

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 successful in excessive increase in iron reserves. An other sensitive method is the nuclear magnetic resonance. It is particularly useful in long-term follow-up.

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## 2.2 Anaemias

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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 periods. 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, sleep disorders, lability of mood, incapability to concentrate may be evident. Anaemia 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 lead 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 microcytic 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

easier detectable than the intricate problems of iron utilization. Increased requirement of iron supply occurs during the period of rapid growth commonly in early postnatal period and during the puberty. The mother's milk contains equal amount of iron like the cow's milk however, newborn is easily able to utilize iron from mother's milk. Iron deficiency in newborn can be manifested by anaemia. Not only anaemia itself, but also the iron deficiency itself influences unfavorable the development of the child. During the puberty is the cereal nutrition insufficient to ensure the necessary iron intake. Parasitic diseases are a further factor inducing the iron loss from organism.

### 2.2.1.1 Decreased (defective) iron absorption

Decrease in iron absorption is observed in several pathological conditions. Following partial or total gastrectomy is the iron absorption impaired, in particular owing to the acceleration of motility and of the food passage in the proximal part of jejunum. In this part of the gut is the site of iron absorption. Achlorhydria is a further factor impairing the iron resorption. Patients with chronic diarrheas and with malabsorption may have disturbances of iron absorption.

### 2.2.1.2 Increased iron loss

Physiological iron loss occurs during menstruation and pregnancy. To maintain the iron balance during the menstruation, double intake of iron is needed. During the pregnancy 500mg of iron passes daily from the mother's organism into the fetal body. The increased requirement is ensured by augmented iron absorption in GIT. An increased supply of iron in food is, as matter of course, needed.

Gastrointestinal disturbances can usually cause increased loss of iron. Blood loss and thus the iron loss is observed in gastric or duodenal ulcers, in gastritis, haemorrhoids and adenocarcinoma of the colon. During chronic intake of salicylates blood loss arises due to the haemorrhagic gastritis induced also by other nonsteroidal antiphlogistic drugs. In all patients with gastrointestinal carcinoma anaemia due to iron deficiency develops.

Gastrointestinal blood loss may be present also in haemorrhagic diatheses and blood coagulation disturbances of various origin. The intravascular haemol-

ysis with haemoglobinuria, pulmonary haemorrhage, bronchiectasias and the blood loss from urogenital system may also be the cause of iron deficiency.

### 2.2.1.3 Iron deficiency consequences

The **cell growth and proliferation disturbances** are generally observed in iron deficiency. Erythrocyte formation is impaired owing to their requirements for the iron supply. The iron deficiency has unfavourable influence on various systems and functions of organism. Infirmity, torpidity, palpitations, dyspnea and very commonly all symptoms of chronic anaemias appear.

Many symptoms of iron deficiency originate in the gastrointestinal tract region. Gingivitis, glossitis with red, smooth and shiny tongue surface is frequently present. Atrophic gastritis with achlorhydria also occurs. In long-lasting iron deficiency the Plummer-Winson's syndrome may occur, with angular stomatitis, hyperkeratosis of oral mucosa, breaking nails, atrophic glossitis, atrophic oesophageal alterations and microcytic, hypochromic anaemia. The nails in addition to the fragility are concave and thin. In women menorrhagia and atrophic gastritis occurs. The iron deficiency may lead to very **specific symptoms** like excessive eating of starch (amylophagia), of ice (pagophagia), earth (geophagia) also of plaster, or even of lead dyes. All these substances bind with iron in the gastrointestinal tract and further impair the iron deficiency. The iron deficiency enhances the lead absorption from gastrointestinal tract in so far that lead intoxication may develop.

The iron deficiency is developing insidiously. In detailed observation firstly a decrease of iron stores without affecting the erythropoiesis is found. In the further stage deficient erythropoiesis is found due to iron deficiency. The end-stage is the iron deficiency anaemia. The **erythrocytes are hypochromic** and small. Bizarre shapes of erythrocytes – the poikilocytes are present. The life span of erythrocytes in the circulating blood is considerably shortened. The reticulocyte number used to be normal, or moderately elevated. It culminates about on tenth day after iron administration.

## 2.2.2 Sideroblastic anaemias

**The sideroblastic anaemia is a hypochromic anaemia** with elevated iron level in serum and enhanced trans-

ferrin saturation. This condition is due to haemosynthesis disturbance. In the peripheral blood sideroblasts are present. Sideroblast is a normocyte containing the non-haemoglobin iron. Mitochondria are localized around the nucleus forming the typical ringlet of sideroblasts. Sideroblasts are regarding the haemoglobin content hypochromic. They are smaller than the mature erythrocytes.

Hereditary sideroblastic anaemia is due to the impaired activity of delta aminolevulinic acid synthetase. This enzyme acts at the beginning of haem biosynthesis. This type of anaemia can often be successfully treated with high doses of pyridoxine.

Acquired sideroblastic anaemia can be induced by isoniazide application or by excessive alcohol intake. In both cases induced disturbance of pyridoxine metabolism is involved. In lead intoxication the haem synthesis is impaired. In about 30 per cent of patients admitted to hospitals for chronic alcohol abuse treatment are sideroblasts present in blood smears. Sideroblastic anaemia can appear in neoplastic diseases usually due to cytostatic treatment. It may occur also in inflammatory conditions (rheumatoid arthritis).

