

ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK

Proteins in Serum and Urine

General Medicine

Lenka Fialová & Martin Vejražka

translated and edited by Jan Pláteník



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1 Proteins in serum

Blood plasma or serum¹ contains many different proteins, originating from various cells. **Biosynthesis** of most of the serum proteins localizes to the liver; small part comes from other tissues such as lymphocytes (immunoglobulins) and enterocytes (e.g. apoprotein B-48). **Degradation** takes place in hepatocytes and the monocyto-phagocytic system, where proteins are degraded mainly following formation a complex (e.g. antigen-antibody, hemopexin-haptoglobin, and lipoproteins). Another way of removal of serum proteins represents their excretion by the kidney and gastrointestinal tract.

The proteins in plasma serve numerous functions:

- keep colloidal-osmotic pressure of the intravascular fluid
- transport of many compounds (e.g. hormones, vitamins, lipids, bilirubin, drugs)
- keep acid-base balance
- nutrition
- blood clotting and fibrinolysis
- defense reactions (humoral immunity):
 - specific immunity (immunoglobulins)
 - non-specific immunity (complement, acute phase response proteins)

	Protein <i>Rel. molecular mass</i>	Concentration in serum (g/l)	Half-life (days)	Function	
	Prealbumin (Transthyretin) <i>54,000</i>	0.2 – 0.4	2	<ul style="list-style-type: none"> • binds thyroid hormones and retinol-binding protein 	↓ malnutrition
	Albumin <i>68,000</i>	35 – 53	15 – 19	<ul style="list-style-type: none"> • the most significant transport protein • keeps osmotic pressure of blood plasma • protein reserve of the organism 	↓ catabolism ↓ hepatopathy ↓ loss of proteins
α₁ region	α₁-lipoprotein <i>180 – 360,000</i>	1.0 – 1.6 (Apo A-I)		<ul style="list-style-type: none"> • high density lipoprotein (HDL) • transport of cholesterol to the liver 	
	α₁-antitrypsin (α ₁ -antiproteinase) <i>54,000</i>	0.9 – 2.0	4	<ul style="list-style-type: none"> • inhibitor of lysosomal proteases (mainly elastase from polymorphonuclear leukocytes) • inherited deficiency can cause disease of lungs (emphysema) and liver (cirrhosis) 	↑ acute inflammation
	α ₁ -acid glycoprotein (orosomukoid) <i>40,000</i>	0.5 – 1.2	5	<ul style="list-style-type: none"> • binds lipophilic substances (e.g. progesterone and some drugs) • involved in regulation of immune response 	↑ inflammation
	α ₁ -fetoprotein <i>69,000</i>	< 7.5 µg/l	3.5	<ul style="list-style-type: none"> • normally produced by fetal liver and yolk sac • major protein of fetal serum • physiologically found in serum of pregnant women 	↑ hepatoma ↑ some gastrointestinal tumors ↑ pregnancy

¹ Recall that *blood plasma* is the liquid circulating in blood vessels *in vivo*. *Blood serum* contains the same proteins as plasma except those involved in blood clotting, especially fibrinogen and prothrombin.

α₂ region	haptoglobin 85,000 – 1,000,000 ²	0.3 – 2.0	2	<ul style="list-style-type: none"> • binds free hemoglobin 	↑ acute inflammation ↓ hepatopathy ↓ intravascular hemolysis (consumption of haptoglobin)
	α₂-macroglobulin 800,000	1.3 – 3.0	5	<ul style="list-style-type: none"> • protease inhibitor (thrombin, trypsin, chymotrypsin, pepsin) • transport of small proteins (cytokines, growth factors) and divalent ions (such as Zn²⁺) • very high molecular weight prevents passage even through damaged glomerular membrane 	↑ acute inflammation
	ceruloplasmin 160,000	0.2 – 0.6	4.5	<ul style="list-style-type: none"> • oxidoreductase activity (oxidation of Fe²⁺ to Fe³⁺) • contains up to 90 % of serum copper 	↓ Wilson disease (hepatolenticular degeneration)
β₁ region	transferrin 77,000	2.0 – 3.6	7	<ul style="list-style-type: none"> • transport and sequestration of free iron 	↑ iron deficiency ↓ malnutrition ↓ hepatopathy ↓ inflammation
	hemopexin 57,000	0.5 – 1.1	3 – 7	<ul style="list-style-type: none"> • binds heme 	
	β-lipoprotein 2,750,000	0.7 – 0.9 (Apo B-100)	3	<ul style="list-style-type: none"> • low density lipoprotein (LDL) • transports cholesterol to peripheral cells • very high molecular mass 	
	C4 component of complement 206,000	0.1 – 0.4	1	<ul style="list-style-type: none"> • part of complement 	↑ inflammation ↓ auto-immune conditions
β₂ region	C3 component of complement 180,000	0.8 – 1.4	1	<ul style="list-style-type: none"> • part of complement 	↑ inflammation ↓ auto-immune conditions
	β ₂ -microglobulin 11,800	0.001 – 0.002		<ul style="list-style-type: none"> • part of leukocytic antigens 	↑ hematologic tumors ↓ disorder of tubular resorption
	fibrinogen 340,000	1.5 – 4.5		<ul style="list-style-type: none"> • part of blood clotting cascade, precursor of fibrin • physiologically only in plasma, absent from serum 	↑ inflammation
	C-reactive protein 111,000	1.5 – 5 mg/l	1	<ul style="list-style-type: none"> • activation of complement 	↑ acute inflammation (bacterial)
γ region	IgG 150,000	8.0 – 18.0	24	<ul style="list-style-type: none"> • late antibodies 	↑ inflammation (chronic)
	IgA³ 160,000	0.9 – 3.0	6	<ul style="list-style-type: none"> • antibodies of mucosal immunity 	↑ inflammations of mucosal membranes and liver
	IgM 900,000	0.6 – 2.5	5	<ul style="list-style-type: none"> • early antibodies 	↑ acute inflammation

Table 1: Overview of serum proteins (arranged according to their electrophoretic mobility)

² Haptoglobin exists in three phenotypes. One of them has a tendency to form high molecular weight polymers.

