Chapter 2

Pathophysiology of blood and haematologic system

2.1 Haematopoiesis

The blood cells circulating in peripheral blood are the end-product of differentiation and maturation of recognizable precursor cells in the bone marrow. In the fetal period of development the formation and maturation of blood cells occurs first in the reticuloendothelial system. The formation of erythrocytes, granulocytes and thrombocytes in adults takes place exclusively in the bone marrow. The lymphocytes in the peripheral blood are already mature, but they can undergo the rapid proliferation as response to various specific stimuli.

The singular types of cells circulating in the blood differ morphologically, as well as functionally. Their origin is extraordinarly closely connected. From the point of view of its architecture the **zone marrow** is in fact an immense network of fibroblasts and endothelial cells forming a framework of progenitors and stem cells, interconnected by fibronectin into a net of tissue macrophages and T-cells. Mature cells have then to pass the slits between the endothelial cells and fibroblasts to reach the sinusoides from where they enter directly the circulation. The outer surface of sinuses is formed by reticular cells. They send projections forming a network with endothelial cells and fibroblasts. The fans of haematopoietic tissue are localized in the network of reticular cells (see Fig. 2.1).

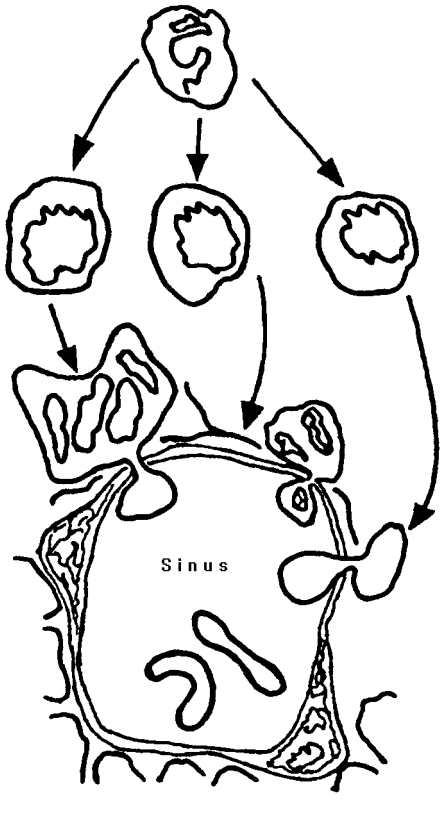

Figure 2.1: Entry of blood cells from bone marrow into the sinuses

Under physiological conditions the cell production must be balanced in speed and rate of their loss. The mean erythrocyte life span in man is about 120

days. The daily amount arising de novo in each microliter of blood is $5 \cdot 10^4$ (which represents 2 per cent of new blood cells). The thrombocyte survival is 7 to 10 days. Their daily production have to reach $2 \cdot 10^4$ of thrombocytes in each microliter of blood. White blood cells (leucocytes) have different kinetics. Granulocytes survive in intravascular milieu about 6 to 12 hours. To ensure their constant number, it must be produced daily about $2 \cdot 10^4$ granulocytes per each microliter of blood. The survival period of lymphocytes is considerably different. Their life duration varies from several months to several years, it means that a complete bone marrow failure in its initial phase is manifested by a fall in granulocyte number. On the contrary, the increased bone marrow activity is manifested by elevated granulocyte number. The bleeding leads rapidly to the fall of thrombocyte number. The information about erythrocyte and thrombocyte production and life- span can be obtained using the $Na_2^{51}CrO_4$ method.