The sideroblastic anaemia appears most frequently without apparent cause, usually in elderly people. The time of cell maturation is always impaired. During the progression of sideroblastic anaemia neutropenia and thrombocytopenia appear associated with bleeding enhancement. Specific treatment of idiopathic forms of sideroblastic anaemias is unknown. Application of erythropoietin, GM-CSF and interleukin 3 might be promising.

### 2.2.2.1 Haemosiderosis due to blood transfusion

Iron level in organism may inadequately rise following repeated blood transfusions in severe anaemias. The symptoms reflect the alterations of organs being the iron depots (heart and liver). The cardiac siderosis is the cause of arrhythmias, conduction defects and the cause of congestive heart failure. **Elevated iron deposits** in the liver impair the hepatocytes, lead to necrosis, fibrosis and cirrhosis. Gonadal dysfunction and ACTH deficiency appear in this condition as a results of iron depositions in pituitary gland.

## 2.2.3 Megaloblastic anaemias

Megaloblastic anaemias are due to the impaired DNA synthesis. The cells with a high metabolic turnover are affected primarily, especially the haematopoietic precursor cells and gastrointestinal epithelial cells. The division of these cells becomes slower, but the cytoplasm development is normal, thus the cell dimensions grow large, they become megaloblastic. The RNA to DNA ratio increases. Megaloblastic erythroid cells tend to desintegration in the bone marrow leading to so-called noneffective erythropoiesis. The megaloblastic anaemias are most frequently due to the vitamin B<sub>12</sub> and / or folic acid deficiency.

### 2.2.3.1 Folic acid

**Folic acid is a trivial term of pteroylmonoglutamate** (pteridine, P-aminobenzoic acid and L-glutamic acid). It is synthesized by many plants and bacteria. Fruits and vegetables contain sufficient amount of folic acid to saturate the normal human demands. However it can be decomposed by cooking. An average consumption is about 50 µg daily. The demands are many times higher in conditions with enhanced metabolism (e.g. during the pregnancy). The folates (salts and esters of the folic acid) are in the intestine converted into the mono- and diglutamates, which are absorbed in proximal jejunum. There are proteins binding with folates present in the blood plasma. The plasmatic folates are present mainly in the form of N-methyltetrahydrofolate. In this form they are transported into polyglutamate form. There is about 5 to 20 mg of folic acid present in the human body. One half of this amount is stored in the liver. The folic acid deficiency is manifested months after its intake with food, or its deficient absorption decreased.

**Folates provide the transfer of methyl, methylene and formyl groups in the process of DNA and RNA synthesis.** During folate deficiency the growth and the maturation of rapidly developing cells in bone marrow are impaired. The thymidylate synthase catalyzes the thymidine synthesis of deoxyuridine and of 5,10-methylenetetrafolate. If this synthesis is impaired, the intracellular concentration of deoxyuridinephosphate rises and is incorporated into the DNA at the site where, under normal circumstances, the deoxythymidinetriphosphate is present.

This DNA has an increased fragmentation, responsible for the cell growth and maturations deviations during the folate deficiency.

### 2.2.3.2 Cobalamin – vitamin B<sub>12</sub>

**It is an organometallic compound with structure similar to porphyrin containing an atom of cobalt.** The human organism is unable to synthesize cobalamin (Cbl). Intake of the daily minimum (2,5 µg of cobalamin) is accomplished by food.

The source of vitamin B<sub>12</sub> is the meat and the milky products. Cobalamin is released from the food in the stomach. The released **cobalamin is bound with gastric R-protein** (R-binding protein), which is present in various synthetase of some glands (saliva, gastric juice, bile, milk), in plasma and in phagocytes. Its precise physiological function is unknown. The R-Cbl complex passes from the stomach into the duodenum, where the R-protein is released and Cbl is bound to the intrinsic factor **IF**. **Intrinsic factor** is a glycoprotein with molecular weight of 50 000 daltons. It is produced in the gastric parietal cells and its production is directly proportional to the hydrochloric acid production. The Cbl-IF complex is resistant to the proteolytic digestion. It enters unchanged the distal ileum. Here are at the mucosal surface receptors present binding with Cbl-IF complex. The complex enters the cells of ileal mucosa where the IF is during several hours destroyed and the free cobalamin is bound to an other transporting protein – the **transcobalamin II** (TC II). The complex cobalamin – TC II enters then the blood circulation and its uptake occurs preferentially in the liver and in the bone marrow. About one half of the cobalamin intake is stored in the liver. The other half is deposited in other tissues and organs including the bone marrow. If the demands for cobalamin are minimal, its stores in organism will be sufficient for some years.

The cobalamin bound to transcobalamin I (TC I) prevails in the blood. The transcobalamin I is a glycoprotein. TC II complex and TC I complex differ from each other by the rate of cobalamin release availability. The organism can obtain cobalamin from TC II complex in a few hours, but from TC I complex even in a few days.