³ Electrophoretic mobility extends also to β-region.

1.1 Albumin

Albumin represents 55 – 65 % of the total serum protein (average albumin concentration in plasma is 40 g/l). It is synthesized in the liver and its production depends on the intake of amino acids.

- Albumin substantially contributes to maintenance of the plasma oncotic pressure. Levels of albumin below 20 g/l are associated with edema.
- It serves as a carrier for transport of bilirubin, heme, steroids, thyroxin, free fatty acids, bile acids, metals, drugs, and many other substances.
- It forms a protein reserve in the body and serves as a source of amino acids, especially the essential ones, for many tissues. In malnutrition the albumin concentration decreases. However, serum albumin is not suitable as an early marker of protein malnutrition, since under the condition of amino acid deficiency also albumin catabolism slows down; and albumin is mobilized from the interstitial space in order to keep the plasma oncotic pressure. From these and other reasons (long half-life, large pool in the body) decrease in albumin does not fully reflect the extent of protein malnutrition.

1.2 Acute phase proteins

The **acute phase response** is a physiologic process that occurs due to local or systemic inflammation, tissue injury due to trauma or surgery, or tumor proliferation. In many other situations the reaction is present in a less pronounced form, such as the condition following an extreme physical exercise, acute heart stroke or around delivery.

Simply stated, the acute phase reaction is triggered by conditions that include destruction of cells, reversible cell damage followed by reparation, or metabolic activation of cells participating in the immune response.

During the acute phase reaction the involved cells produce many mediators and signaling molecules, which in the liver (and to a lesser extent elsewhere) induce rapid changes in protein synthesis. The proteins, whose plasmatic concentrations change markedly (over 25%) under this condition are called the **acute phase proteins (acute phase reactants, APRs)**. Some proteins increase in plasma (positive acute phase reactants); whereas others decrease (negative acute phase reactants).

The APRs represent a heterogeneous collection of proteins. Nevertheless, most of them can be classified according to its function to one of the following groups:

- **Components of immune reactions:** Some APRs directly participate in removal of the agent that caused inflammation. Other proteins function in removal of damaged cells, or modulate the immune reaction. The examples are C-reactive protein, the proteins of complement, especially C3 and C4, tumor necrosis factor α (TNF- α), interleukin 1 (IL-1) and interleukin 6 (IL-6).
- **Protection from collateral tissue damage:** During the acute phase the phagocytes and dying cells release many substances with a potential to destroy the causative agent and 'dissolve' the affected tissue. Proteolytic enzymes and reactive oxygen species are the main substances acting in this manner. Their effects must be limited to keep the collateral damage to healthy tissue as low as possible. That is why the APRs include **protease inhibitors** (α_1 -antitrypsin, α_1 -antichymotrypsin, α_2 -macroglobulin), and also **proteins that inhibit production and availability of reactive oxygen species**, very often by

binding and stabilizing transition metals and their complexes (haptoglobin, hemopexin, ferritin, ceruloplasmin).

- **Transport of waste by-products of inflammation:** in addition to haptoglobin and hemopexin mentioned above probably also serum amyloid A (SAA) protein would be in this category.
- **Coagulation factors and proteins participating in tissue regeneration:** e.g. fibrinogen.

Function of some of the positive acute phase proteins remains unknown, although the protein may be significant in clinical examinations (e.g., procalcitonin, PCT).

The positive acute phase proteins can be divided according to the time dynamics of their increase into three groups:

- **Early response acute phase proteins** have very short biological half-life; changes in their levels begin to appear 6 – 10 hours after the primary insult, and peak during the second or third day. The typical representatives are C-reactive protein (CRP) and serum amyloid A protein (SAA). More recently, procalcitonin (PCT) is examined.
- **Intermediate response acute phase proteins** increase 12 – 36 hours after beginning of the disease, and peak by the end of the first week. α_1 -acidic glycoprotein (orosomucoid), α_1 -antitrypsin, haptoglobin, and fibrinogen belong to this group.
- **Delayed response acute phase proteins** are represented by C3 and C4 proteins of the complement and ceruloplasmin. Changes in their levels are observable 48 – 72 hours after the insult and reach maximum in 6 – 7 days.

The **C-reactive protein (CRP)** is one of the most significant APR's. This protein serves as an opsonin. It has been named after its ability to precipitate with C-polysaccharide of *Pneumococcus*. The CRP increases as early as in **4 hours** following the acute phase onset and during the first two days its concentration may rise more than **100times**. Maximal CRP concentration is reached in 24 – 48 hours; its half-life is also about 24 hours.

The physiological concentration of CRP in plasma is usually below 2-8 mg/l. Rapid and robust CRP increase, typically above 60 mg/l, occurs especially in **acute bacterial infections**. In contrast, viral infections show relatively low levels of CRP (usually below 40 mg/l). Estimation of plasmatic CRP levels thus helps in decision whether an antibiotic therapy should be started. Successful antibiotic therapy should lead to fast fall of CRP level; in contrast, persistent infection causes lasting CRP elevation.

Examination of CRP can reveal an **infection following surgery**. The third day after an operation the CRP should rapidly normalize. Persistent elevation or only a partial decrease followed by a new elevation suggests presence of an infection or another inflammatory complication.

A moderate increase in CRP is also associated with **heart stroke**. Slightly elevated CRP levels (usually around 10 mg/l) are considered as a sign of risk of cardiovascular disease. CRP monitoring is also useful in **auto-immune diseases**.

A disadvantage of CRP is its low specificity. Unlike procalcitonin it does not inform about severity of organ damage, but only about presence of the infection. The two markers thus complement each other.