2.1.1 Development of blood cells

The blood cell development and formation occurs in several consecutive periods. Moving back from the mature cell we get to the basis of the haematopoiesis. Its earliest stage is represented by the **pluripotent stem cells – PSC**. They are the basis for the differentiation of myeloid, as well as of lymphoid cells. The lymphoid stem cells give rise to precursor and mature progenitor cells of T- and B-cells. **The basal three-lined myeloid stem cells** are termed unit forming cells – spleen colony forming units CFU-S. During in vitro cultivation of the bone marrow cells three-lined myeloid stem cells are capable to form colonies of identical cells. These colonies were originally discovered in the spleen of lethal doses irradiated mice to whom the bone marrow of histoidentical donors was applied. The spleen colonies contain megakaryocytes, granulocytes and erythroid precursors.

The CFU-S further differentiate in three lines.

1. CFU-E (colony forming unit – erythroid)
   CFU-Meg (colony forming unit – megakaryocyte)
   CFU-B (colony forming unit – basophil)
2. CFU-G (colony forming unit – granulocyte)
   CFU-M (colony forming unit – macrophage)
3. CFU-Eo (colony forming unit – eosinophil)

There are few pluripotent cells in the bone marrow, hence it is difficult to isolate them. The blood cell maturation is maintained by a group of glycoproteins called the **haematopoietic growth factors – HGF**. Except the blood cell maturation they also influence their function. Under their influence the growth of CFU cells becomes enhanced. Thus, these growth factors are called also the colony stimulating factors (CSF) for granulocytes G-CSF (granulocyte-colony-stimulating factor) and for macrophages M-CSF (macrofage-colony-stimulating factor) or GM-CSF for both of them. Except for lymphocytes, all other development lines need the presence of IL-3 or GM-CSF for maturation. Generally IL-3 and GM-CSF stimulate proliferation and differentiation of progenitor cells. The stem cells can be stimulated by at least three interleukins (IL-1, IL-4, IL-6). Interleukin 6 in combination with IL-3 accelerate the dividing of the stem cells.

The localization and the maturation of lymphocyte precursors is in comparison with other blood cells more complex. The primary precursors of B-cells originate from bone marrow, spleen and lymph nodes. The differentiation and maturation of B-cells occur in lymph nodes. The primary precursors of T-cells are in the bone marrow, from where they are travelling into the thymus, where their further differentiation follows. Then they get into the spleen, the bone marrow and lymph nodes. Here is their "residence" and the site, where they obtain the necessary functions. T- and B-cells pass then into the blood and the tissues, where they are needed. The circulating lymphocytes are only one very small part of the global lymhocyte number division (see Fig. 2.2).

The differentiation of progenitor cells is significantly influenced by monocytes and T-cells. The monocytes produce IL-1 and TNF as a response to the bacterial products action (IL-1 is a cytokine influencing many functions in organism:, TNF – tumor necrosis factor, named also cachectin). IL-1 and TNF stimulate fibroblasts and endothelial cells to produce haematopoietic growth factors (HGF with the exception of IL-3 and IL-5). The antigens of various types stimulate the T-cells to IL-3, IL-5 and GM–CSF production. These haematopoietic growth factors act on progenitor cells, thus ensuring the blood cells development. Fibroblasts and endothelial cells have an important function in forming a layer where the progenitor cells adhere and where