**There are two active forms of cobalamin present in human organism: methyl-cobalamin and adenosylcobalamin.** Cyanocobalamin is converted into active forms before entering the tissues.

**Methylcobalamin** is an essential cofactor in homocysteine conversion into the methionine. Foliates are involved in this process. The methionine production from homocysteine is catalysed by methionine synthetase requiring the presence of methylcobalamin and the transfer of methyl-group from CH<sub>3</sub> tetrahydrofolate to the homocysteine. Methionine and tetrahydrofolate arise. In methylcobalamin and in 5-methyltetrahydrofolate (CH<sub>3</sub>-tetrahydrofolate) deficiency homocysteine is accumulated. Impairment of homocysteine conversion to the methionine can be responsible for neurologic complications during cobalamin deficiency. Methionine is needed for choline and choline containing phospholipids production and for methylation of myelinic protein.

**Adenosylcobalamin** is important for conversion of methylmalonylcoenzyme A (CoA) into the succinyl-coenzyme A. Adenosylcobalamin deficiency leads to increase of methylmalonyl-CoA and its precursor CoA in tissues resulting in fatty acids alterations and disturbance of neuronal lipids.

### 2.2.3.3 Types of megaloblastic anaemias and their incidence and prevalence

Four types of megaloblastic anaemias exist:

1. due to cobalamin deficiency,
2. due to deficiency of folic acid,
3. induced by some drugs,
4. seldom occurring forms of enzymic disturbances.

The first two types may be due to low cobalamin or folic acid intake, to impaired absorption of these substances or their utilisation.

Drugs which can induce megaloblastic anaemia belong to the groups of purine or pyrimidine antagonists, or to the substances inhibiting the DNA synthesis.

Megaloblastic anaemia in cobalamin deficiency is often termed pernicious anaemia. This term should be used for designation of the megaloblastic anaemia due to a lesion of gastric mucosa leading to the intrinsic factor deficiency. The term pernicious anaemia has been accepted because until 1926 this type of anaemia was frequently fatal.

In folic acid deficiency the megaloblastic anaemia occurs less frequently. Its occurrence is observed in chronic alcoholism.

The chemotherapy of malignancies and disorders of immune system may also lead to megaloblastic anaemia.

At present the cobalamin deficiency occurs more frequently in vegetarians and especially in vegans.

Megaloblastic anaemias in cobalamin deficiency are manifested by haematologic, gastrointestinal and neurologic alterations.

**The anaemia prevails** in blood smears. **Thrombocytopenia** is sometimes observed. Symptoms of anaemia include weakness, bradypsychia, dizziness, tinnitus, palpitations, angina pectoris, and signs of congestive heart failure. The patient is pale, his skin and sclerae are slightly subicteric. Heart rate is accelerated. The heart is usually enlarged and systolic murmurs are present. Liver and spleen may be also enlarged. Subfebrility can be present.

**The gastrointestinal alterations** are observed in the mouth. The tongue is usually smooth, red and often painful. Anorexia is present, leading to body weight loss. Decreased intake of nutrients is often complicated by frequent diarrhea.

**Neurological disorders** are very variable. They begin with demyelination and continue with axonal degeneration and finally end with complete loss of neurons. These alterations attack first the peripheral nerves, the medulla dorsalis and cerebellum. The disturbances of sensitivity appear first, then anaesthesia and weakness. Later ataxia and disorders of movement coordination are found. Disturbances of sphincter functioning are also observed. Patient is irritable, or dementia can develop. The anaemia is usually tremendous during the examination. The patient tolerates this condition quite well, because of very slow progress of the anaemia.

### **Pernicious anaemia**

This disease is characterized by the presence of macrocytes in peripheral blood and of megaloblasts in the bone marrow. The asynchronous maturation of erythroblasts is due to the impaired DNA synthesis. The cytoplasm of macrocytes contains more haemoglobin than under physiological circumstances. Owing to this, and to the impairment of nucleus development, the erythrocytes exhibit deformations (anisocytosis, poikilocytosis). The neutrophils use to be hypersegmented.

Pernicious anaemia is due to **cobalamin deficiency**, which itself is caused by the absence of intrinsic fac-

tor, most frequently due to the gastric mucosa atrophy. It is not a simple atrophy of mucosa. Very frequently a further pathological condition is found, e.g. thyroidal disease, suprarenal cortical atrophy, or insulin-dependent diabetes mellitus. A polyendocrinopathy is often involved. Antibodies against the adrenal, thyroid, parathyroid glands, and against the stomach are detected.

During the lymphocyte analysis a significant decrease in the circulating suppressor cell number is found. In the bioptic examination of gastric mucosa a high number of lymphocytes is observed. Some lymphocytes bind directly with the intrinsic factor in gastric mucosa. Parietal cells of gastric glands produce intrinsic factor and the hydrochloric acid. The circulating antibodies to the gastric parietal cells are found in 90 per cent of patients with pernicious anaemia. The antibodies react with the membranes of parietal cells. The parietal cells are progressively destroyed, the production of hydrochloric acid and of intrinsic factor decreases and the atrophic gastritis is developing. Antibodies to the intrinsic factor are found in serum and in gastric juice in approximately 70 per cent of patients. The underlying cause of parietal cells destruction is the activation of inflammatory reaction components, triggered of antibodies or immune complexes. The histological examinations show the cellular atypia.

