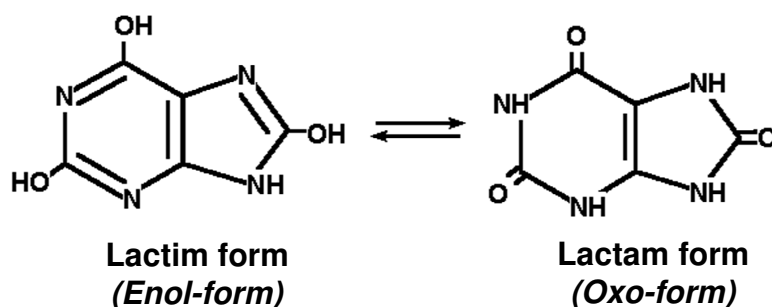
Lactam form
(Oxo-form)

Fig. 5: Uric acid

Total amount of uric acid in the body is approximately 1 g. Uric acid originates from three sources: food nucleotides, degradation of tissue nucleotides and biosynthesis. Uric acid is not only a waste product but it also protects cells from reactive oxygen species.

75 – 80 % of uric acid is eliminated by kidneys (see later). The rest (20 – 25 %) is eliminated by ***gastrointestinal tract*** where it can be further degraded to NH_3 and CO_2 by bacterial flora (Fig. 7).
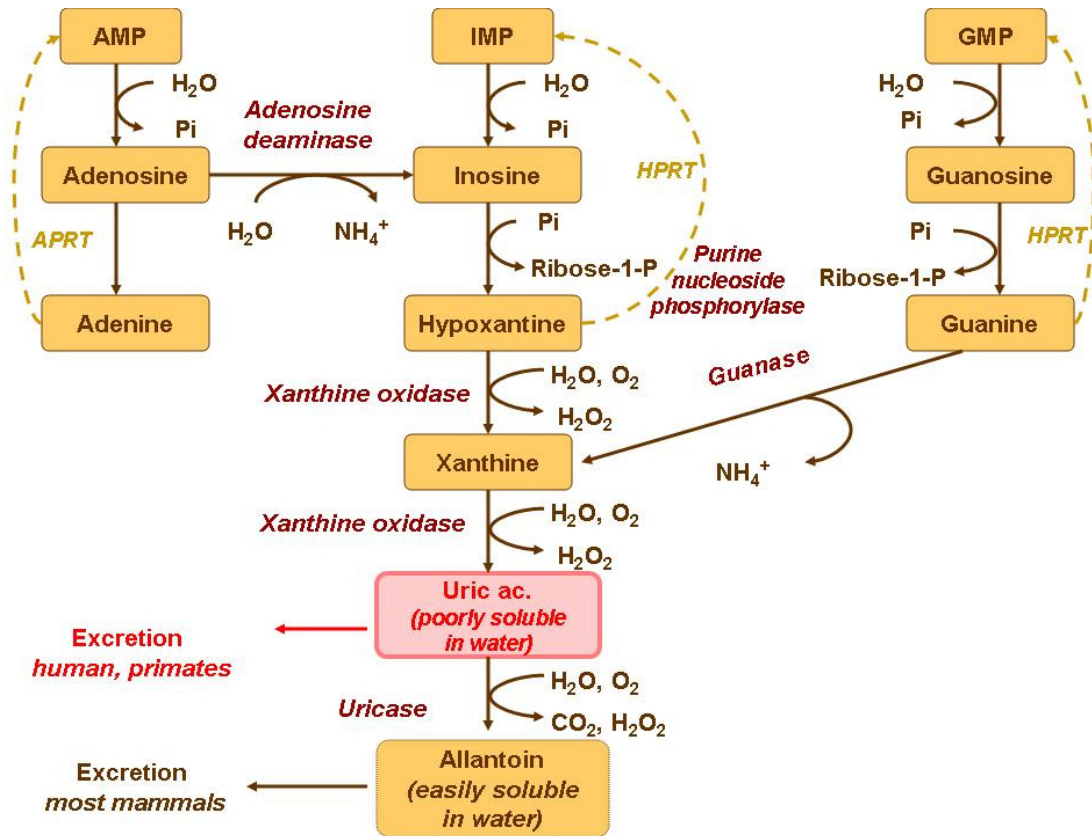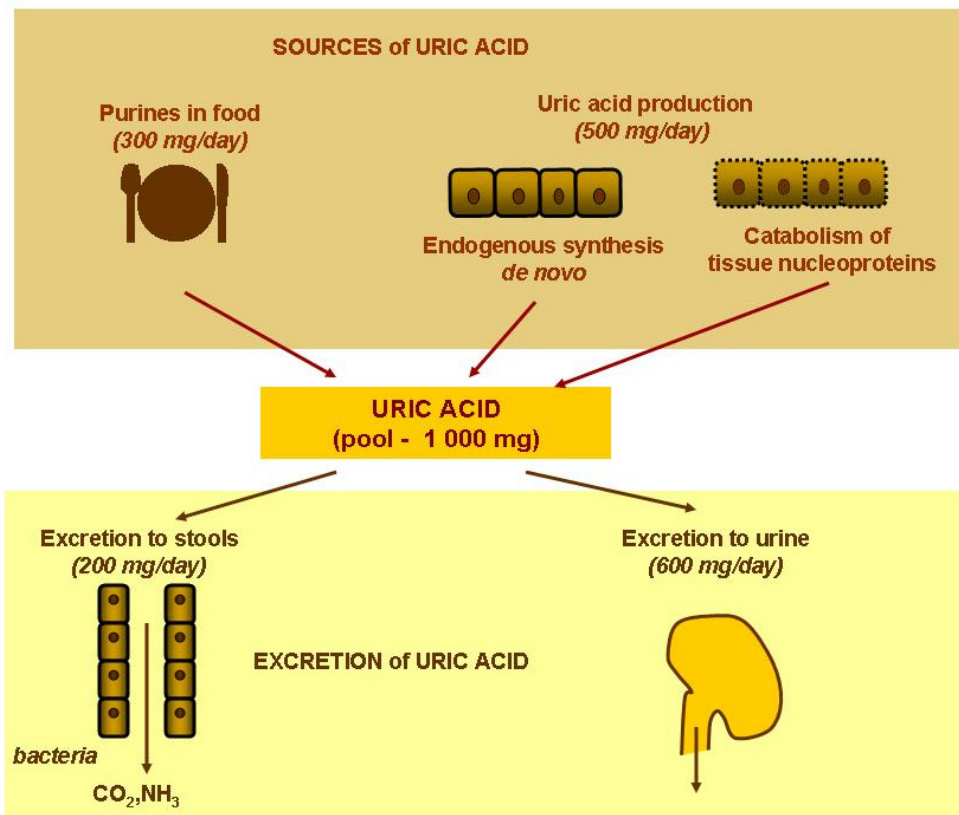
Fig. 6: Degradation of purine nucleotides