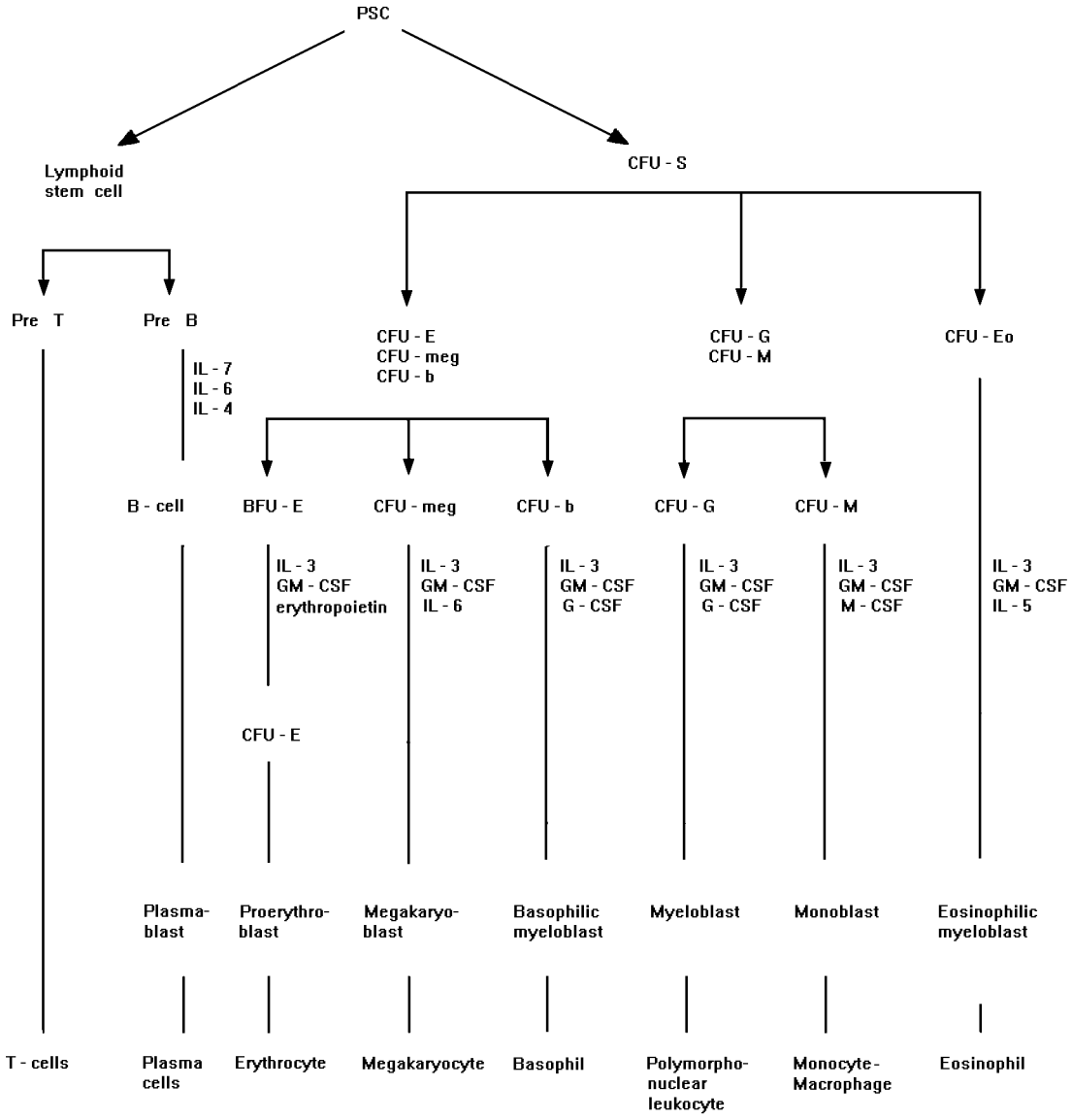

Figure 2.2: Development of blood cells

their differentiation occurs. The progenitor cells are bound to this layer with fibronectin receptors.

The haematopoietic growth factors are perspective for clinical use. GM–CSF is promising for use in aplastic anaemias in children, in granulocytopenia due to chemotherapy. Erythropoietin can partially substitute the numerous transfusions in patients chronically dialysed for renal failure. Understanding of the mechanisms having a central position in cell production is necessary for exact diagnosis and therapy.

The erythrocytes are the "descendents" of the

pluripotent stem cell. **The progenitor cell of the erythrocytes is the erythroid burst-forming unit (BFU)**, sensitive to erythropoietin and further haematopoietic factors. More mature cell is the erythroid colony-forming unit (CFU-E) producing smaller colonies of erythroid cells. In cell cultures it lasts about four to seven days. The erythroid cell production is higly sensitive to erythropoietin.