Following **total gastrectomy** without vitamin B<sub>12</sub> intake megaloblastic anaemia appears in every case. Megaloblastic anaemia may occur also in jejunal strictures and diverticles. In these conditions can a large number of microorganisms colonize the small intestine and destruct the present cobalamin. Complicated enteritis may also be the underlying cause of cobalamin deficiency. The jejunum resection and malabsorption are commonly the cause of vitamin B<sub>12</sub> deficiency. An inborn disturbance of vitamin B<sub>12</sub> resorption associated with proteinuria may infrequently be involved.

Megaloblastic anaemia may develop in association with **cytostatic treatment**, especially if agent influencing the DNA synthesis are used. Substances with antiviral effects can also induce megaloblastic anaemia.

Macrocytes are found in peripheral blood in megaloblastic anaemia. There is decrease in the thrombocyte and leucocyte count and the erythrocytes exhibit various shapes and size. The **anisocytosis** and

**poikilocytosis** is usually associated with **ovalocytosis** and with the presence of nucleus in macrocytes. The presence of six or more lobed neutrophils found in blood examination is commonly sufficient to identify the megaloblastic anaemia. The bone marrow is hypercellular. Erythrocyte precursors are very large. The ineffectiveness of the erythropoiesis is manifested by possible destruction even of 90 per cent of erythrocyte precursors in bone marrow. Abnormal mitoses and giant metamyelocytes occur. Megakaryocyte number is reduced and they have abnormal shape.

The identification of the actual pathogenesis of megaloblastic anaemia requires the serum cobalamin level determination, the GIT investigation and microscopic examination of bone marrow.

## 2.2.4 Haemolytic anaemias

**The haemolytic anaemia is due to the increased erythrocyte destruction.** The bone marrow is capable to increase the erythrocyte production even up to the eight fold values. If anaemia appears during increased erythrocyte destruction it is the sign that the compensatory capacity of bone marrow has been exceeded. Under physiological circumstances the enzymic activity in erythrocytes falls progressively and they become not easily deformable. The erythrocytes fulfil their functions in organism 120 days in average.

Their life-span is substantially shortened in haemolytic anaemia. It can last only several days or even several hours.

From pathophysiological point of view haemolytic anaemias may be caused by

- abnormalities of erythrocyte membrane
- enzymic deviations
- haemoglobin alterations
- interaction of erythrocyte membranes with antibodies
- effects of toxins or microbial products
- high temperature or mechanical injury

In haemolytic anaemia erythrocytes with normal or abnormal shape may occur. **The reticulocyte number** in peripheral blood is elevated. Erythrocytes with the rests of nuclei may occur. Bilirubin

and LDH are increased in plasma so is the urobilinogen in urine. The life-span of erythrocytes in peripheral blood is significantly shortened. Splenomegaly and hepatomegaly and cholelithiasis are frequently present in chronic haemolytic anaemia. The skin is icteric and pale. Erythroid hyperplasia is present in the bone marrow.

The haemolysis can be extra- or intra-vascular. Haemolysis occurring in the reticuloendothelial system is termed extravascular haemolysis.

### 2.2.4.1 Intravascular haemolysis

**The intravascular haemolysis appears following transfusion, in patients with artificial valve, or as a result of paroxysmal cold or nocturnal haemoglobinuria, or as cold-agglutinins or by clostridial infection induced haemolysis.** Haemoglobin is actually released from intact erythrocytes in these cases. The released haemoglobin is bound to haptoglobin, haemopexin, or to albumin in blood plasma. The uptake of haptoglobin-haemoglobin and haemopexin-haemoglobin complexes runs rapidly in the liver. The haem-albumin complexes (methaemalbumin) circulate in the blood during some days. If the binding capacity of plasmatic proteins is exceeded, haemoglobinuria appears. The haemoglobinuria itself does not damage kidneys, nevertheless, other components (stroma) of disturbed erythrocytes exert a very unfavorable effect on kidneys. The accumulation of "these rests" of erythrocytes in kidneys can induce acute renal failure. Chronic haemoglobinuria results in iron deficiency. Renal tubules participate in this process. During haemoglobinuria the tubular cells become destructed because they are "overfilled" with haemosiderin originating from iron absorbed from haem. There is a significant fall of plasmatic haptoglobin level and elevation of lactate dehydrogenase (LDH) values, originating from haemolysed erythrocytes in acute intravascular haemolysis.

### 2.2.4.2 Extravascular haemolysis

The extravascular haemolysis represents a premature elimination of erythrocytes from circulation. Erythrocytes are sequestered by fixed phagocytes in spleen and in liver. Only a very little amount of haemoglobin gets into the plasma. The iron recirculates and is used for further erythropoiesis. During the haemolysis its plasmatic level rises, the hap-

toglobulin level falls, but nevertheless haemoglobinuria is not present. The values of LDH, originating from destructed erythrocytes, are elevated. The level of conjugated bilirubin depends on haemolysis and the functional capacity of liver. During noneffective erythropoiesis the intramedullar haemolysis appears being also an extravascular haemolysis.

