Blood plasma and blood cells

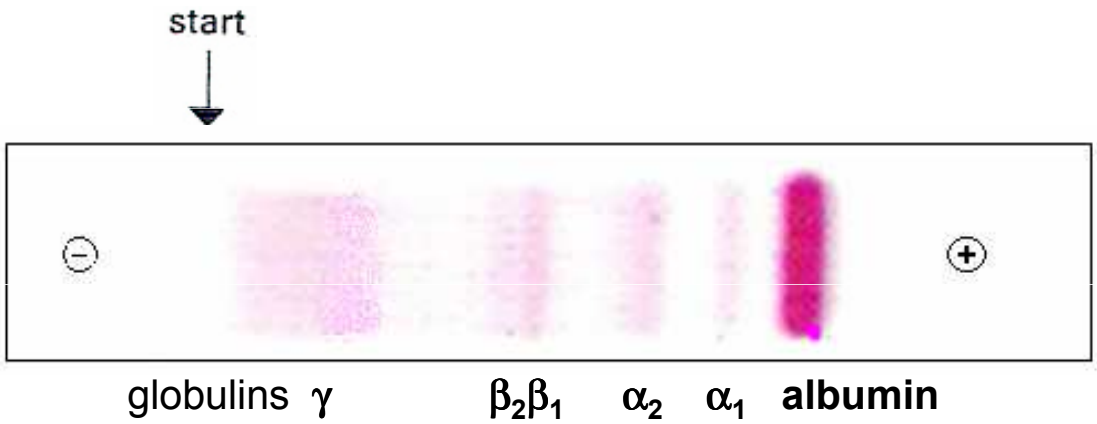
Biochemistry II
Lecture 11

2008 (J.S.)

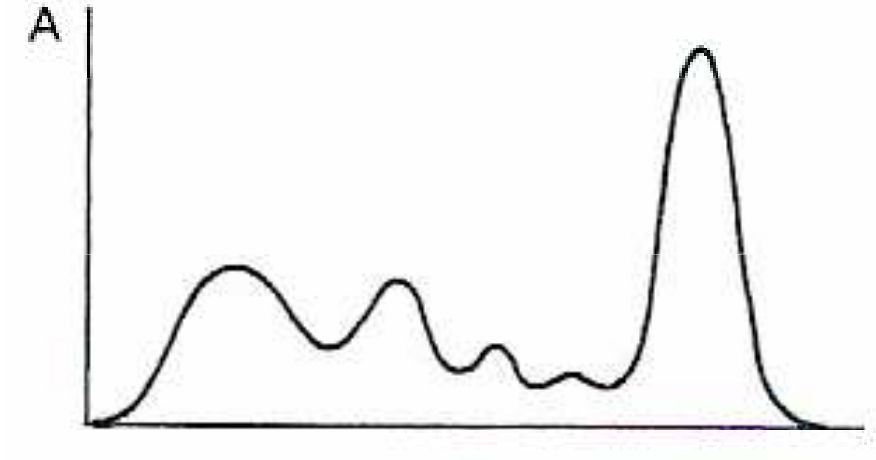
Blood serum proteins- the six main fractions

Total serum proteins 62 – 82 g / l

Electrophoretic separation on a cellulose acetate strip (pH 8.6)



Densitogram:



Normal values
(mass fraction of total proteins)

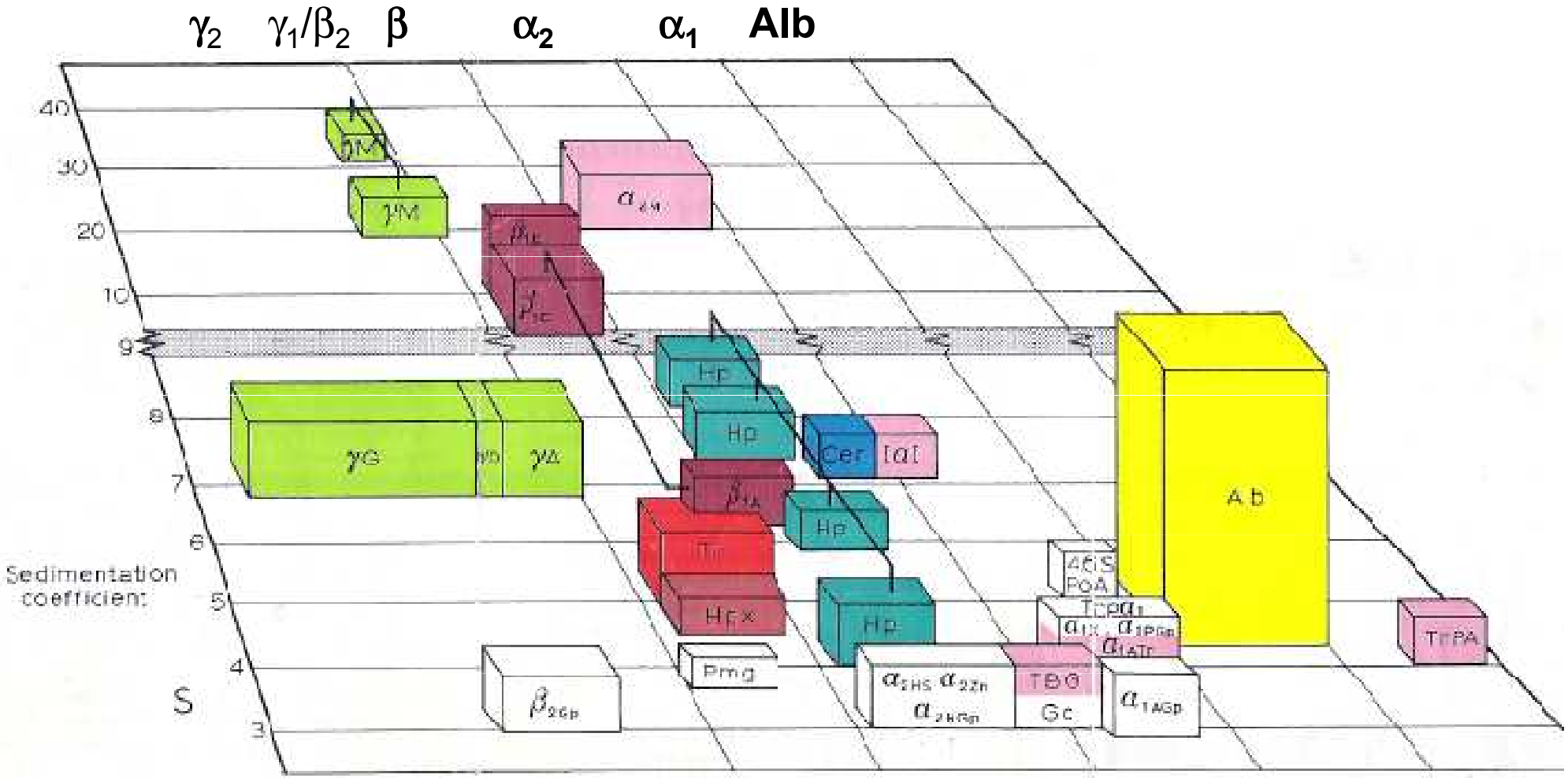
Albumin	0.50 – 0.62	0.55
α_1-Globulins	0.03 – 0.06	0.05
α_2-Globulins	0.07 – 0.13	0.10
$\beta_1\beta_2$-Globulins	0.09 – 0.15	0.12
γ-Globulins	0.14 – 0.22	0.18