Procalcitonin (PCT) is an acute phase protein that has recently come into focus both in research and clinical practice. It is a protein that has 116 amino acids (MW 13,000) and is physiologically formed by C cells of thyroid gland as a precursor of calcitonin. Especially

during generalized bacterial infections it is also produced by other cells, mainly monocytes, macrophages and neurocrine cells, and its plasmatic concentration swiftly rises. The exact physiological significance of procalcitonin is rather unclear; it probably participates in regulation of inflammation and has analgesic effects. Half-life of procalcitonin is one day and after an immune stimulation its serum concentration rises about 20times during 2-3 hours. An increase is observed only in **generalized bacterial, mycotic and protozoic infections**, but not in viral infections. A less pronounced elevation is seen in polytraumatic injury, burns and large abdominal surgery.

Negative acute phase reactants are proteins whose levels decrease during an acute stress decrease. Albumin, prealbumin and transferrin behave in this manner. For monitoring of the acute phase response, they are less significant than the positive reactants; rather, these proteins are examined as markers of liver protein synthesis and malnutrition.

1.3 Immunoglobulins

Antibodies (immunoglobulins) are specific proteins of blood plasma with electrophoretic mobility $\beta - \gamma$. They are produced by plasmatic cells as humoral immune response to a particular **antigen**. The immunoglobulin molecule is able to bind specifically the antigen against which the immunoglobulin was directed; an immune complex results. Other functions of immunoglobulins encompass fixation of complement, binding neutrophilic leukocytes and macrophages, and activation of phagocytosis. There are 5 classes of immunoglobulins: **IgG, IgM, IgA, IgD, and IgE**. The basic structure of immunoglobulin molecule consists of **two identical heavy (H) chains**, designated according to the classes as $\gamma, \mu, \alpha, \delta,$ and ϵ ; and **two light (L) chains** κ and λ that all the classes have in common. Each immunoglobulin molecule contains the light chains of either κ or λ type.

The primary infection with bacteria or protozoa elicits during 2 – 3 days production of IgM antibodies, which is 5 – 7 days later switched to production of IgG of the same specificity. A repeated infection causes a rapid increase in IgG but only a small elevation of IgM.

Marked changes in the amount and spectrum of plasma immunoglobulins are best revealed by electrophoresis; and can manifest as:

- **Hypogammaglobulinemia:** occurs due to high loss of immunoglobulins in urine or intestine. Another relevant cause is a decrease in immunoglobulin production, which can affect one or all the immunoglobulin classes. These defects in humoral immunity can be primary or secondary and result in severe immune deficits manifesting themselves as severe recurrent infections.
- **Hypergammaglobulinemia:**
 - **Polyclonal:** develops as a response to antigen stimulation, in which several clones of plasmatic cells are activated producing more immunoglobulins of one or more classes. It is also called **polyclonal gammopathy**. It is found in chronic infections, liver and auto-immune diseases. In electrophoretic examination a broad blurred band in the γ -globulin region is found.
 - **Monoclonal:** a single clone of plasmatic cells produces monoclonal immunoglobulin (paraprotein) formed by a single type of heavy and the light chain. The physico-chemical homogeneity of the paraprotein leads to a marked sharp narrow band on electrophoresis, usually in the region of β - γ globulins. Monoclonal immunoglobulins most often belong to the IgG and IgM class. They are produced either as a complete immunoglobulin molecule, or as separated light

chains (the **Bence-Jones protein**), or heavy chains. The Bence-Jones protein easily passes through glomerular filter to the urine. Finding of monoclonal immunoglobulin is symptomatic for a **malign disease plasmocytoma** (multiple myeloma, tumor originating from a single clone of plasmatic cells), or for a **benign monoclonal gammopathy**.

1.4 Laboratory investigation of serum proteins

The basic examination of proteins in serum or plasma is an estimation of their total concentration, called '**total serum protein**'. If a pathologic value is found, or in other indicated cases, further detailed examination follows, encompassing **electrophoresis of serum proteins**, **immunofixation** and targeted estimation of **concentration of selected proteins**.

1.4.1 Total serum protein

Estimation of total protein is a common and affordable test providing basic information on protein synthesis, utilization and excretion. Many diseases alter the spectrum of serum proteins, but only some of them alter also the total protein level.

Changes in total serum protein:

Hypoproteinemia (serum protein is low):

Absolute: amount of serum protein is decreased due to

a) *high loss by:*

kidney

gastrointestinal tract (e.g. intestinal inflammation)

skin (burns)

bleeding

to the 'third space' (e.g. abdominal cavity in ascites)

b) *low protein biosynthesis* (chronic liver diseases)

c) *insufficient intake of protein* (malnutrition)

Relative: in **hyperhydration** state the actual amount of serum protein is actually unchanged, but the protein is diluted due to retention of water and salts. Concentrations of all particular proteins are decreased proportionally.

Hyperproteinemia (serum protein is high):

Absolute: usually caused by an increased production of certain specific proteins, such as immunoglobulins in plasmocytoma.

Relative: comes in **dehydration** state due to insufficient intake or excessive loss of fluids (diarrhoe, vomiting). The total amount of protein is preserved, but apparent concentrations of all particular proteins are proportionally increased.

Principle of total protein estimation:

The biuret reaction is used. Proteins form a violet complex with **cupric salts in alkali**, suitable for photometric estimation. The resulting complex of Cu^{2+} ions with peptidic bonds strongly absorbs light at 540-560 nm. The color intensity is proportional to the concentration of protein. In general, substances containing at least two groups $-\text{CO}-\text{NH}_2$ or $-\text{CO}-\text{NH}-$ give the biuret reaction, i.e. the reaction is not specific for proteins. The simplest compound

giving the reaction with cupric salts in alkaline medium is biuret $\text{NH}_2\text{-CO-NH-CO-NH}_2$ (dimer of urea), hence the name of the reaction. Amino acids and dipeptides do not react.

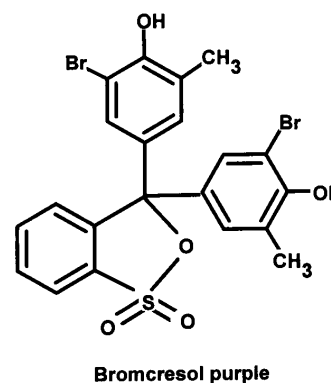
Reference values (S-protein): 65 – 85 g/l

1.4.2 Albumin

Estimation of serum albumin is a common test, suitable especially in diseases of the liver, kidney and assessment of nutritional state. Low serum albumin can accompany chronic inflammations and increased catabolism in some disease states. Other causes of low or high albumin are similar as for total serum protein.