Erythropoietin is a glycoprotein with a molecular weight of 34 000 daltons. Cloning of erythropoietin gene enables to produce great amount of active erythropoietin. Erythropoietin is primarily produced in kidneys as a response to hypoxia. Its plasmatic level is directly proportional to the degree of hypoxia. Erythropoietin is bound to the specific receptor on the surface of erythroid stem cells. After the binding it induces the differentiation of these cells to proerythroblasts. The proerythroblasts are the first differentiated precursor cells of erythrocytes. Under the physiological conditions the evolution from proerythroblast to the completely mature normoblast last about 4 days. During this period the nucleus becomes progressively smaller and the haemoglobin produced in cytoplasm raises. The last step is the extrusion of the pycnotic nucleus out of the normoblast. In this way the normocyte changes into the reticulocyte remaining still 2 to 3 days in bone marrow. The reticulocytes are released into the circulating blood about 24 hours before their mitochondria and ribosomes disappearing. Thus, they become mature red blood cells.

The erythrocyte precursors from pronormoblast to reticulocyte contain a specific surface receptor for iron-transferrin complex, enabling to incorporate a sufficient amount of iron for haemoglobin formation.

The bone marrow is able to enhance the erythrocyte production three to five times during one to two weeks if it is stimulated by high erythropoietin level. In chronic haemolytic anaemias is the erythropoiesis five to seven times enhanced.

2.1.2 Haemoglobin biosynthesis

Haemoglobin is a tetrameral compound composed of two pairs of peptide chains (α, β, γ, δ), connected by covalent bonds to the haem group. The globin synthesis is controlled by relevant gene. Haemoglobin makes up about 98 per cent of cytoplasmatic proteins in circulating erythrocytes. There is 97 per cent of haemoglobin of type A ($\alpha 2$, $\beta 2$) in adults. The remaining 3 per cent of haemoglobin is of type A2 ($\alpha 2$,$\delta 2$). Fetal haemoglobin (HbF or $\alpha 2$, $\gamma 2$) does not exceed more than 1 per cent of the total haemoglobin amount in healthy adult persons. HbF persists in congenital haemolytic anaemias in adult age.

The first step of haem production in precursor cells is the condensation of succinyl-coenzyme A (CoA) with glycine to form delta-aminolevulinic acid. This reaction occurs in mitochondria and requires the glycine activation by pyridoxine phosphate. Therefore in sideropenic anaemias with impaired haem production the treatment with pyridoxine is sometimes successful. The second step of haem synthesis occurs in cytosol. Two molecules of delta-aminolevulinic acid form the porphobilinogen ring. Further reactions proceed again in mitochondria. Iron is incorporated into the protoporphyrin IX forming the haem. The haem is bound to globin in cytosol forming the haemoglobin.

2.1.2.1 Role of haemoglobin

The primary role of red blood cells is **the oxygen transport** from lungs to the tissues and the carbon dioxide transport in the opposite direction. The tridimensional structure of human haemoglobin optimizes its perfect function. During the blood flowing through the lung capillaries the oxygen binds with haemoglobin. On each gram of haemoglobin 1,34 ml of O_2 is bound. The haemoglobin affinity to oxygen is modified by three intracellular factors:

1. the hydrogen ion
2. the carbon dioxide
3. 2,3-diphosphoglycerate (2,3-DPG)

Increase in concentration of any of these factors shifts the oxygen dissociation curve to the right. The 2,3-DPG molecule binds to the beta-chain of deoxyhaemoglobin. In this way it reduces the oxygen affinity to haemoglobin. The consequence of this fact is an increased oxygen release.

The oxygenation of tissues depends on three main factors:

1. the blood flow
2. the oxygen capacity of blood
3. the haemoglobin affinity to oxygen.