Blood plasma proteins

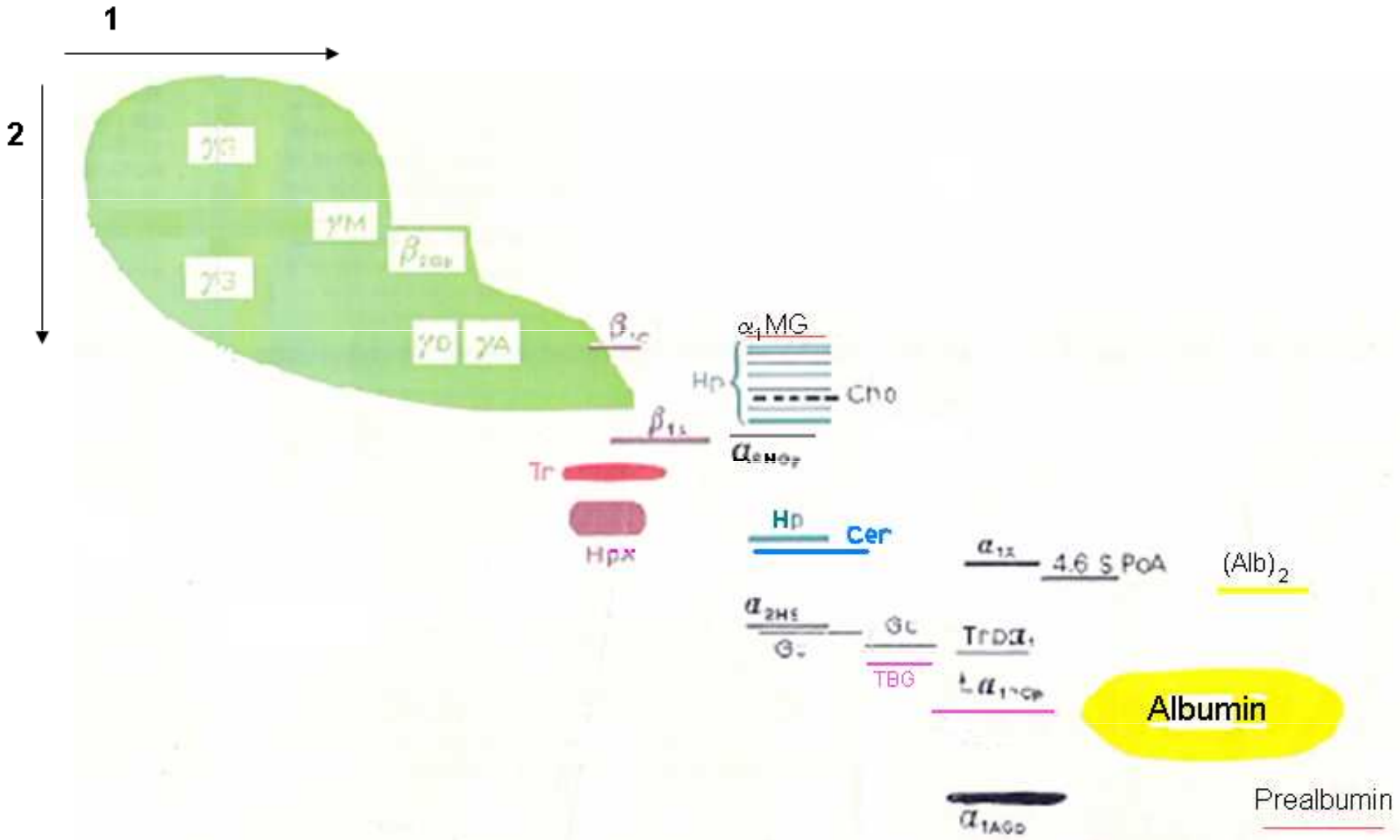
About 10 000 proteins were estimated, from which **22 high abundance proteins** represent approximately 99 % of total protein in human plasma.

	Transthyretin (prealbumin)
Albumin	
α_1-Globulins	acid α_1-glycoprotein
	α_1-antitrypsin
	antithrombin III
	apolipoprotein A I, A II
α_2-Globulins	α_2-macroglobulin
	C3, C4-components
	haptoglobin
	ceruloplasmin
	plasminogen
β_1-Globulins	transferrin
	haemopexin
	fibronectin
	apolipoprotein B_{100}
β_2-Globulins	fibrinogen
	C1q-component
γ-Globulins	immunoglobulins G, A, M, D

Separation of fractions in ultracentrifuge

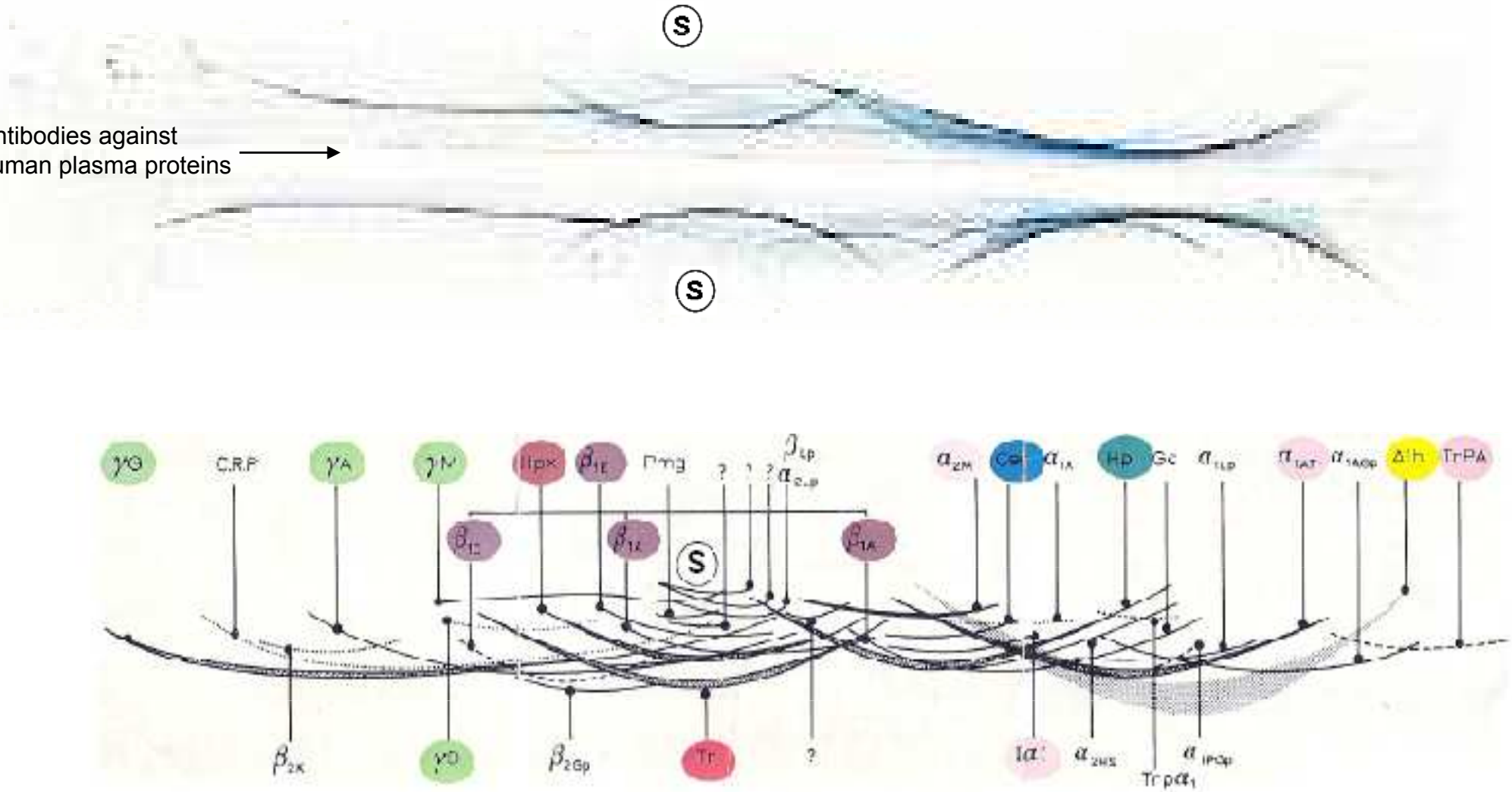


Starch-gel electrophoresis (two-dimensional)



Immunoelectrophoresis

Antibodies against
human plasma proteins



Most of the high abundance plasma proteins, except for immunoglobulins, are **synthesized in the liver**.