### 2.2.4.3 Underlying causes of haemolytic anaemias

**The causes leading to haemolytic anaemias can be various.** To understand the etiopathogenesis of haemolytic anaemias a classification according to the underlying cause is needed. The two basic groups of haemolytic anaemias in this classification form the inborn and the acquired haemolytic anaemias. The underlying cause may be the abnormalities of erythrocytes or the alterations in plasma, or in circulation. The factors participating in the etiopathogenesis may be various: antibodies, mechanical injury or infections or toxic substances. According to the disease course and duration the haemolytic anaemia may be acute or chronic. The antibodies can be localized at the erythrocyte surface or they can be present in the plasma.

Haemolysis of erythrocytes can appear owing to the **intracorpuseular abnormalities**. There are frequently defects of erythrocyte membrane, of haemoglobin, or of enzymes. Sclerocytosis is the manifestation of the most common **membrane defect**. Erythrocytes with spheric shape are present in the peripheral blood. The mean haemoglobin content in this type of erythrocytes is elevated. The osmotic resistance is decreased and the life-span of erythrocytes in the circulating blood is shortened. The splenomegaly, bilirubin concrements in the gallbladder are present and intermittent jaundice often occurs. Icterus appears especially following stress, infections and pregnancy. During infections the aplastic crisis may appear. It is usually due to the bone marrow depression, and to the enhanced haemolysis of spherocytes. The basis of the haemolysis is the sequestration of erythrocytes with reduced deformability. These erythrocytes require extremely high ATP supply to maintain the membrane flexibility. The molecular basis of the spheric shape of erythrocytes is not understood precisely. A complex of several causes is probably involved. The most important are those concerning the proteins forming the cy-

toskeleton and the alterations of spectrin. The spectrin deficiency correlates usually with the severity of anaemia. A structural anomaly of ankyrin may be sometimes present. In addition to this, the sodium permeability of spherocytes is enhanced and the requirement of ATP supply is increased to ensure the cation pumps functioning. If not, water accumulation in the cell appears.

**Hereditary spherocytosis** is an autosomal dominant inborn disorder characterized by production of abnormal erythrocytes which are precociously destroyed in the unaltered spleen. Clinical manifestations of the hereditary spherocytosis are the anaemia, splenomegaly and jaundice. This condition was formerly termed the inborn haemolytic icterus. It is impossible to prevent the enhanced haemolysis of spherocytes. The splenectomy – removal of the organ, where the erythrocytes sequestration occurs the most intensively, may be the only way of the effective treatment.

There exist similar membrane abnormalities, with occurrence of eliptocytes or stomatocytes in the peripheral blood. The stomatocytes have atypical darker central area. They occur also in hereditary spherocytosis and can be observed in blood smears in alcoholism, in hepatic cirrhosis and sodium pump defects. Some drugs and considerable pH change can induce a similar deformation of normal erythrocytes. The hereditary spherocytosis should be distinguished from the spherocytosis in haemolytic anaemia, which is due to antibodies.

The most common enzymic disturbance is the **glucose 6-phosphate dehydrogenase (G6PD) deficiency**. There are also other enzymic defects but their occurrence is very uncommon (pyruvate kinase and hexokinase deficiency). Normal erythrocytes use the glucose to form ATP and 2,3-diphosphoglycerate (2,3-DPG). ATP is necessary for maintenance of the membrane flexibility and the cation pumps functioning. 2,3-DPG influences the oxygen binding with haemoglobin and especially the oxygen uptake in tissues. About 10 per cent of glucose is metabolized in the hexose monophosphat shunt where NADPH is formed. NADPH is necessary for regeneration of glutathione protecting the haemoglobin against denaturation, and the sulphhydryl groups in cellular membranes. So the integrity of cellular membrane is maintained. NADPH detoxicates also the hydrogen peroxide and oxygen radicals. G6PD deficiency leads

to oxidative impairment of erythrocytes. There is an inherited disturbance involved having several forms. The variant occurring in blacks is milder. Another variant the so-called mediterranean (occurring in the region of Mediterian Sea) is a condition with very low values of G6PD, reaching only 5 per cents of normal values. G6PD deficiency is the cause of the haemolysis during hepatitis, during high intake of vicia faba or of drugs with oxidative effects. A further form of G6PD deficiency is observed in some intestinal disorders associated with an increase in number of bacteria and in uraemia.

## 2.2.5 Acquired haemolytic disorders

The life-span of erythrocytes may be shortened in infectious diseases, in malignant processes, in cardiovascular and immune disorders. The cause of haemolysis is the most frequently apart from erythrocytes themselves. The acquired haemolytic anaemias can be classified into two categories according to the positivity or negativity of the Coomb's test.