The oxygen release of haemoglobin is expressed by the dissociation curve of haemoglobin. pH elevation, temperature fall and decrease in 2,3-DPG lead to oxygen affinity enhancement (the shift to the left). Fall in pH, elevation of temperature and of 2,3-DPG reduce the oxygen affinity (shift to the right). The shift of dissociation curve to the left represents simultaneously the decrease of oxygen release. Shift of dissociation curve to the right occurs under unfavorable conditions in organisms. It means also increased oxygen release into the tissues.

2.1.3 Metabolism of erythrocytes

During their release from bone marrow the erythrocytes lose the nuclei, ribosomes and mitochondria. Thus they lose the ability of proteosynthesis and of oxidative phosphorylation. The glucose, entering the erythrocytes by diffusion, is converted into glucose 6-phosphate. About 80 to 90 per cent of it is converted into lactate. During the metabolization of two molecules of glucose two molecules of ATP are generated. About 10 per cent of intracellular glucose 6-phosphate is oxidized in the hexose-monophosphate shunt in which glutathione is produced, protecting the haemoglobin sulphhydryl groups and membranes against oxidation by peroxides and superoxides and against effects of certain drugs and toxins.

The considerable part of ATP, generated by glycolysis, **is spent for the functioning of Na-K-pump**. This pump is required to maintain the ionic balance in cytoplasm. The erythrocyte survival in the circulation is determined by its membrane integrity and its flexibility. The membrane of erythrocytes contains 50 per cent of proteins, 40 per cent of lipids and 10 per cent of carbohydrates. The bilayer membrane contains phospholipid and cholesterol molecules in the ratio of 1,2 to 1. The molecules are arranged in the membrane with the hydrophobic chains oriented inside the membrane and the polar groups to the outer plasmatic surface of membrane and to the cytoplasmatic surface of membrane. The outer (plasmatic) part is richer in lecithin and sphingomyelines. The inner part consists of relatively more phosphatidylserine and phosphatidylethanolamine. The lipids of the outer part are freely exchangable with plasmatic lipids.

The cell membrane of erythrocytes contains 8 types of fundamental proteins. Some polypeptides are located in the membrane so that one end is oriented to the outer surface of the membrane and the other to its inner surface. So is the glycoforin situated, containing polysaccharidic antigens of blood groups, similarly situated is the protein 3 forming channels through which anions can pass into and out of erythrocytes. Other proteins are only at the inner surface of membrane: several enzymes a structural proteins, like **spectrin** and **actin**, being interconnected. They form a network giving the inner structure of the membrane. Tropomyosin and protein 4.1 also participate in the structure formation. The protein **ankyrin** binds spectrin and a fraction of proteins involved on ionic transport. Protein 4.1 binds glycoforin, actin, tropomyosin and spectrin (Fig. 2.3).

The erythrocyte are destroyed when their membrane have lost its flexibility. This quality is needed to enable the erythrocytes to pass across the capillaries and especially across the splenic sinuses. The erythrocyte membrane alteration arises by successive ATP depletion in erythrocytes leading to erythrocyte shape change and to forming of sharp projections at the membrane surface. The ATP depletion causes breaking of the connections in the spectrin and actin network. Thus, the localization of proteins at the inner surface of membrane is changed, and irregular protein aggregation arises.

If the continuity of erythrocyte membrane is impaired the released components are metabolised. The haem is catabolised by the microsomal oxidative system. The porphyrin cycle is converted into the bile pigments. The released iron is incorporated into the ferritin or it is transported by transferrin into the erythrocyte precursors. Iron reversibily binds the oxygen. Except for this it participates in several oxidation-reduction reactions. Substantially the anorganic ion of iron is extremely toxic. Under physiological circumstances is the iron homeostasis strictly controlled and balanced.