Fig. 7: Sources and excretion of uric acid

4.1 Properties of uric acid

Uric acid is ***poorly soluble in water***. At pH values below 5.5, as typical for urine, most molecules of uric acid exist in the non-dissociated and thus less soluble form. Uric acid can then form crystals or concrements. Coolness contributes to insolubility of uric acid. The solubility of uric acid rises with increasing pH value. At physiological blood pH 7.4 the ionized form prevails and more soluble sodium or potassium urate is formed.

Oxidative cleavage with concentrated nitric acid can be used for the uric acid detection. Imidazole ring of purine is opened and two molecules join, forming purpuric acid. Salts of purpuric acid are colored. Addition of ammonia yields murexide (ammonium salt of purpuric acid, Fig. 8). Murexide reaction is employed in urine stone analysis.
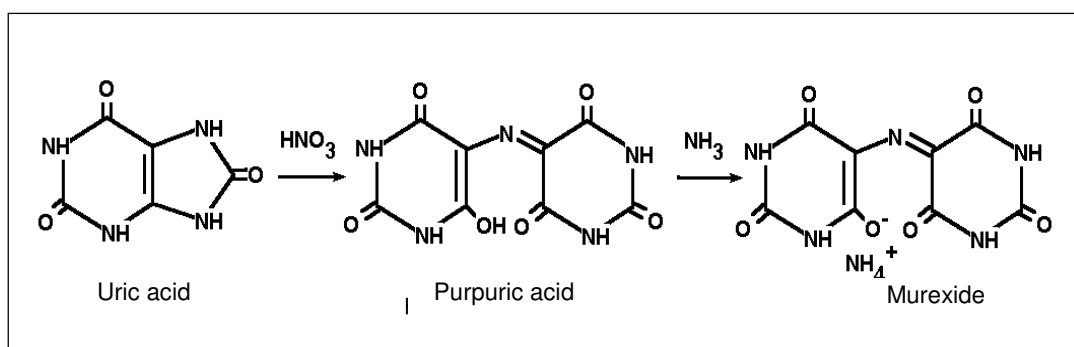