Albumin

is the major plasma protein, normal concentration **35 – 53 g / l**; about 10 – 12 g albumin are produced daily. $M_r \approx 67\,000$ (585 amino acid residues).

Albumin is essential for the maintaining of **oncotic pressure** in capillaries.

Because of a negative net electric charge (~ 12 mmol/l), it acts as an important **buffer base** and in binding of Ca^{2+} (about 50 % of total calcium).

Hydrophobic areas of the surface of albumin molecules provides the **transport** of free fatty acids, bilirubin, and also, weakly and non-specifically of steroid and thyroid hormones, and numerous drugs (e.g., salicylates, penicillins, sulfonamides, and barbiturates).

Hypoalbuminaemia occurs in liver diseases, nutritional depletion, due to losses in renal diseases, chronic intestinal inflammations, vast burns, as well as in hyperhydration. A reduction in plasma oncotic pressure results in oedema.

Transthyretin (prealbumin)

is a tetrameric protein, M_r 50 000. Serum concentration 100 - 400 mg/l. Biological function – binding of thyroxin and retinol-binding protein. Due to its very short biological half-life (2 days) it serves as a marker of malnutrition, impairment or recovery of liver proteosynthesis.

Haptoglobin (Hp)

is an α_2-sialoglycoprotein with **haemoglobin-binding capacity** that prevents both iron loss and kidney damage during haemolysis; the complexes Hb-Hp are rapidly captured by the reticuloendothelial system. Concentration in serum of adults 0.4 – 2.1 g/l, it falls in haemolysis.

Molecular **polymorphism** of Hp exists: there are three major phenotypes - Hp1-1, Hp 2-2, and the heterozygous Hp 2-1. Molecular mass of Hp 1-1 is 86 000, that of Hp 2-2 from 170 000 to 900 000.

Hp also protects against free radicals in haemolysis and exhibits an antiinflammatory action by inhibition of prostaglandin synthesis.

Haemopexin

β_1-Glycoprotein, $M_r \approx 70\,000$, **binds free haem**, if it appears in the plasma, so that it may be captured by the liver cells (receptor-mediated endocytosis).

Transferrin

β_1- Glycoprotein, $M_r \approx 79\,000$, serum concentration **2.5 – 4.0 g / l**.
It transports Fe^{3+} ions. Molecule of transferrin can bind two ferric ions, Under normal conditions, about 1/3 of the total iron-binding capacity is saturated.

In iron deficiency, the synthesis of transferrin is stimulated.

In chronic alcoholism, glycosylation of transferrin is impaired and detection of carbohydrate-deficient transferrin (CDT) may serve as a marker of chronic alcohol abuse.

Ceruloplasmin

is an α_2-globulin, a blue protein, because of firmly bound 8 Cu^{2+} ions.
M_r 132 000. Serum concentration 150 – 600 mg / l.

Even though ceruloplasmin contains about 90 % of copper plasma content, it doesn't take part in Cu^{2+} transport.
Biological function of ceruloplasmin is the **ferroxidase activity** that prevents the occurrence of Fe^{2+} ions and possible Fenton reaction.
Thus it is viewed as one of endogenous antioxidants.

α_1-Antitrypsin (α_1AT, α_1-proteinase inhibitor)

is an α_1-glycoprotein, normal serum concentration about **2 – 4 g / l**. $M_r \approx 54\,000$. This protein inhibits proteinases released from polymorphonuclear leukocytes (namely elastase) and other proteinases, which may occur in blood plasma and attack the elastin between alveoli in the lung.

α_1-Antitrypsin deficiency is one of the common inborn error. Individuals with the genotype ZZ produce less than 15 % on usual amounts of α_1AT and they are exposed to a high risk of **pulmonary emphysema** due to enzymatic degradation of elastin in the lungs, with consequent reduction of the surface area available for gas exchange.

Smokers also risk an insufficient effectivity of α_1AT: Components of tobacco smoke oxidize the sulfide group of methionyl residue in position 358 of α_1AT (which takes part in interactions with proteinases) to sulfinyl group that disables the interactions. In addition, the smoke irritates the tissue and increased occurrence of leukocytes results in a higher local activity of proteinases.

The acute phase proteins (APP)

Positive acute phase proteins

Their hepatic synthesis is induced by numerous cytokines that enter the circulation as products of, e.g., macrophages, epithelial cells, and fibrocytes.

C-reactive protein, CRP	response time 6 – 8 h
Serum amyloid A protein	increase 10 – 100 times
Acid α_1-glycoprotein	response time 24 h
α_1-Antitrypsin	increase 2 – 4 times
Haptoglobins	
Fibrinogen	
Ceruloplasmin	response time 48 h
C3 and C4 components	increase by 50 %

APP type I

stimulated by TNF-α, IL-1, and IL-6

- CRP
- acid α_1-glycoprotein
- haptoglobins
- haemopexin

APP type II

stimulated by IL-6 and glucocorticoids

- fibrinogen
- α_2-macroglobulin
- α_1-antitrypsin and other serpins
- ceruloplasmin, hepcidin

Negative acute phase proteins

Their synthesis in the liver is decreased in the catabolic state:

Transthyretin (prealbumin)	response time < 24 h
Transferrin	24 – 48 h
Albumin	> 48 h

Biological half-lives of some plasma proteins

	in days
Albumin	17 – **19** – 23
Immunoglobulins G	15 – **18** – 26
Transferrin	7 – **8.5** – 10
Immunoglobulins A	**5.5**
Acid α_1-glycoprotein	**5.2**
Fibrinogen	4 – **4.5** – 5.5
Haptoglobins	**4**
Immunoglobulins M	**4**
Transthyretin (prealbumin)	**2**

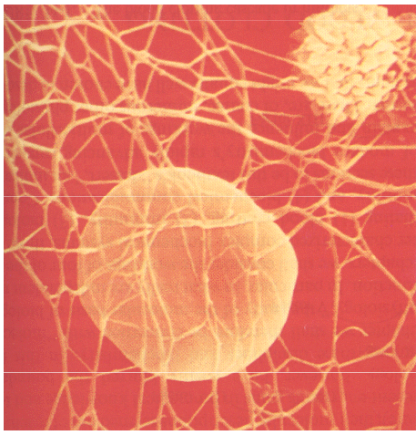
Haemostasis

If the smaller vessels are injured by traumas, the leakage of blood is discontinued normally in few minutes due to a series of interactions between the vessel wall, blood platelets, coagulation factors, and the fibrinolytic system.

The initial step in haemostasis is arteriolar **vasoconstriction**, which temporarily reduces local blood flow.

Blood platelets adhere then to the vessel wall at the site of injury, aggregate to each other, forming so the initial, unstable **primary platelet plug** ("white thrombus").

Vascular injury also activates coagulation factors that form thrombin, which converts plasma fibrinogen to insoluble, crosslinked fibrin and relatively resistant - the **secondary, platelet-fibrin plug** ("red thrombus").



The blood cells are caught in a network of fibrin.

Local formation of fibrin activates local generation of plasmin, an enzyme of the **fibrinolytic system**, which digests fibrin plugs (in parallel with tissue repair processes).