### 2.2.5.1 Anaemia mediated by antibodies

This group comprises conditions where the **antibodies are directed straight against the antigens of erythrocytes** and are directly or indirectly responsible for the haemolysis. The antibodies formed following the blood transfusion or by sensitization to "foreign" cells during pregnancy are termed alloantibodies. The antibodies formed without the sensitization are the autoantibodies representing the deviations of the immunity regulation itself. Presence of the antibodies at the erythrocyte surface decreases their flexibility and make them more sensitive to the phagocytosis performed by macrophages in the spleen and the liver. Macrophages destroy and "engulf" the portion of membrane covered with antibodies or the complement. Erythrocytes which have lost a part of their membrane become spherical, their flexibility is decreased and their life-span in the circulating blood is shortened.

**Autoimmune haemolytic anaemias (AIHA)** are characterized by antibodies against the own antigens of erythrocytes. Autoantibodies arise spontaneously or as response to a situation where an happen (often a drug) is present. A manifestation of a systemic disease is involved about in 40 per cent of cases. Diseases and disorders of immune system

like collagen-vascular diseases, chronic inflammatory processes of intestine, chronic lymphatic leukaemia or lymphomas are frequently accompanied with autoimmune haemolytic anaemias. Autoantibodies increase with age. Haemolytic anaemia need not to be always present. The haemolysis itself does not depend on the presence of antibodies. A complex of factors as density of antibodies at the erythrocyte surface, implication of complement and tendency to phagocytize the erythrocytes by macrophages in the spleen are involved in these processes. Autoimmune haemolytic anaemias (AIHA) can be divided into two groups:

1. the warm-antibody induced haemolytic anaemias,
2. the cold-antibody induced haemolytic anaemias.

### 2.2.5.2 Warm-antibody induced haemolytic anaemias

**These conditions are mediated by IgG antibodies bound with erythrocytes at normal body temperature.** They can fixe also the complement. This group of anaemias is usually associated with collagenoses, lymphomas, it occurs in chronic lymphocytic leukaemia, in ovarial teratomas or in colitis ulcerosa. More than one half of cases are of unknown origin, nevertheless the **idiopathic haemolytic anaemia** can be a sign of later appearing systemic diseases. The clinical manifestation of anaemia may be various. It can be a form manifested by moderate asymptomatic jaundice and splenomegaly. The haemolysis is very prominent and the signs of erythrocyte loss compensation by bone marrow can be observed in severe cases. If the level of antibodies is high they are detectable by positive indirect Coomb's test in plasma. Coomb's indirect and direct antiglobulin test is used to detect the antierythrocytic antibodies or other plasmatic proteins participating in the erythrocyte agglutination. The agglutination is used as evidence of the antibody presence at the surface of erythrocytes. There are components of complement C3b and C3d.

**Thrombocytopenia** is often observed in AIHA arising also on immune basis. The number of reticulocytes in peripheral blood is usually increased. The haptoglobin level in serum is commonly low. Spherocytes are present sometimes in peripheral blood. Their number is variable. Bilirubin level raises.

The possible therapeutic intervention is to influence the binding and the production of antibodies. The splenectomy is an extreme solution. Immunosuppressive drugs may be useful in refractory forms of disease. The blood transfusion is inefficient because the transfused blood corpuscles are more rapidly destroyed than the own erythrocytes of the patient. The treatment with steroids reduces the antibody binding with the fixed macrophages and thereby the rate of erythrocyte destruction becomes decreased.

### 2.2.5.3 Cold-antibody induced haemolytic anaemias

**Cold agglutinins** inducing the haemolytic anaemias have a large temperature range. They are bound with erythrocytes at the temperature up 4°C to about 30 to 32°C. This temperature (30 to 32°C) occurs in acral parts of organism during exposure to moderate cold. In low temperature reacting IgM antibodies may be produced in organism during the infectious mononucleosis, cytomegaloviral infections and mycoplasmal pneumonia or during protozoal infections. The produced antibodies may survive in organism days and weeks.

An independent **idiopathic by cold agglutinin induced syndrome** is characterized by the presence of monoclonal IgM antibodies. It can precede the Waldenstrom's macroglobulinaemia, but it can persist years without malignant transformation. The polyclonal cold antibodies IgM are formed immediately after the infection. They may not persist long time in organism, in the contrary to the monoclonal cold-antibodies IgM persisting years in organism. Cold-IgM antibodies are bound with the erythrocytes during the organism exposure to cold. They fix the complement at the surface of erythrocytes. When the erythrocytes become rewarmed, the antibodies dissociate and only the activated complement remains at the erythrocyte surface. The fixed C3b component of the complement is sufficient to make erythrocyte being removed by macrophages in the spleen. The C3 component fixed to the erythrocyte membrane is converted into the inactive C3d form. This influence the further binding or activation of C3. These processes are responsible for the rate of haemolysis.

The patients with haemolytic anaemia induced by cold-antibodies have various kinds of pains. Their acral parts become blueish if exposed to cold – what

is called acrocyanosis. This condition can be positively influenced by minimalization of the exposure to the cold.

### 2.2.5.4 Paroxysmal cold haemoglobinuria

It is an infrequent condition. **Under the influence of cold the polyclonal IgM antibodies are bound with erythrocytes fixing the complement.** During the re-warming the complement cascade becomes activated and a very intense intravascular haemolysis arises. This condition is associated with syphilis or with viral infections.