Principle:

In a weakly acidic environment albumin behaves as a cation. It can react with anionic dyes yielding an **albumin-dye complex**. Binding of the dye is associated with a change in color. The reaction takes place in weakly acidic medium in the presence of surface-active substances. Organic dyes are used that react with albumin much faster than with other proteins. They usually contain $\text{-SO}_3\text{H}$ group. **Bromocresol purple** or **bromocresol green** can serve as examples.



Reference values (S-Albumin): 35 – 53 g/l

For estimation of albumin in other fluids, such as urine or cerebrospinal fluid, more sensitive immunochemical techniques are used (most often immunoturbidimetry).

1.4.3 Electrophoresis

In case of pathologic value of total serum protein, or if a more detailed information is needed, electrophoresis of serum protein should be performed.

Electrophoresis is based on the movement of charged particles in an electric field. The assayed substances must have character of ions or ampholytes. Proteins are typical ampholytes that can carry positive or negative charge depending on pH of buffer in which the electrophoresis takes place.

Electrophoretic mobility of proteins depends on:

- properties of protein molecules (charge, shape and size of the molecules, the relative molecular mass);
- properties of the medium in which electrophoresis is performed (pH, ionic strength, voltage, current).

Positively charged protein molecules tend to adsorb more than the negatively charged ones, hence in protein electrophoresis the negative charges are generally used. Isoelectric point of most serum proteins lies in the weak acidic range of pH 5 – 6. Therefore, in an alkaline buffer of pH 8.6, the serum proteins behave as polyanions and migrate towards the anode (+). Albumin bears the highest negative charge among serum proteins, and so it moves to the anode with the highest speed.

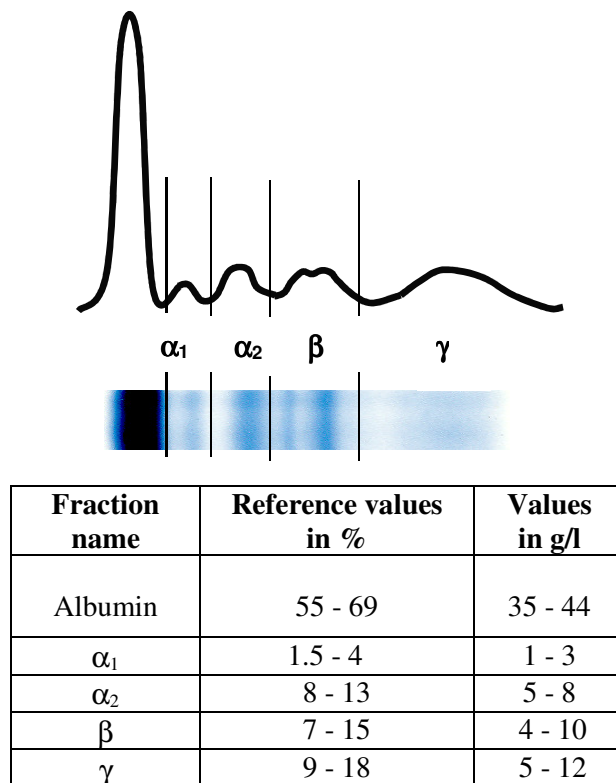
Electrophoresis can be performed on various kinds of mechanical support. Historically, the earliest support used was a chromatographic paper. Nowadays the protein electrophoresis in clinical laboratories is most often done on **cellulose acetate sheets** or in an **agarose gel**. In case of electrophoresis on paper or cellulose acetate the proteins migrate in a buffer solution that is outside the support structure; therefore, the protein molecules separate according to their charges. Likewise, in agarose gel of rather low concentration the protein separation would be based on charge.⁴

Typically, the electrophoresis of serum proteins yields 5 – 6 fractions: **albumin, α_1 , α_2 , β , and γ** (Fig. 1). The β fraction is sometimes resolved to β_1 and β_2 . Except for the albumin, each fraction consists of a group of proteins with approximately equal electrophoretic mobilities.

Electrophoresis of serum proteins is especially useful for investigation of:

- **Dysproteinemia** (alteration of spectrum of serum proteins)
- **Paraproteinemia** (presence of monoclonal immunoglobulin)

Fig 1: Scheme of an electrophoreogram of serum proteins together with a densitometric recording



⁴ In more concentrated gels, however, the electrophoretic separation of proteins would be different due to 'molecular sieving': the larger the protein molecules, the more are retarded by the gel mesh; the separation then depends on the protein molecular mass rather than charge. Such electrophoretic separation is used e.g. in examination of urinary proteins (q.v.).

Description of particular fractions on electrophoresis of serum proteins

- **Prealbumin zone**

In this region *prealbumin (transthyretin)* is found; but its band is very faint and difficult to evaluate.

- **Albumin zone**

Albumin forms a remarkable, rather wide, and well delineated zone. It appears weaker if albumin concentration falls below 30 g/l. Seldom observed doubling of the albumin band can be symptomatic of a genetic structural aberration of albumin in heterozygotes – *bisalbuminemia* – or happens due to binding of an exogenous substance, such as penicillin, to albumin.

- **Interzone between albumin and α_1 -globulins**

Faint homogeneous staining of this area is caused mainly by *α -lipoproteins (HDL)*. The *α -acidic glycoprotein* displays the same electrophoretic mobility, but its contribution to the interzone staining is minimal.

- **Zone of α_1 -globulins**

The α_1 -globulin zone is affected predominantly by presence of *α -antitrypsin*. Acute inflammations cause visible thickening of this zone. Genetic variability of α_1 -antitrypsin is clinically significant, and can manifest also in electrophoresis as weakening or even disappearance of this zone and/or alteration of its position.