2.1.4 Absorption of iron

The absorption of iron occurs mainly in duodenum and in the upper part of jejunum. Anorganic iron salts contain bivalent (Fe^{2+}) or trivalent (Fe^{3+}). The iron enters the organism with food, mainly in form of trivalent ion. The salts containing Fe^{3+} are insoluble. The acid gastric juice enables the absorption. The low pH value makes the iron salts solubles. The intake of iron by food is 10–20 mg daily. About 10 per

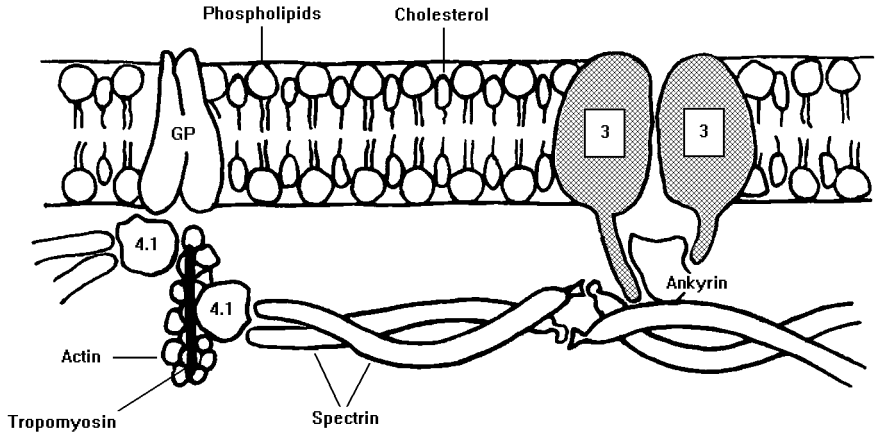

Figure 2.3: Structure of the red blood cell membrane

cent of this amount is absorbed. The haem present in the food is more easily absorbed than the anorganic iron. **The iron is absorbed by epithelial cells in the upper part of gastrointestinal tract**. The components of the tea, particularly tannin, but also the phosphates and several other substances, inhibit the iron resorption. The iron absorption is connected with absorption of heavy metals like lead, mercury, cadmium and strontium. Increased iron absorption is associated with increased absorption of these metals.

The precise mechanism by which the iron passes through the epithelial barrier is not understood. It is known that the serum glycoprotein-**transferrin** (m.w. 80 kDa) transports iron into the tissues. One molecule of transferrin can bind two atoms of iron. All binding-sites together represent the total binding capacity for iron – TIBC (total iron-binding capacity). Under normal circumstances 20 to 45 per cent of this capacity is occupied by iron. Specific receptors at the cells catch up the transferrin from which the iron is released into the cytoplasm of these cells. Precursors of erythrocytes in the bone marrow have a high density of transferrin receptors.

Excess of iron in organism is deposited in form of **ferritin** or **haemosiderin**. The iron in ferritin is provided with protein coverig and forms iron-apoferritin complex, in which iron is present as trivalent ion (Fe^{3+}). The apoferritin synthesis is stimulated by iron. Under physiological circumstances a close correlation exists between the plasmatic ferritin concentration and iron stores in organism. Ferritin may be absorbed (swallowed up) by lysosomes, catabolized to haemosiderin and decomposed to protein, lipid and iron. **Daily loss of iron from organism is 1 to 2 mg**. Most frequently the loss originates, under physiological conditions, **in desquamation of cells of gastrointestinal tract**. This loss is compensated by adsorption of iron from food. If the iron loss raises, its absorption from gastrointestinal tract becomes enhanced proportionally. The iron absorption can be increased by 30 to 40 per cent. So it is ensured that the enhanced iron loss is compensated by its proportionate absorption. There is not any mechanism to remove the excessive iron and to attain the necessary balance available.

About 80 to 90 per cent of resorbed iron gets into bone marrow and is available for erythropoiesis. The half-time between the plasmatic level of iron and its concentration in bone marrow is about 75 minutes. The iron is incorporated into the haemoglobin. The iron stores are created in the bone marrow. **The richest iron stores are deposited in the cells of MPS (mononuclear phagocytic system) in the bone marrow, in extrahepatocytic structures of the liver, in the spleen and hepatocytes**.