Vasoconstriction

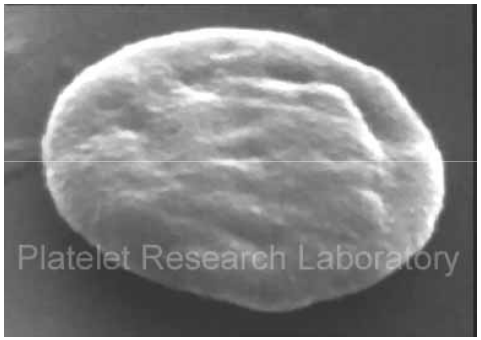
is either a reflex to an injury or the result of stimulation by serotonin, thromboxane TXA_2, and platelet derived growth factor (PDGF), which are released from activated platelets.

Injury of endothelial cells

enables the contact blood with subendothelial collagen fibres and endothelial cells begin to secrete **von Willebrand factor** (**vWF**), a large protein, which is the carrier for coagulation factor VIII and promotes platelet adhesion to collagen.

Blood platelets

adhering to collagen are activated – they change their shape to spherical and form pseudopodia.



http://www.platelet-research.org/

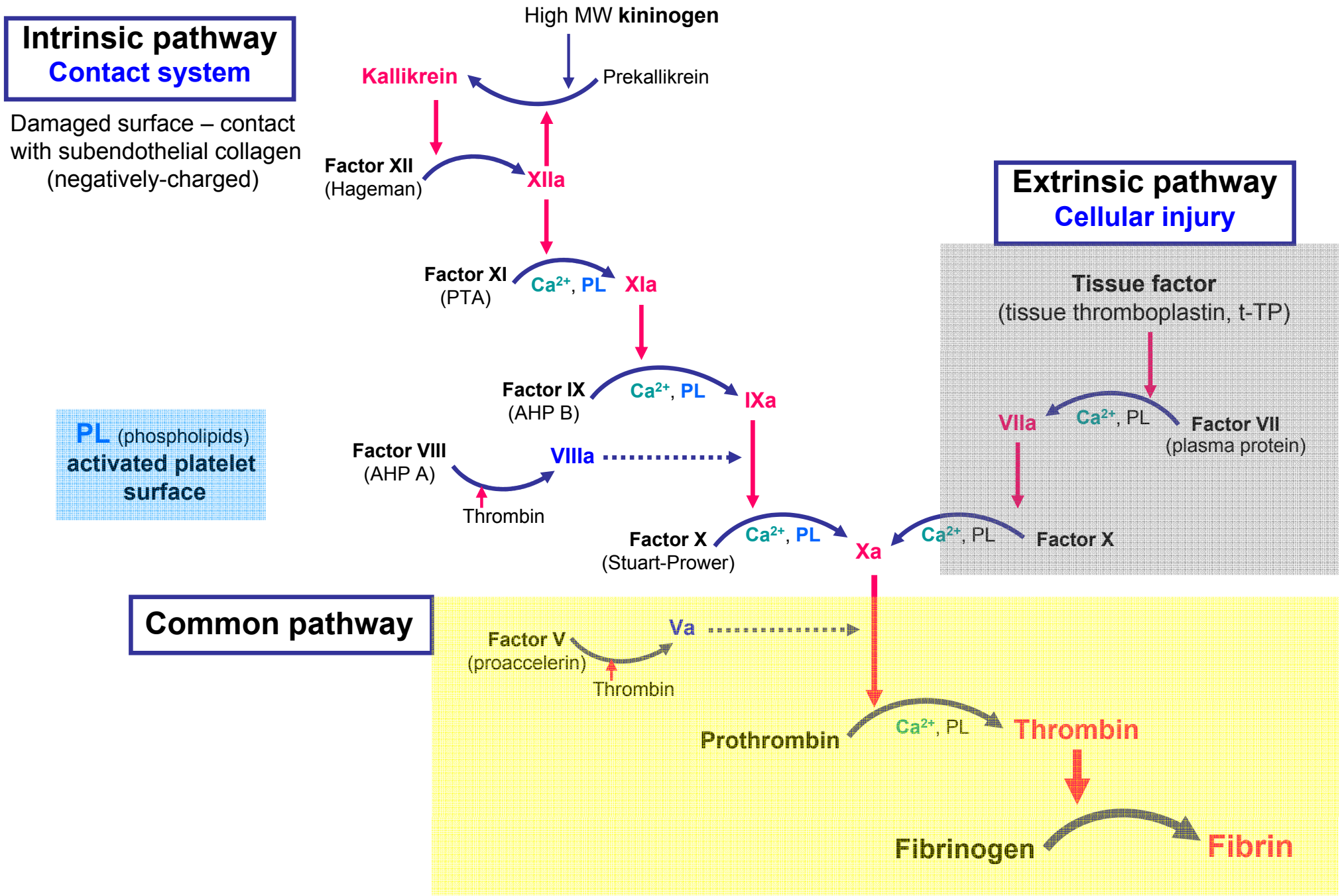
Activated platelets release from their granules compounds that stimulate **aggregation of platelets** and thus formation of the **primary platelet plug**:

serotonin (5-hydroxytryptophan),
ADP,
thromboxane TXA_2,
fibronectin,
platelet derived growth factor (PDGF), and
platelet activating factor (PAF, an 1-*O*-alkylglycerophospholipid).

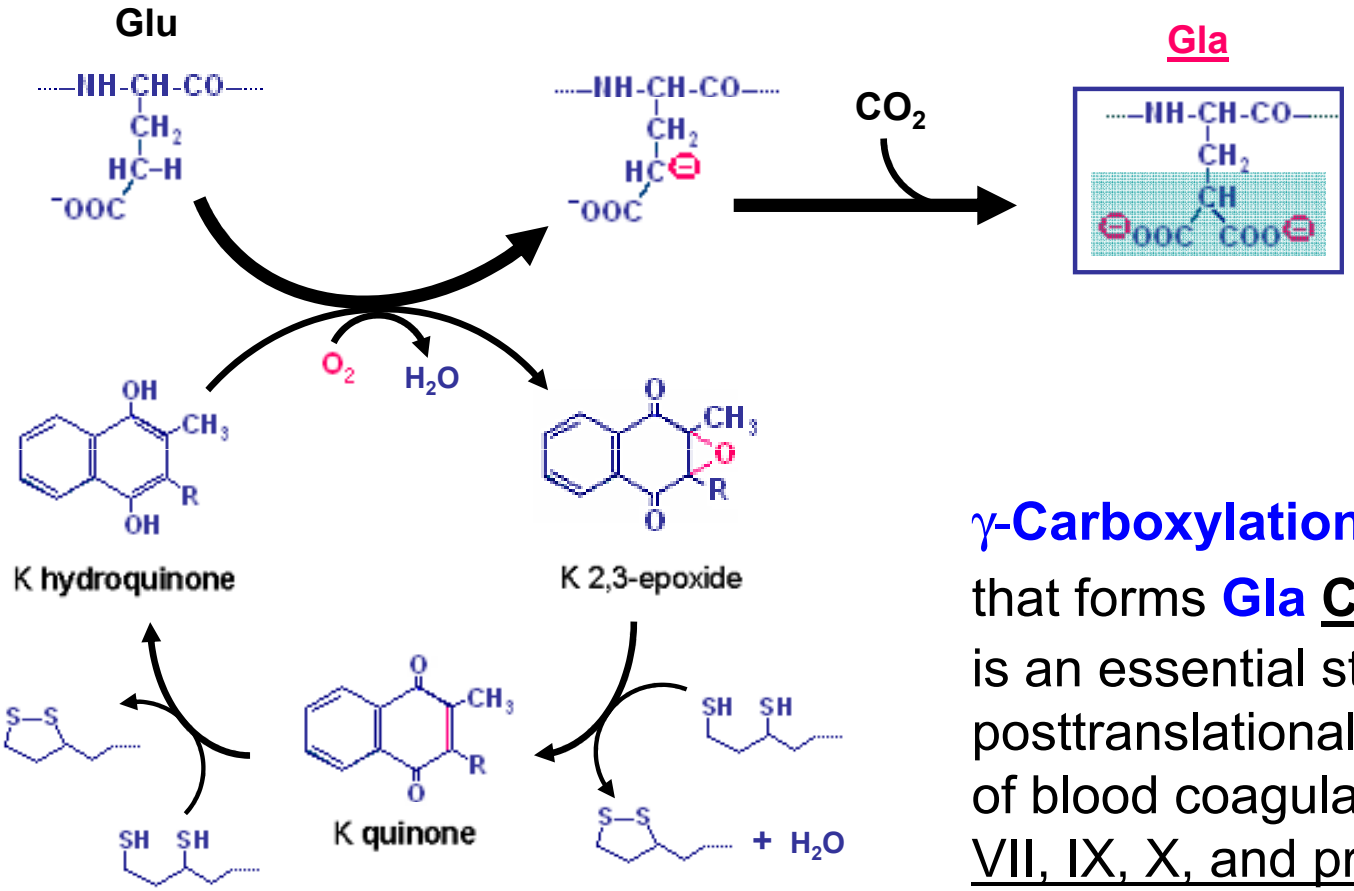
Aggregation of platelets is supported also by