Fig. 8: Murexide reaction

4.2 Estimation of uric acid concentration

Most state-of-art techniques for measuring uric acid concentration employ ***uricase***, an enzyme converting uric acid into allantoin, hydrogen peroxide and carbon dioxide (Fig. 9). Decrease in uric acid concentration in the reaction mixture may be determined by ***direct photometry at 290 – 293 nm***, because uric acid and allantoin differ in their absorption spectra. Uric acid has an absorption peak at this wavelength while allantoin does not absorb light in this range.

Another choice is ***indirect measurement*** in which hydrogen peroxide formed in the uricase reaction is used in a coupled reaction catalyzed by ***peroxidase***. A ***quinonimine dye*** is formed by ***oxidative coupling*** of (usually) 4-aminoantipyrine and a suitable derivative of phenol (e.g. N-ethyl-N-(2-hydroxy-2-sulphopropyl)-m-toluidine)). Intensity of the resulting color is proportional to the concentration of uric acid in solution. Ascorbic acid interferes with this method; therefore ascorbate oxidase is added to eliminate such an influence.
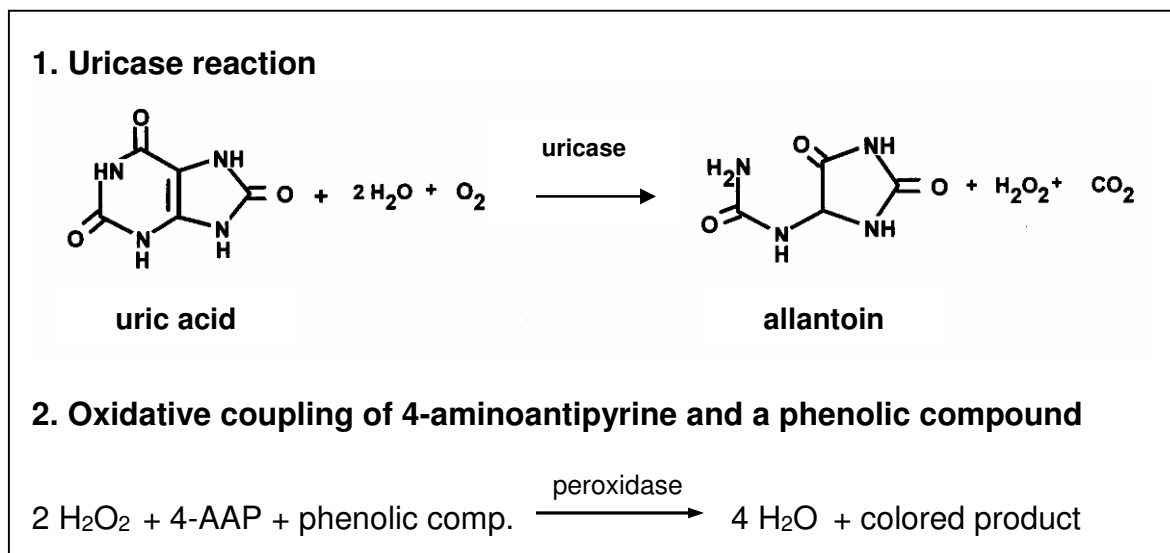
1. Uricase reaction

uricase

uric acid   allantoin

2. Oxidative coupling of 4-aminoantipyrine and a phenolic compound

peroxidase

$2\ H_2O_2$ + 4-AAP + phenolic comp. $\longrightarrow$ $4\ H_2O$ + colored product

Fig. 9: Estimation of uric acid

4.3 Uric acid in blood serum

Concentration of uric acid in blood depends on intake of purines in food, intensity of its production and on its excretion. Especially increased levels of uric acid are of clinical significance.