- **Zone of α_2 -globulins**

This zone consists mainly of two proteins - *α_2 -macroglobulin* and *haptoglobin*.

Changes in concentration of α_2 -macroglobulin are of minor diagnostic significance. Haptoglobin forms 6 phenotypes that differ in electrophoretic mobility. The electrophoretic examination of serum proteins, however, does not allow identification of particular haptoglobin phenotype.

- **Interzone between α_2 and β_1 -globulins**

Normally this zone is only faintly stained. In case of hemolysis the resulting *complexes of hemoglobin-haptoglobin* form a band in this region.

- **Zone of β_1 -globulins**

Shape and intensity of staining of the β_1 -globulin zone is affected almost exclusively by the presence of *transferrin*. The intensity of this zone correlates well with total iron binding capacity of blood plasma. In case of anemia due to iron deficiency as well as in pregnancy the synthesis of transferrin increases and so also the β_1 -globulin zone intensity seen on serum electrophoreogram. Another protein with β_1 -electrophoretic mobility *hemopexin* is poorly stained with the commonly used protein stains and so changes of its concentration do not affect appearance of serum electrophoreogram.

- **Interzone between β_1 and β_2 -globulins**

The *immunoglobulin IgA* is found here, causing homogeneous staining of this region. Next, *β -lipoprotein (LDL)* forms a distinct band in this zone, whose visibility depends on its concentration.

- **Zone of β_2 -globulins**

- The *C3 component of complements* contributes to this zone. The staining intensity of β_2 -globulins band, however, is a poor indicator of C3 concentration.

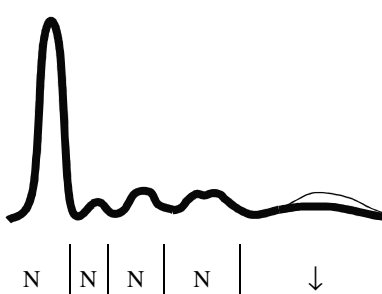
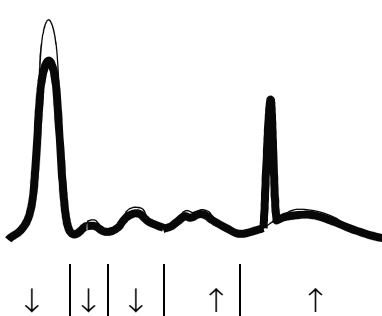
- **Zone of γ -globulins**

Appearance of the γ -globulin zone is affected by concentrations of the four subclasses of *immunoglobulin IgG*. Elevation of IgG manifests as more intense staining and widening of this zone. *Immunoglobulin IgM* is found rather close to the start. Increase in IgM concentration, isolated or accompanying the IgG elevation, cannot be seen on electrophoresis.

Tab. 2 Changes of serum electrophoreogram in several pathologic conditions

(↓-decreased, N-normal, ↑ - increased fraction)

Type of electrophoreogram	Comments	Alb.	α_1	α_2	β	γ	Occurrence (example)	
Acute inflammation	<ul style="list-style-type: none"> positive acute phase reactants increased (α_1antitrypsin, orosomuroid, haptoglobin, ceruloplasmin, CRP, C3), negative acute phase reactants decreased 		↓ or N	↑	↑	N	<ul style="list-style-type: none"> acute phase of infectious diseases acute tissue damage (myocardial infarction, surgery) large burns 	
Chronic inflammation	<ul style="list-style-type: none"> polyclonal amplification of immunoglobulins 		↓ or N	N	N	N	↑	<ul style="list-style-type: none"> recovery after infectious disease some rheumatic diseases
Chronic active inflammation	<ul style="list-style-type: none"> elevation of α-globulins indicates activity of the inflammatory process 		↓	↑	↑	N	↑	<ul style="list-style-type: none"> chronic active rheumatoid arthritis
Hepatic type	<ul style="list-style-type: none"> impaired proteosynthesis in hepatocytes excessive production of immunoglobulins sometimes β and γ fractions unseparated ('β - γ bridge') - due to elevation of IgA 		↓	↓	↓	↓	↑	<ul style="list-style-type: none"> chronic liver diseases - liver fibrosis and liver cirrhosis
Nephrotic type	<ul style="list-style-type: none"> severe loss of protein into urine (renal loss of albumin prevails) increase in proteins with the highest MW α_2-macroglobulin and β-lipoprotein 		↓	N	↑	↑	↓ or N	<ul style="list-style-type: none"> nephrotic syndrome

<p>Hypogammaglobulinemia</p>	<ul style="list-style-type: none"> decrease in the γ globulin region 		<ul style="list-style-type: none"> primary defect in antibody production secondary defect in antibody production loss of immunoglobulins
<p>Monoclonal gammopathy</p>	<ul style="list-style-type: none"> homogeneous peak anywhere in region β to γ 		<ul style="list-style-type: none"> benign monoclonal gammopathy malign monoclonal gammopathy (malign myeloma)

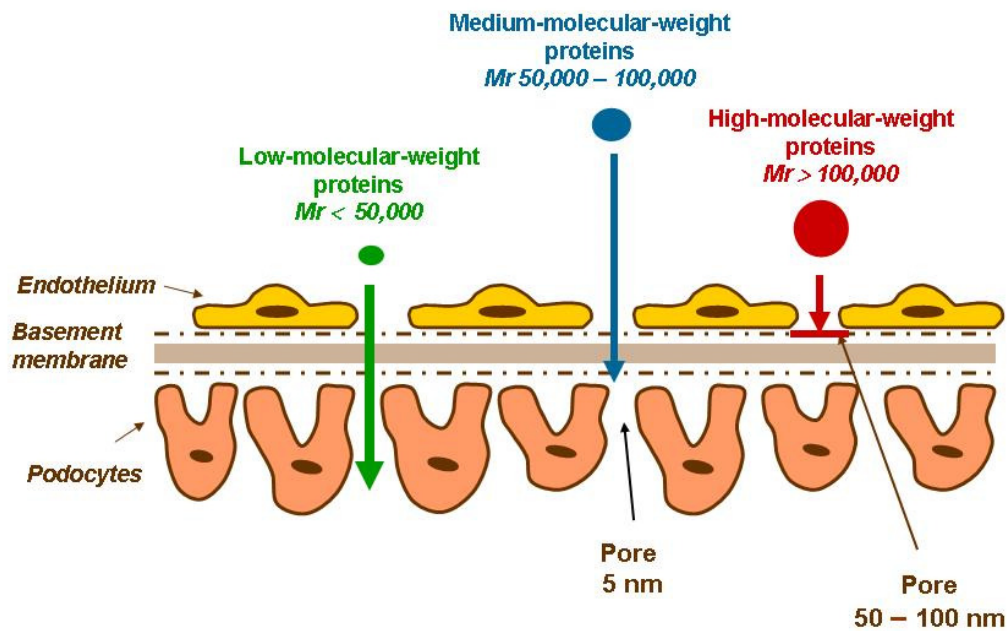
2 Protein in urine (proteinuria)