von Willebrand factor (vWF produced by endothelial cells),
thrombin, and
fibrin which originate as products of coagulation cascade from plasma proteins prothrombin and fibrinogen.

The blood clotting cascade



Vitamin K metabolic cycle in the liver cells

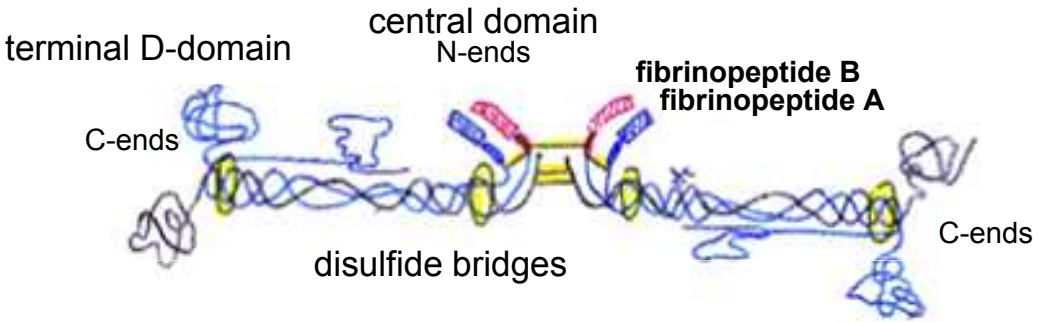


γ-Carboxylation of Glu residues
that forms **Gla** **Ca²⁺-binding centres**
is an essential step of
posttranslational processing
of blood coagulation factors
VII, IX, X, and prothrombin.

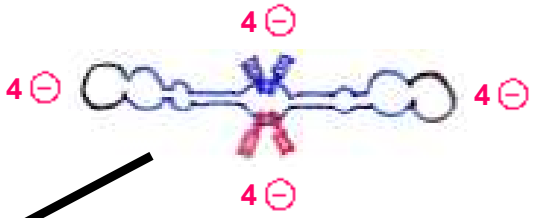
The two stages of reduction of vitamin K epoxide to the hydroquinone are **inhibited** by **coumarin anticoagulants** warfarin or dicoumarol (analogues of vitamin K) used as inhibitors of blood clotting in the treatment of thrombosis.

Fibrinogen

Glycoprotein, 330 kDa
6 chains – (**A**α **B**β γ)$_2$

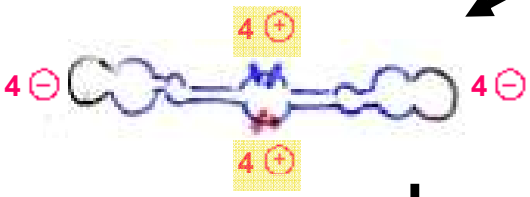


1.5 – 4 g/l (plasma β$_2$-globulin fraction)



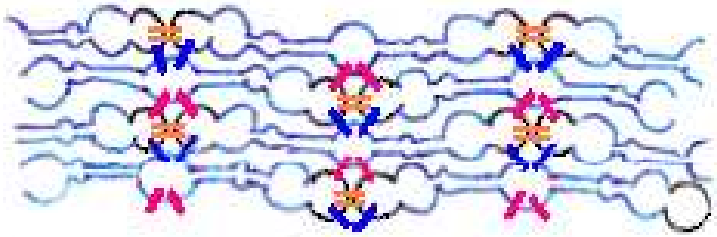
thrombin

Fibrin monomer (α β γ)$_2$



+ 2 fibrinopeptides A (16 AA)
+ 2 fibrinopeptides B (14 AA)

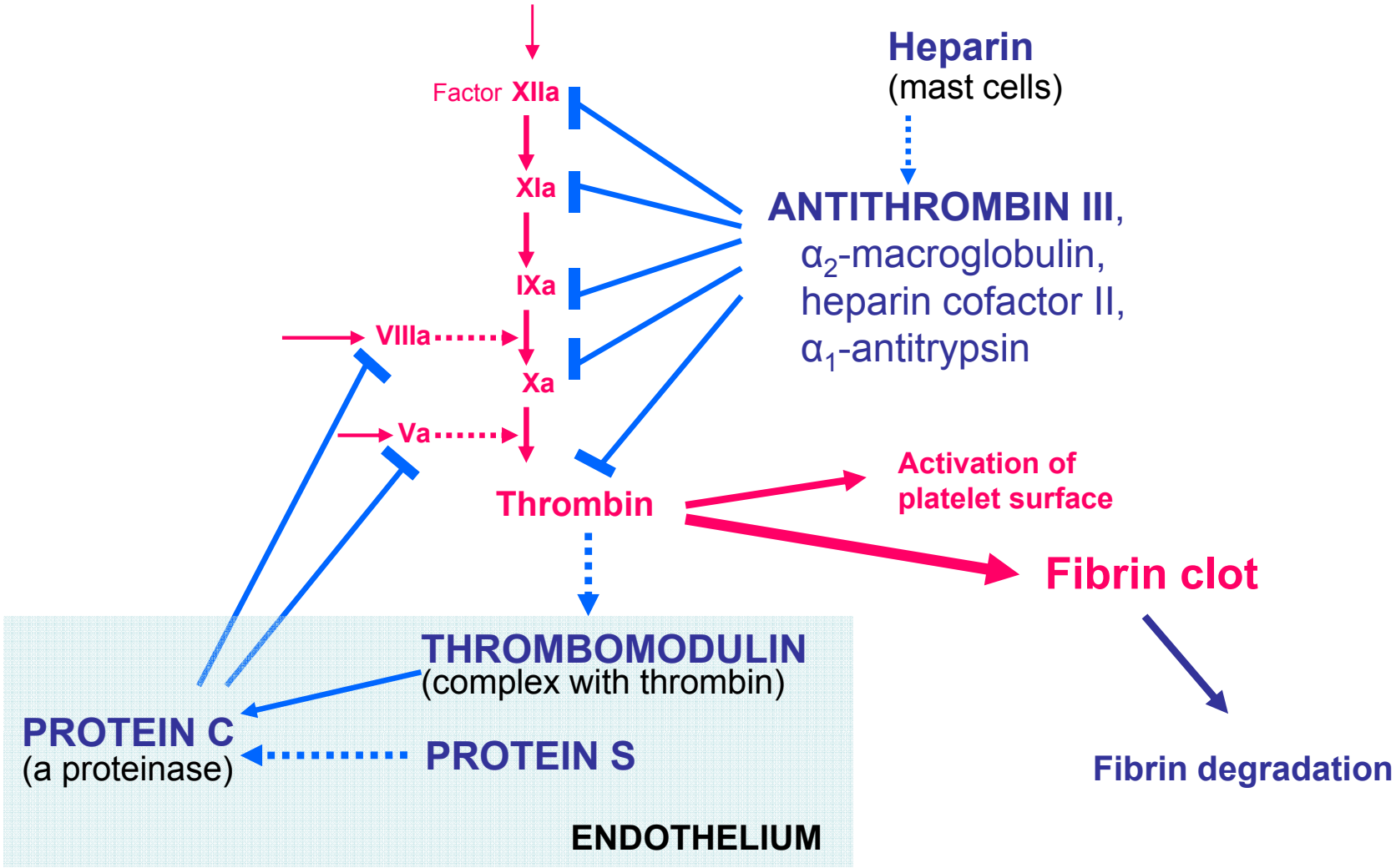
Fibrin "soft" clot (electrostatic interactions)