Hyperuricemia results from ***overproduction or decreased excretion of uric acid***. Concentration of urates can exceed their solubility in hyperuricemia.

Overproduction of uric acid

- Excessive *de novo* synthesis of purines associated with hyperuricemia is found in some ***genetically determined impairments of purine metabolism***. Partial or complete ***defect of hypoxanthine-guanine phosphoribosyltransferase (Lesch-Nyhan's syndrome)*** may serve as an example. Re-utilization of purine nucleotides is impaired and purines are therefore degraded to uric acid. Another genetic disorder leading to increased production of uric acid is ***increased activity of phosphoribosylphosphate synthetase***.

- ***Anticancer therapy*** (cytostatic chemotherapy, radiotherapy) induces extensive cell lysis and leads to increased formation of uric acid. Purine nucleotides are released from degraded nucleic acids and metabolized to uric acid. Some hematological diseases associated with excessive production (polycythemia vera) or degradation of cells (leukemia, hemolytic anemia) are, in a similar way, joined with hyperuricemia.

- ***Increased intake of foodstuffs rich on purines*** (offal, meat, legume, in smaller extent also chocolate, cocoa and coffee) also leads to overproduction of uric acid. Kidneys may fail to compensate the overload with uric acid and uricemia grows up.

- ***Alcohol*** increases the level of uric acid. Increased production of lactate inhibits renal secretion of uric acid. The decreased excretion of uric acid is later replaced with high uricosuria.

Decreased excretion of uric acid

Decreased excretion of uric acid is one of the most frequent causes of hyperuricemia.

- *Decreased tubular secretion* of uric acid is common in patients with hyperuricemia. The cause of this disorder is unknown.

- Excretion of uric acid is lowered in *states associated with decreased glomerular filtration and impaired tubular function*.

Reference values (fS-UA):	Women	140 – 340 µmol/l
	Men	220 – 420 µmol/l

4.4   Uric acid in urine

Majority of uric acid (75 – 80 %) is eliminated by kidneys. It is freely filtered in glomeruli (uric acid is only minimally bound to proteins) and then most of it is reabsorbed in the proximal tubuli. Next, uric acid is again secreted in the distal part of proximal tubuli and once again reabsorbed. Approximately *0.6 g of uric acid per day* (3.6 mmol/day) is excreted under purine-free diet. Normally, with a common diet, these values are higher – about 0.8 g/day (5.0 mmol/day, Fig. 10). Tubular secretion of uric acid is inhibited if other organic ions (e.g. acetoacetic acid, β-hydroxybutyric acid, lactate or some drugs) are also excreted to a high extent.
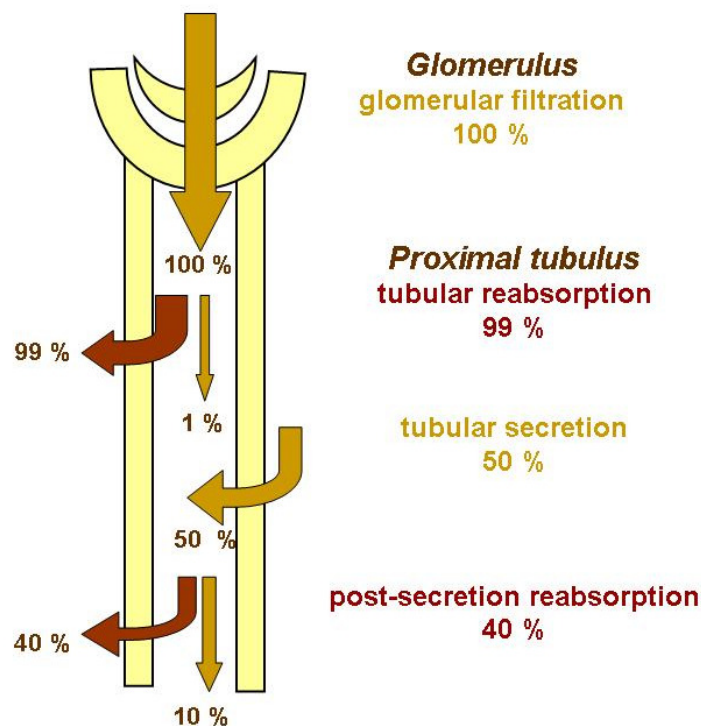