A healthy adult keeping usual physical activity excretes into urine about **40 – 60 mg of protein per 24 hours**, with a **maximum of 96 mg/m² (i.e., about 150 mg) of protein per 24 hours** ('physiological proteinuria'). About 40 % of these proteins are plasmatic proteins, while the remaining 60 % comes from the kidney (the Tamm-Horsfall uromucoid) and the urinary tract (secretory IgA, other undefined glycoproteins and glycopeptides).

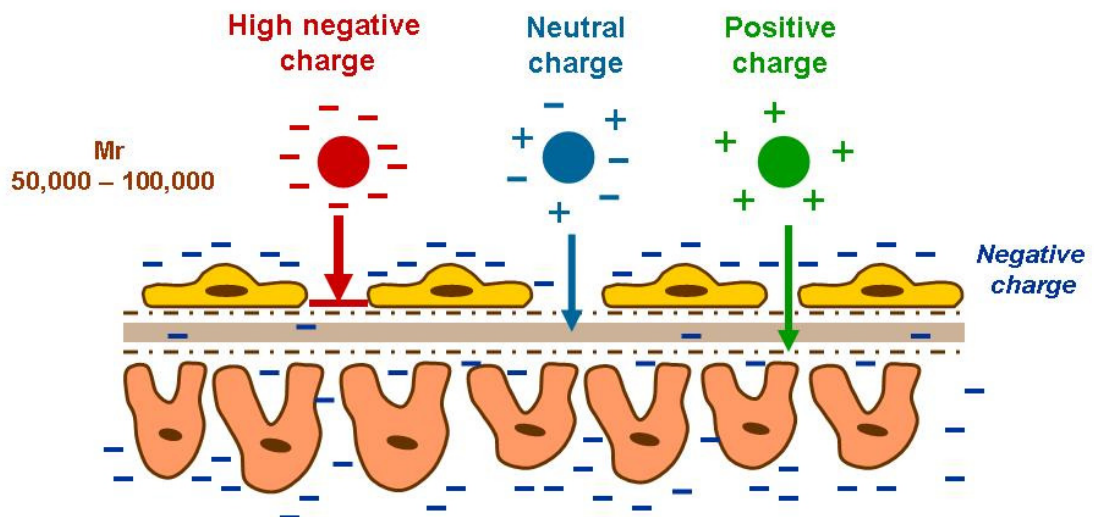
The amount and composition of proteins in urine depend on their filtration through glomerular membrane, reabsorption in tubules and renal hemodynamics. Under normal condition the glomerular membrane is impermeable for substances having molecular weight above 60,000. In contrast, low-molecular-weight plasma proteins pass the glomerular membrane freely, and are subsequently reabsorbed and catabolized in proximal tubuli.

Two major factors determine the ultrafiltration through glomerular membrane:

1. **Microporous structure** of glomerular basal membrane prevents passage of proteins of MW above 100,000 – 150,000 (e.g. IgG, IgA). It is **selectivity with respect to the size**.



2. **Electrostatic repulsion** effect results from properties of all components of the glomerular wall. Hydrated polyanionic macromolecules of the glomerular wall bear predominantly negative charges causing repulsion of the plasma proteins that are also negatively charged. This mechanism prevents passage of proteins of MW 60,000 – 70,000 (e.g. albumin, transferrin). Neutral or positively charged molecules pass the glomerular filter much more easily. This is **selectivity with respect to charge**.



2.1 Classification of proteinuria

Occurrence of proteins in urine in the amount exceeding 150 mg per 24 hours is called a **proteinuria**. It is a common sign of kidney diseases and classification of proteinuria is of high diagnostic value. Proteinurias can be divided into three basic groups:

- **Pre-renal proteinuria** (“overflow” proteinuria) originates at high concentrations of low-molecular-weight plasma proteins that even under normal condition pass into the ultrafiltrate. Glomerular permeability for proteins need not be impaired. Tubular reabsorption may be intact as well, but due to protein over-load some proteins escape into urine. Pre-renal proteinuria occurs e.g. in cases of severe **intravascular hemolysis**, when hemoglobin appears in the urine (hemoglobinuria); or in the **crush syndrome** (traumatic damage to skeletal muscles), when myoglobin is found in urine (myoglobinuria). Presence of immunoglobulin light chains (Bence-Jones protein) points to **myeloma**. The pre-renal proteinuria often accompanies some acute inflammatory and necrotizing diseases. Tissue degradation products as well as some small acute-phase reactants (e.g. orosomucoid) are excreted.
- **Renal proteinuria** is further divided to:
 - **Glomerular proteinuria** results from an increased permeability of the glomerular membrane for protein. In **selective glomerular proteinuria** the negative charge of glomerular membrane is lost, which results in increased excretion of the middle-size proteins of MW 70,000 – 100,000, such as albumin and transferrin, while large proteins are preserved. In **non-selective glomerular proteinuria** the selectivity with respect to the size is impaired as well, and in addition to the middle-size proteins also species with MW above 100,000, such as IgG, penetrate into urine. In glomerular proteinuria the daily loss of protein usually exceeds 2 g.
 - **Tubular proteinuria** is caused by decreased reabsorption of normally filtrated proteins. High excretion of low-molecular-weight proteins (microproteins) of MW 10,000 – 70,000 (β_2 -microglobulin, α_1 -microglobulin, free Ig light chains), which are normally reabsorbed in tubules, is found. A common cause is **damage to tubular cells by some nephrotoxic drugs** (cytostatics, some antibiotics) or **heavy metals** (Hg, Pb, Cd). Tubular proteinuria can also accompany some prerenal proteinurias (paraproteinuria, myoglobinuria) due to competition of the pathologic protein and other filtrated proteins for tubular transport processes. Tubular proteinuria usually results in only moderate loss of protein, typically 0.3 – 1.5 g/24 hours.
 - **Mixed proteinuria** is a combination of non-selective glomerular and tubular proteinuria. It is found in advanced stages of kidney diseases as a sign of damage to all parts of the nephrons.
- **Post-renal proteinuria** results from **bleeding, tumors** and inflammations in the urinary tract; the urine is directly contaminated by blood plasma. This condition is reliably identified by presence of plasma proteins of very high molecular weight (α_2 -macroglobulin, IgM), which do not pass the glomerular membrane even in the non-selective glomerular proteinurias.