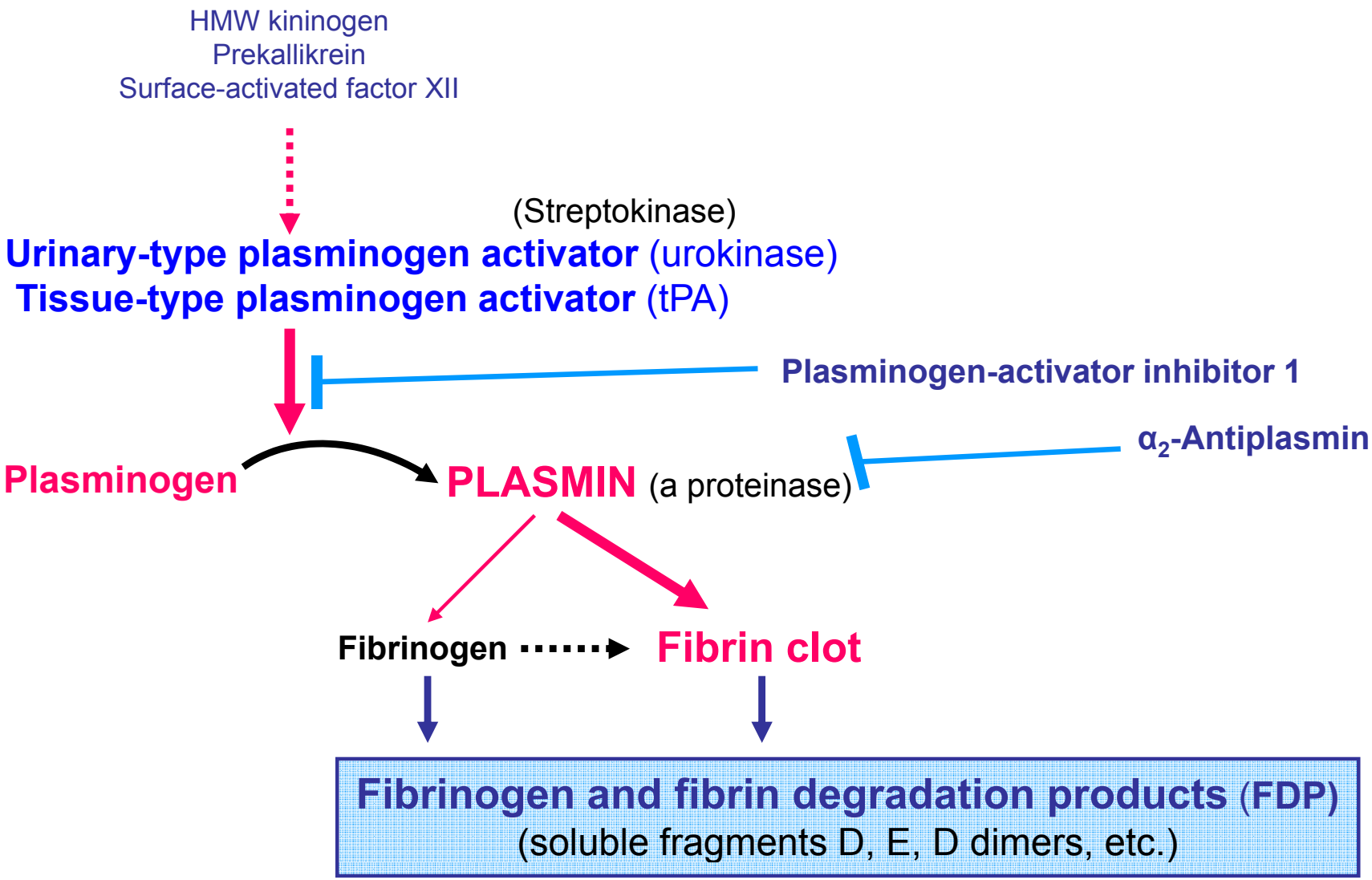
Fibrin "hard" clot – factor **XIIIa** (***transglutaminase***, fibrin ligase) catalyses formation
; of **covalent cross-links** (isopeptide bonds) between side chains of glutaminyl and lysyl residues

The cascade of the clotting system permits **enormous amplification** of its triggering signals.

Factors limiting clot growth:



The fibrinolytic system



Thrombolytic treatment in myocardial infarction or embolism

are effective, if administered early enough, before irreversible damage of the tissue occurs.

Urokinase
is an proteinase that activates plasminogen directly. It is secreted by epithelial cells of renal tubules.

Streptokinase
is a plasminogen activator produced by β-haemolytic streptococci.

Tissue-type plasminogen activator (**t-PA**, alteplase) and other thrombolytic drugs (streplase, saruplase) are produced by recombinant gene technology.

Red blood cells - erythrocytes (RBC, Ercs)

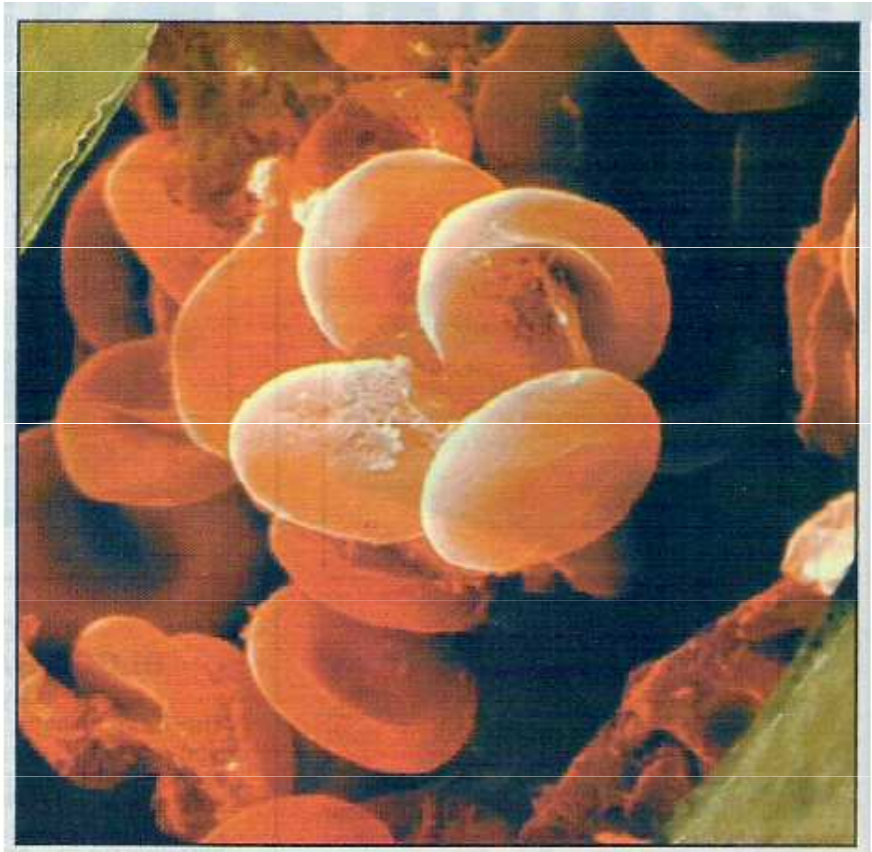
Biconcave shape, diameter 8 μm, deformations are possible. High surface-to-volume ratio facilitates gas exchange.