Fig. 10: Excretion of uric acid

Uric acid is a considerable risk factor for both urinary tract and renal parenchyma.

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If the excretion of uric acid is increased there is a higher risk of ***urate urolithiasis***. Especially important is this risk in individuals with permanently acidic and concentrated urine. Urate concrements are usually made of pure **uric acid**, sometimes of **sodium urate**. **Ammonium urate** may precipitate in weakly alkaline urine, typically in case of urinary infection.

Crystals of sodium urate can ***precipitate even in renal interstitium*** and cause inflammatory response (chronic interstitial nephritis).

Acute renal failure is a relatively rare disorder that results from sudden increase of uric acid in blood (e.g. cytostatic therapy in patients with leukemia) in case that urine is concentrated and acidic at the same time (dehydration). Crystals of uric acid are formed in distal tubuli and collecting ducts under these conditions and drainage of urine is blocked (acute urate nephropathy).

Examination of uric acid in urine is especially important in patients with increased blood concentration of uric acid and in patients with urolithiasis.

The amount of uric acid in urine can be expressed in several ways:

a) ***Concentration in the morning sample of urine***. According to results of this test, the amount and type of beverages is adapted in order to decrease the urate concentration.

b) ***The amount of uric acid excreted in 24 hours***. Urine is collected for 24 hours and a mixed sample is analyzed. This method can distinguish between hyperuricemia from increased production and decreased excretion.

c) ***Uric acid/creatinine ratio*** in a random sample of urine. Results give information similar to the previous technique but it is not necessary to collect urine for 24 hours.

d) ***Clearance of uric acid*** may be calculated according to formula

$$Cl_{UA} \text{ (ml/s)} = \frac{U_{UA} \times V}{P_{UA}}$$

U_{UA}: concentration of uric acid in urine (mmol/l)
P_{UA}: concentration of uric acid in serum or plasma (mmol/l)
V: volume of urine in ml/s

e) ***Fractional excretion of uric acid***. This parameter gives summary information on transport of uric acid in renal tubuli. The role of dysfunction of renal tubular cells on hyperuricemia may be assessed using this value. It can be calculated for randomly taken sample of urine and blood in which the concentration of uric acid and creatinine is examined. Fractional excretion of uric acid is calculated using the formula:

$$FE_{UA} = \frac{U_{UA} \times P_{Cr}}{P_{UA} \times U_{Cr}}$$

U_{UA}: concentration of uric acid in urine (mmol/l)
P_{Cr}: concentration of creatinine in serum or plasma (mmol/l)
P_{UA}: concentration of uric acid in serum or plasma (mmol/l)
U_{Cr}: concentration of creatinine in urine (mmol/l)

Reference values:

Uric acid excreted per day:	**1.5 – 4.5 mmol/day**
Clearance of uric acid	**0.07 – 0.22 ml/s**
Fractional excretion of uric acid:	**0.04 – 0.12**

4.5   Gout

Gout is an important symptom of disorders of uric acid metabolism. Concentration of uric acid is increased in extracellular fluid and in tissues. If the solubility of urates is exceeded the urate crystals precipitate from solution to tissues with scarce blood circulation – e.g. the soft tissues of joints. Inflammatory response is started, resulting in degenerative damage to the joint. Gouty tophus is a small knot-like formation characteristic for chronic gouty arthritis. Crystals of urate are deposited in the centre, surrounded with inflammatory cells and fibrous tissue.

Recurring attacks of acute arthritis are another typical feature of gout. Crystals of sodium urate can be found in leucocytes of synovial fluid during the attack.