<i>Type of proteinuria</i>	<i>Finding in urine</i>	
	<i>Characteristic proteins in urine</i>	<i>Comments</i>
Renal: Glomerular selective	<ul style="list-style-type: none"> • Albumin • Transferrin (MW 70,000 – 100,000)	Small increase in albumin (microalbuminuria) 30 – 300 mg/day is an early and valuable sign of kidney damage in diabetes mellitus and hypertension
Renal: Glomerular non-selective	<ul style="list-style-type: none"> • Albumin • Transferrin • IgA, IgG (MW > 70,000)	Plasmatic microproteins are not elevated. Macromolecular proteins are absent.
Renal: Tubular	<ul style="list-style-type: none"> • α_1-microglobulin • β_2-microglobulin • Light Ig chains (MW 10,000 – 70,000)	The whole spectrum of microproteins of MW 10,000 – 70,000 is present.
Renal: Mixed glomerulo-tubular	<ul style="list-style-type: none"> • Albumin • Transferrin • IgA, IgG • α_1-microglobulin • β_2-microglobulin 	All types of plasma proteins except the macromolecular ones are present
Pre-renal	<ul style="list-style-type: none"> • Light Ig chains: monoclonal gamopathy • Hemoglobin: intravascular hemolysis • Myoglobin: crush syndrome 	Presence of proteins that are not normally found in plasma.
Post-renal	<ul style="list-style-type: none"> • Hemoglobin • All types of plasma proteins including macroproteins (α_2-macroglobulin, IgM, apolipoprotein A1) 	Exudation of plasma proteins into urinary tract

Table 3: Types of proteinuria

In addition to pathologic proteinuria, sometimes also **intermittent proteinurias**, elicited by unusual physical exercise, cold, or febrile state, occur in clinical practice. In some adolescents a proteinuria that appears only in upright position, called **postural or orthostatic proteinuria**, is observed. It is explained as a result of vasoconstriction at lumbal hyperlordosis and represents the commonest cause of proteinuria in children. Most of these proteinurias spontaneously disappear if their causes subside.

Microalbuminuria. Physiological excretion of albumin into urine does not exceed 30 mg per day. Microalbuminuria is defined as **daily excretion of albumin from 30 to 300 mg**. Such a small amount of albumin is below sensitivity of ordinary test strips, but there are special strips for the screening purposes; a positive finding must be subsequently confirmed by quantitative estimation of urinary albumin (sensitive immunochemical techniques are used). Estimation of microalbuminuria is of great significance in **diabetic** and **hypertonic patients** as it is an early sign of incipient diabetic or hypertonic nephropathy. If microalbuminuria is found, it is usually a reason for a more intense therapeutic regime, which can slow down or even halt the progression of kidney damage.

2.2 Laboratory examination of protein in urine

The laboratory examination of proteinuria aims at

1) Qualitative demonstration of protein in urine. In practice two methods are in use:

- Diagnostic strips
- Test with sulfosalicylic acid.

2) Quantitative estimation of protein in urine. If a pathologic proteinuria is found, a daily loss of proteins in urine is measured. The urine is collected for 24 hours, mixed and a sample is taken in which the protein concentration is measured. The urine protein concentration in g/l multiplied by volume of urine in liters gives the daily loss of protein.

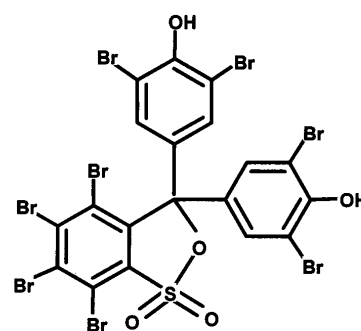
Evaluation of daily protein loss:

<i>Protein in urine:</i>	<i>Degree of proteinuria:</i>
< 0.150 g/24 hours	Physiological proteinuria
< 1 g/24 hours	Moderate proteinuria (most often tubular)
1.0-3.5 g/24 hours	Intermediate proteinuria
> 3.5 g/24 hours	Severe proteinuria
> 10 g/24 hours	Proteinuria associated with severe nephrotic syndrome

3) Determination of the type of proteinuria. Electrophoretic techniques that resolve urinary proteins according to their molecular weights provide semiquantitative information on the spectrum of excreted proteins, enabling classification of the proteinuria. Quantitative estimation of selected particular proteins in urine, such as albumin, α_1 -microglobulin, β_2 -microglobulin, and IgG, is also valuable.