The reserves of iron in liver can be quantitatively determined by virtue of modern methods (atomic absorption spectroscopy). Information about the iron stores provides the serum ferritin level. The fer-

ritin concentration in serum raises according to iron amount present in organism and in turn, it falls when the iron amount decreases. However, the serum ferritin level is elevated in inflammation in oncologic conditions and in hepatic diseases. During the iron deficiency protoporphyrin IX is accumulated in erythrocytes, because it cannot be converted into the haem. Computerized tomography (CT) of the liver enables to reveal the iron stores, this method is especially succesful in excessive increase in iron reserves. An other sensitive method is the nuclear magnetic resonance. It is particularly useful in long-term follow-up.

2.2 Anaemias

Anaemia is one of the most frequent manifestations of diseases. About one third of patients admitted to the hospital exhibits anaemia. Frequently the urgent symptoms dominate at the admission and anaemia remains unobserved, shifted out of sight on later periode. Nevertheless, it could play a key role in solving of the given status. It is sometimes incorrectly considered to be the consequence of pathologic alteration although the contrary can be right.

Anaemia is a condition with haemoglobin reduction in blood below the physiological value relevant to the given person according to the sex, age, external conditions of life and important circumstances in organism in the given situation. Reduced concentration of erythrocytes is frequently accompanied with decreased haemoglobin. If the changes of water content or its shift in organism are not involved, the haemoglobin level correlates with the haematocrit value (during dehydration even in an anaemic patient are normal haemoglobin and erythrocyte values due to the plasma volume decrease).

The important role of erythrocytes is to transfer the oxygen in organism. When the amount of haemoglobin falls the oxygen supply of tissues becomes disturbed, therefore the most severe consequence of anaemia is the hypoxia of tissues.

If the anaemia is developing slowly **the compensatory mechanisms begin to function helping to supply tissues with oxygen**. The formation of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes is elevated during anaemia. 2,3-DPG binding with haemoglobin results in the oxyhaemoglobin curve shift to the right. It means, that in tissues is more oxygen released from haemoglobin.

The periphery responds to anaemia with compensatory dilatation and fall in peripheral resistance of vessels. Owing to this change, cardiac output rises with an increase in stroke volume and mild increase in heart rate. Therefore the pulse pressure is elevated and later the systolic pressure falls. Even in a patient with hypertension normal values of systemic arterial pressure can appear during development of anaemia. The anaemia treatment may result in reappearing of hypertension, similarly during the treatment with erythropoietin. Decreased oxygenation in coronary vessels impairs the myocardial contractility.

The hypoxia in organism is the cause of development of several symptoms. Dyspnea following exercise, headache, tinnitus, palpitations, syncopes, decreased libido, sleepdisorders, lability of mood, incapability to concentrate may be evident. Anemia impairs considerably existing angina pectoris in elderly patients with vessel diseases. The long-lasting untreated anaemia may unmarked premature dementia and claudicatio intermittens. Anorexia appears. Further impairment of anaemia may be associated with tachycardia. A systolic murmur is often heard over the precordium owing to the changes in blood viscosity and blood flow rate changes. Oedemas of extremities appear. Further progression of anaemia is sometimes associated with thrombocytopenia which may land to haemorrhage from various organs, retinal haemorrhage occurs frequently.

2.2.1 Anaemia due to iron deficiency

The iron deficiency is the most frequent underlying cause of the classic hypochromic microtic anaemia. The haemoglobin content in erythrocytes is decreased. The hypochromic anaemia without microcytosis occurs in bone marrow dysplasia. Microcytosis without hypochromia appears sometimes in anaemias developing during chronic diseases. Hypochromia associated with microcytosis most frequently occurs in anaemias due to iron deficiency.

The iron deficiency is due to the iron loss from the organism or its utilization. Enhanced loss of iron is