Nonnucleated, no cellular organelles, cytoskeletal components.

Concentration of haemoglobin in RBC is about 330 g / l (~ 95 % of all proteins).

Production of erythrocytes from red cell progenitors is located in the bone marrow and regulated by erythropoietin synthesized mainly by the kidney.

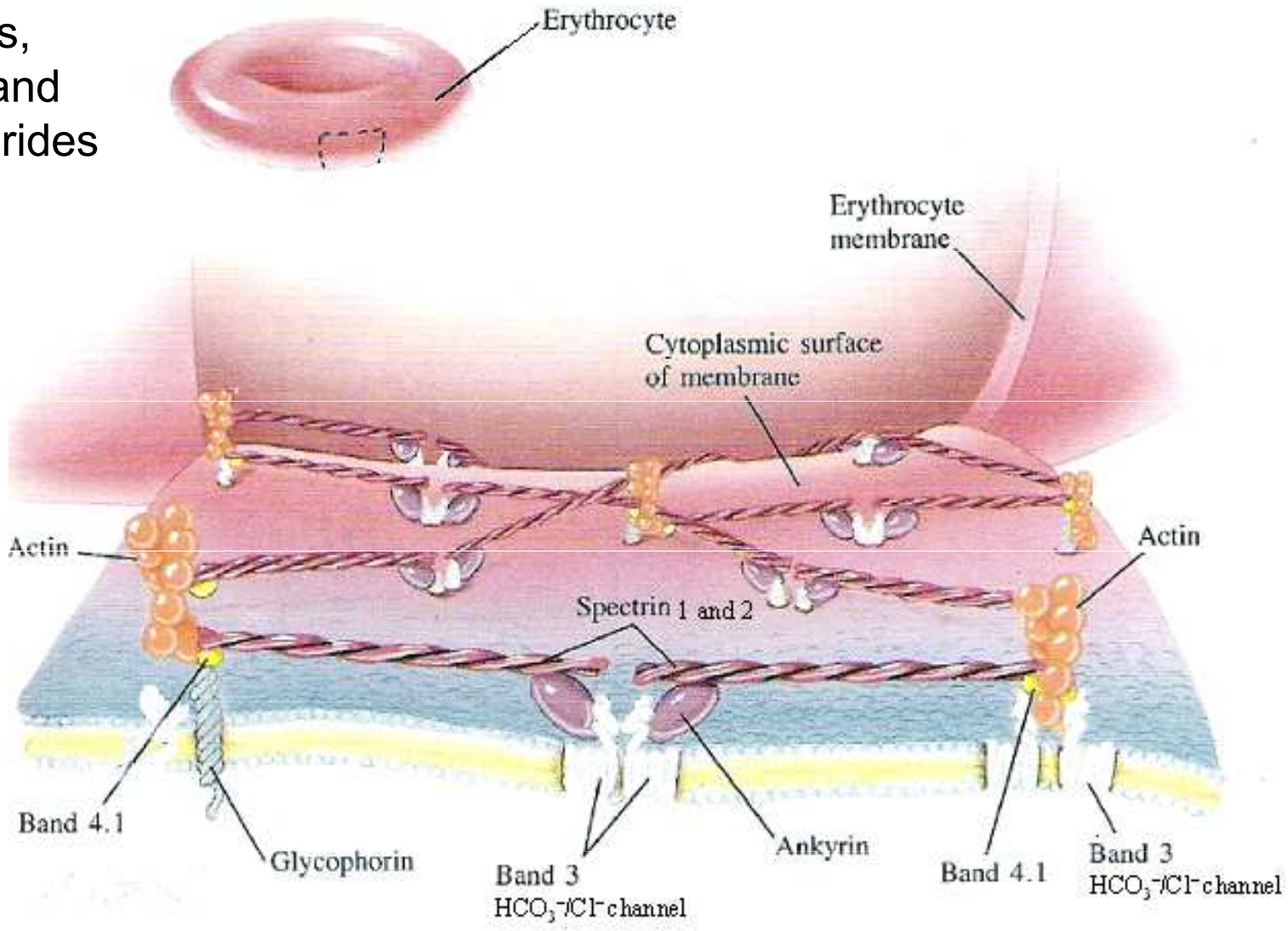
Reticulocytes still containing ribosomes and elements of ER are released into the circulation where they transform into adult red blood cells.



Colored scanning electron micrograph (SEM) of red blood cells flowing through a blood vessel (Photo by Dr. Philippa Uwins, Whistler Research).

Erythrocyte membrane

52 % proteins,
40 % lipids, and
8 % saccharides



Aquaporins, glucose transporters as well as other membrane proteins are not shown.

Glycophorins

are transmembrane single-passing glycoproteins. The saccharidic component (60 % by mass) consists of numerous oligosaccharides. It is highly sialylated and represents the major part of the glycocalyx on the outer surface. The negative electric charges prevent agglutination of RBC.

Polymorphism of glycophorin A in its amino acid sequence denotes the MN blood groups of individuals´ erythrocytes.

Cytoskeletal proteins

are fixed to the inner surface of the membrane and help determine the shape and flexibility of the RBC.

Spectrin

is the major cytoskeletal protein. It consists of two long polypeptide chains that form a loosely coiled dimer; two dimer form a tetramer, on which are binding sites for other cytoskeletal and membrane proteins (ankyrin, actin, protein 4.1).

Spherocytosis is a hereditary deficiency in the amount of spectrin or abnormalities of its structure. The spherocytes are more susceptible to osmotic lysis than are normal Erc.

dimer of spectrin

Metabolism of the red blood cell

Anaerobic glycolysis, producing lactate, is the energy source.
The synthesis of 2,3-bisphosphoglycerate, closely associated to glycolysis, affects the affinity of haemoglobin for dioxygen.

The pentose phosphate pathway is efficient, it metabolizes up to 10 % of the total flux of glucose. NADPH produced is required for the reduction of oxidized glutathione and methaemoglobin

In the adult RBC, glycogenesis, synthesis of fatty acids, cholesterol, proteins, and nucleic acids cannot occur, as well as catabolism of fatty acids and ketone bodies.
Some lipids (e.g. phospholipids, cholesterol) from the red cell membrane can exchange with corresponding lipids of plasma lipoproteins.

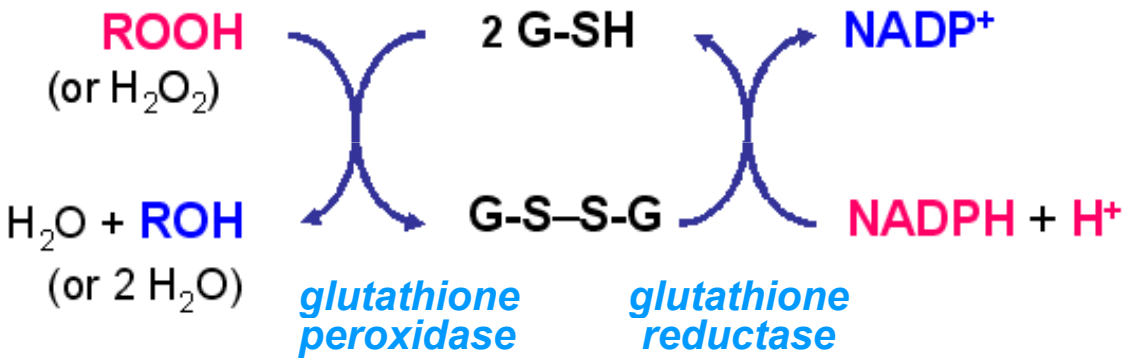
Erythrocytes and oxidative stress

High partial pressure of O_2 and the presence of Fe^{II} in haemoglobin represent a menace to processes and structures within erythrocytes.