### 2.2.5.5 Drug-induced haemolytic anaemias

Are relatively common conditions. According to the type of haemolysis three forms can be distinguished:

#### 1. *The haptent type*

**The drug binds with the erythrocyte membrane forming a neoantigen against which antibodies are produced.** The presence of the drug is needed for arising haemolysis. Examples of haptent drugs are most commonly penicillins and cephalosporines.

#### 2. *The "innocent" harmless type*

**The drugs binding to the plasmatic proteins stimulate the antibodies forming the immune complexes and activate the complement.** To the erythrocytes as with – the innocent passangers – the complement is bound. These erythrocytes can haemolyze intra- or extravascularly. The rate of haemolysis depends on the speed of complement activation and inactivation. At the erythrocyte surface is only the complement present. This type of haemolysis occurs following sulfonamide-, phenothiazid-, quinine- and isoniazid- treatment.

#### 3. *The alpha-methyldopa type*

Haemolytic anaemia develops **in chronic treatment with alpha-methyldopa.** Alpha-methyldopa probably modifies the antigen system of erythrocytes. Levodopa and antiinflammatory drugs may also induce a similar type of haemolysis.

### 2.2.5.6 Hypersplenism

This term designates a condition with massive sequestration of erythrocytes, leucocytes and thrombocytes in abnormally functioning spleen. Under pathological circumstances the aging erythrocytes are taken up by spleen in its convoluted poorly oxygenated sinusoids. In hypersplenism **young active erythrocytes are taken up by the spleen** and so are unaltered leucocytes and thrombocytes. Therefore pancytopenia appears, however also bicytopenia – the decrease of two types of blood cells, or only of a single type of blood cells may be present. Hypersplenism is characterized by the presence of reduced number of blood elements in the circulating blood and of a large number of them in the bone marrow.

Decreased number of a certain type of blood elements in peripheral blood is associated with compensatory hyperplastic haemopoiesis in the given line of differentiation and with consequent disorder of maturation. Hypersplenism may develop in chronic haemolytic anaemias due to whatever causative factor, during chronic infections, leukaemias and myeloproliferative diseases, lymphomas, rheumatoid arthritis and in lipid metabolism disturbances.

### 2.2.5.7 Paroxysmal nocturnal haemoglobinuria

This condition is an acquired disorder associated with enhanced sensitivity of erythrocytes to complement – mediated impairment. Also the precursors of erythrocytes may be involved. This condition occurs in bone marrow aplasia and leukaemia. Apart from erythrocytes also the granulocytes and thrombocytes exhibit enhanced sensitivity to the lytic effect of the complement. The binding of the complement component C3 to erythrocytes and granulocytes is increased. The paroxysmal nocturnal haemoglobinuria may occur also independently, not only in bone marrow aplasia or leukaemia. It can result in leukaemia. In the bone marrow is usually a compensatory hypercellularity found. Iron deficiency is uncommon.

### 2.2.5.8 Haemolytic anaemias due to chemical substances, toxins and parasites

The underlying cause of haemolytic anaemia can be caused by various anorganic substances and complexes of organic compounds, toxins, venoms and

heavy metals. Arsenic and copper bind to sulphhydryl groups of various endogenous compounds (e.g. glutathion, coenzyme A etc.). The venoms (snake venom) contain lysolecithinase inducing the lysis of erythrocytes destroying directly their membranes. In patients with advanced hepatic cirrhosis the haemolytic anaemias may develop because the erythrocyte membranes absorb an immense amount of cholesterol. The most common causative factor of parasitic diseases it is malaria.

### 2.2.5.9 Haemolytic anaemias induced by trauma of erythrocytes

**During extracorporeal circulation** the erythrocytes may be mechanically impaired. This is the so-called fragmentation haemolysis. The plastic heart valves or vascular shunts can be in the development of haemolytic anaemia implicated. Fragments of erythrocytes are found in peripheral blood. The remaining erythrocytes are small spherical microspherocytes with increased polychromia. The level of plasmatic haptoglobin is decreased and the values of bilirubin and of LDH are elevated. If the conditions duration is very long, iron deficiency can develop owing to the haemosiderinuria. The erythrocyte fragmentation arises also **in extensive burns**. Intravascular haemolysis without fragmentation of erythrocytes arises following extreme physical exertion. It is commonly described in Marathon runners and during or after long-lasting marches.

### 2.2.5.10 Microangiopathic haemolytic anaemia

This condition occurs when fibrin is formed inside the vessels and the small vessels are partially occluded by microthrombi. Normal erythrocytes are fragmented in this condition. The microangiopathic haemolytic anaemia develops during the disseminated intravascular coagulation (DIC), in thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome, in malignant hypertension and in transplant rejection. The thrombocytopenia is present owing to the simultaneously occurring intravascular coagulation in these conditions. The thrombotic condition occurring in malignant tumors (particularly in the mucous-producing adenocarcinomas of GIT) associated with the occurrence of thrombosis is termed Trousseau's syndrome. DIC may be only one of the

haemostasis alterations which are their paraneoplastic manifestation.