2.2.1 Diagnostic strips

The diagnostic strips for detection of protein in the urine are based on the so called **protein error of acid-base indicators**. The test zone contains an acid-base indicator, such as **tetrabromophenol blue**, which is yellow at pH up to 3.5, while at higher pH it turns green or blue. The strip reaction zone also contains a buffer that dissolves upon contact with the examined urine sample and keeps pH in the range 3.0 – 3.5, therefore the indicator has a yellow color. If a protein is present in the sample, it binds with its amino groups to the indicator. It changes the indicator properties: at the constant pH 3.0 – 3.5 the protein-bound indicator takes green or greenish-blue color, i.e. as if the indicator were in higher pH (that is why ‘protein error’). The indicator color changes from green to blue; the color intensity depends on the protein concentration and is evaluated visually or instrumentally.



Tetrabromophenol blue

With very alkaline urine samples (pH above 8), or if the urine is highly concentrated, the test can give false positive results (due to exhaustion of the buffer in the strip reaction zone). In these cases the sample should be acidified with diluted acetic acid to pH 5 – 6 and the test repeated. A false positive result can also appear due to high concentration of some substances with amino groups (contamination of the sample vessel with some disinfectants) that bind on the indicator in a similar way like the proteins do.

A disadvantage of the diagnostic strips is their different sensitivity towards particular proteins: the strips react very well with albumin, indicating its presence in urine from 0.1 – 0.5 g/l; whereas their sensitivity to globulins, glycoproteins and the Bence-Jones protein is much lower. These strips are not suitable for detection of microalbuminuria, in which albumin concentration ranges between 20 and 200 mg/l (daily albumin loss 30 – 300 mg/24 hours). Special test strips for screening of microalbuminuria are available; they are based on an immunochemical reaction.

2.2.2 Test with sulfosalicylic acid

Addition of sulfosalicylic acid to protein-containing urine sample results in protein denaturation and cloudy appearance or even precipitate. The reaction is fairly sensitive as it detects proteinuria from 0.1 – 0.2 g/l. Differences in sensitivity towards particular proteins are not so pronounced as in the diagnostic strips; sulfosalicylic acid precipitates globulins as well.

The test gives false positive results in cases of urinary excretion of some X-ray contrast substances, penicillin, sulfonamides, salicylic acid, and antidiabetic drugs.

Semiquantitative evaluation of the sulfosalicylic acid test:

<i>Appearance:</i>	<i>Evaluation</i>	<i>Approximate protein concentration:</i>
Opalescence	Traces	0.05 – 0.1 g/l
Slight turbidity (transparent, a text behind the tube is legible)	+	0.1 – 0.2 g/l
Opaque turbidity (not transparent, without flakes)	++	0.5 – 1.0 g/l
Milky turbidity with flakes	+++	2.0 – 5.0 g/l
Cheese-like precipitate	++++	above 5.0 g/l

2.2.3 Quantitative estimation of protein in urine

The quantitative measurement of protein in urine is technically rather demanding. Three groups of techniques are used:

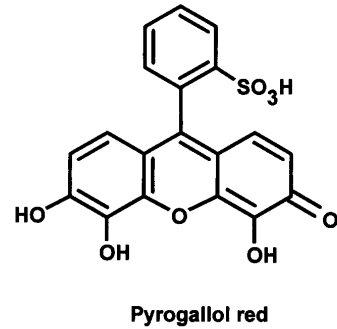
1. Denaturation of proteins e.g. with trichloroacetic acid, sulfosalicylic acid, followed by **turbidimetric measurement** of the degree of turbidity.

2. **Colorimetric methods** with or without denaturation, e.g. the biuret method.
3. Methods based on **dyes that bind to proteins** in the sample, such as Coomassie Brilliant Blue G250, or pyrogallol red.

Nowadays, the turbidimetric and pyrogallol red methods are preferred, since they are suitable for automatic analyzers.

Estimation of proteinuria with pyrogallol red:

The pyrogallol red forms with molybdate a pink complex having absorption maximum at 470 nm. Binding of proteins to this complex in acidic medium deepens the color with shift of the absorbance maximum to 600 nm. The absorbance of the reagent- protein complex displays a linear relationship to protein concentration in the range 0.06 – 2.0 g/l.



2.2.4 Determination of type of proteinuria using electrophoresis

In order to determine the type of proteinuria it is necessary to know the spectrum of proteins excreted to urine. Electrophoretic techniques are generally used. Separation of urinary proteins according to their molecular weight enables a semiquantitative evaluation of particular diagnostically significant proteins and classification of proteinuria. Electrophoresis in agarose or polyacrylamide gel has become a method of choice for the analysis of urinary protein.

The separation of proteins on the basis of their size, rather than charge, can for instance be achieved by using a polyacrylamide gel whose density increases, i.e., its pores become gradually smaller, from cathode to anode. Small molecules get farther than large ones in this type of gel.

Another, more often used possibility is treatment of the sample with a detergent **sodium dodecyl sulphate (SDS)**. The negatively charged anionic SDS binds to proteins rendering their original charges insignificant. The resulting complexes have approximately equal charges (more precisely: surface charge density). If the electrophoresis runs in a relatively dense gel, the SDS-treated proteins separate according to their molecular weight: small molecules migrate faster than the large ones ('molecular sieve'). With a proteinuric sample, β_2 -microglobulin migrate the farthest, while albumin (MW 70,000) is found in the middle of the lanes and between start and the albumin band proteins of MW > 70,000 would be located.

Evaluation of electrophoresis of urinary protein:

In **glomerular proteinurias** the electrophoreogram contains a band of albumin and other proteins between the albumin and the start (i.e. having MW > 70,000).

Proteins found in glomerular proteinurias:

	MW	Glomerular proteinuria	
Albumin	68,000	Selective	Non-selective
Transferrin	77,000	Selective	Non-selective
IgG	150,000		Non-selective
IgA	160,000		Non-selective
Haptoglobins	85,000-1,000,000		Non-selective

Tubular proteinurias display bands between the position of albumin and the anode end of the electrophoreogram, i.e. proteins of MW < 70,000.

In case of a **mixed proteinuria**, both the proteins found in glomerular and tubular proteinuria would be found on both sides from the albumin position.

The presence of α_2 -macroglobulin (MW=800,000) together with a picture similar to mixed proteinuria suggests a **post-renal proteinuria**.