Efficient antioxidants protect RBC from damage caused by oxidative stress.

- ***Superoxide dismutase*** and ***catalase***
  decompose superoxide anion and hydrogen peroxide.

- ***Glutathione peroxidase***
  catalyzes reduction of hydrogen peroxides by GSH (reduced glutathione). GSH is regenerated by NADPH in the reaction catalyzed by ***glutathione reductase:***



NADPH is required for regeneration of glutathione to its reduced form GSH. NADPH is generated in two reaction of the **pentose phosphate pathway** catalyzed by ***glucose-6-P dehydrogenase*** and ***6-phosphogluconate dehydrogenase***.

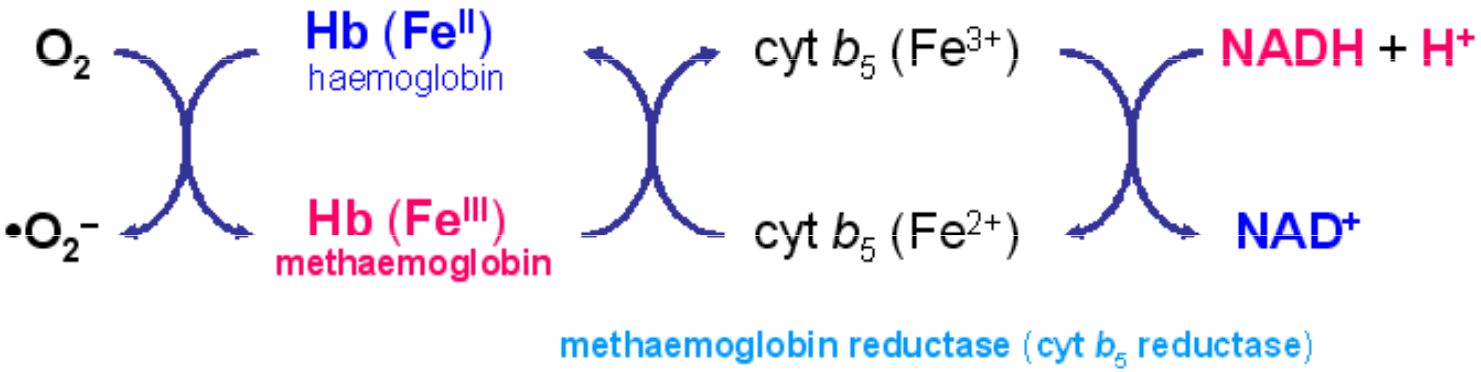
Deficiency of glucose-6-phosphate dehydrogenase

is the most common of all inherited enzymopathies, caused by point mutations within the gene located in chromosome X. It is extremely frequent in some regions of the world: in tropical Africa, the Mediterranean, in certain parts of Asia, and, for example, among Afroamericans (11 % incidence).

The deficiency is quite benign in the absence of oxidative stress. However, an exposure to oxidants (e.g. drugs - antimalarial pamaquine, sulfonamides, chemicals - naphthalene, consumption of fava beans, some infections) may result in a **severe attack of haemolytic anaemia**, because namely RBC are sensitive to increase in production of oxygen radicals and peroxides.

On the other hand, **this enzyme deficiency protect against falciparum malaria.** The parasites causing this disease require reduced glutathione and the products of the pentose phosphate cycle for optimal growth.

– ***Methaemoglobin reductase*** (cytochrome b_5 reductase) is a component of the NADH-cytochrome b_5 methaemoglobin reductase system, which reduces methaemoglobin-Fe^{III} back to haemoglobin-Fe^{II} that is able to transport dioxygen.



In the blood of healthy individuals, less than 1 % of total haemoglobin is present in the form of methaemoglobin.

Inherited methaemoglobinaemia – inherited deficiency of MetHb reductase.

Acquired methaemoglobinaemia occurs after ingestion of certain drugs (e.g. sulfonamides) or chemicals (e.g. aniline, nitrites, in sucklings also nitrates). Evident cyanosis appears usually when more than 10 % of total haemoglobin is oxidized to methaemoglobin.

Polymorphonuclear leukocytes (PMN)

Neutrophils

are the most numerous circulating leukocytes (50 – 70 %).
They have an important role in **non-specific defence** mechanisms – they can move along a chemical gradient of leucotactic substances to the site of a tissue injury or bacterial infection. Neutrophils are **microphages**.

Metabolism

Considerable activities of glycolysis, glycogenesis, and the pentose phosphate pathway.

Due to low number of mitochondria, only slight activity of the citric acid cycle and oxidative phosphorylation.
The proteosynthetic apparatus is developed less perfectly than in other cells.

Some special enzyme activities e.g. NADPH oxidase and myeloperoxidase.

The biological half-life of neutrophils is about 6 -7 hours in the blood, a few days in the connective tissue.
Neutrophils can survive even under anaerobic conditions.

Phagocytosis

- the role of neutrophils in antibacterial defence

After bacterial invasion into a tissue, neutrophils begin migration from the capillaries to the site of infection.

Their movements are initiated and directed by **chemotaxis**. Leucotactic substances (attractants) are, for example, various complement components, small bacterial peptide fragments, and eicosanoids, namely leukotriene LTB_4.

Neutrophils adhere to endothelial cells of the capillary wall, the process supported by membrane proteins integrins and selectins is called **margination of neutrophils**, and penetrate through the capillary wall – **diapedesis** – to the site of infection.

Then they actively engulf microorganisms or other small particles by **phagocytosis**. Bacterium or a foreign particle is encompassed by pseudopodia and phagosome originates after complete closure.

Phagosome fuses with lysosomes and specific granules into phagolysosome, vacuolar H^+-ATPase maintain the content at pH about 4, and hydrolases catalyze digestion of organic components.

Examples of important proteins in neutrophils:

Primary granules (lysosomes)

Hydrolases

cathepsin B – an acid proteinase
elastase – a neutral proteinase able to split elastin
β-glucuronidase – an acid specific glycosidase, absent in other cell types
lysozyme – splits muramic acid, a peptidoglycan of bacterial walls

Myeloperoxidase – catalyzes formation of HClO from peroxide and chloride

Defensins – small basic peptides that easily invade into lipid dilayers

Secondary (specific) granules

Hydrolases

collagenase – a metalloproteinase hydrolyzing collagen
lysozyme – muramidase

Lactoferrin – a protein that binds firmly ions of iron

The respiratory burst of phagocytic cells

is the sole profitable utilization of reactive oxygen species production – it helps kill bacteria engulfed by phagocytic cells.

Interaction of neutrophils with bacteria, binding of chemotactic factors or immunocomplexes onto specific receptors in plasma membrane activate motility of neutrophils, secretion of granules, and the activity of an membrane enzyme ***NADPH oxidase*** (a flavoprotein) and ***cytochrome b_{558}*** that initiate the respiratory burst:

$$2\ \mathbf{O_2} + \mathrm{NADPH} \xrightarrow[\text{cyt } b_{558}]{\text{NADPH oxidase}} 2\ \mathbf{\bullet O_2^-} + \mathrm{NADP^+} + \mathrm{H^+}$$

The consumption of O_2 by the cell rises steeply due to superoxide production, which results in formation of hydrogen peroxide (a spontaneous dismutation of superoxide anion):

$$\mathbf{2\ \bullet O_2^-} \longrightarrow \mathbf{H_2O_2} + O_2$$

Myeloperoxidase catalyzes the production of **hypochlorous acid** – an effective microbicidal agent

$$H_2O_2 + Cl^- + H^+ \longrightarrow \mathbf{HClO} + H_2O$$

In a similar way, **peroxynitrous acid** HO-O-NO is formed from nitroxide NO.