### 2.2.6 Haemoglobinopathies

Haemoglobin is an allosteric protein responsible for the O<sub>2</sub>, CO<sub>2</sub> and H<sup>+</sup> transport. It is the substantial protein of erythrocytes. Haemoglobin is comprised of four polypeptide chains containing more than hundred aminoacids. The iron present in the haem group is responsible for the reaction with the oxygen. The chains are denoted as alpha, beta and delta chains. The haemoglobin of healthy adults (adult haemoglobin) contains two alpha and two non-alpha chains (beta or delta). The basic configuration of chains is linear, the alpha chain contains 141 and the remaining chains 146 aminoacids. One portion of haemoglobin forms in the chain alpha helix, another portion is three dimensional and the further part is of tetrahedral shape.

**The haemoglobin molecule operates as an important component of buffer system.** The imidazole group at the histidine operates as acceptor of protons with dissociation constant within the physiological range of the blood pH. Haemoglobin exhibits a large buffering capacity (about 53 mmol/l) what enables to minimize the pH value changes in blood during the O<sub>2</sub> binding and CO<sub>2</sub> release in lungs, and vice versa in tissues.

The oxygen saturation of haemoglobin falls from 100 to 75 per cent during **the oxygen transport** from the lungs (pO<sub>2</sub> 100 mmHg) into the tissues (pO<sub>2</sub> 40 mmHg). The oxygen release is enhanced by 2,3-diphosphoglycerol acid (2,3-DPG) binding with haemoglobin. 2,3-DPG is a product of glycolysis. The pH changes and CO binding also participate in oxygen release from haemoglobin. Structural changes of globin may be the cause of altered haemoglobin affinity for oxygen molecule.

**Mutations in DNA sequences**, controlling the globin synthesis, may produce abnormal haemoglobins (the haemoglobinopathies), or cause a decrease of the haemoglobin synthesis rate (thalassemia). The term haemoglobinopathy is used for structural anomalies of haemoglobin associated with its altered function. Thalassemia represents a mutation resulting in decreased synthesis of haemoglobin of a certain type. Many similar mutations may occur, however, only some of them are the underlying cause of altered haemoglobin function concerning the oxy-

gen transport or anaemia. The mutations concerning haemoglobin function may be manifested by impairment of its solubility, by instability or by changes in affinity to oxygen. The impaired globin synthesis is secondarily connected with disorders in haem synthesis leading usually to haemolytic anaemia.

The altered structure is accompanied with abnormal haemoglobin function.

#### 2.2.6.1 Sickle cell anaemia (drepanocytosis)

**This condition is a single base mutation of DNA molecule leading to the substitution of valine for glutamic acid.** This type of haemoglobin exhibits decreased solubility. If the HbS is less saturated with oxygen it polymerates in erythrocytes (drepanocytes). The alterations arise in the membrane of erythrocytes, cellular dehydration develops and their deformability becomes decreased. The altered erythrocytes may cause occlusion in the microcirculation resulting in microinfarctions of vital organs. **Crises arise** characterized by bone pains, spleen autoinfarctions and renal damage. HbS gene is detected in about 10 per cent of American blacks and in 25 per cent blacks in Africa. The only advantage of this hemoglobinopathy is, that the patients with this condition are resistant against malaria caused by *Plasmodium falciparum*. 90 per cent of haemoglobin in homozygotes is HbS, the remainder is HbF or HbA<sub>2</sub>. This condition is clinically manifested already early, after the birth, when the HbF begins to decrease physiologically. Main symptoms are **oedemas of extremities, splenomegaly, infarctions of lungs and bone marrow and cerebrovascular complications**. Further organs may be also impaired. Intrahepatic disturbance of microcirculation may be the underlying cause of hepatic dysfunction and of hyperbilirubinaemia. The intrarenal disturbance of microcirculation may lead to papillary necrosis and haematuria accompanied with decrease in renal concentration capacity. Impairment of skin microcirculation is the underlying cause of necrotic ulcerations, localized most commonly around the ankles. Severe retinal alterations may occur. Microinfarctions in the bone marrow and in bones are commonly leading to an aseptic necrosis forming favorable conditions for osteomyelitis development. Growth disturbances are observed. The extremities are long and the habitus is asthenic. The chronic haemolysis may be the underlying cause of frequently occurring

cholelithiasis with bilirubin concrements. There is a tendency to folic acid deficiency, to impairment of immunity partially due to the splenic tissue loss and to decreased IgM production. Infections and cardiomyopathy occur frequently in these disorders.

In sickle cell anaemia the haemoglobin values about 6 to 8 g/dl, the haematocrit is usually only 18–24 per cent. The number of reticulocytes used to be elevated above 20 per cent. Erythrocytes containing nuclei are present in the peripheral blood. On the blood smear sickle shaped erythrocytes are found. Their number can be more precisely determined on wet smear under anaerobic conditions. Precise identification may be performed by using the haemoglobin electrophoresis. The contribution of HbS in sickle cell anaemia reaches 90 to 95 per cent. The plasmatic level of direct reacting bilirubin raises, the haptoglobin in serum is decreased, the values of LDH are elevated. Evidence of sickle cells in the peripheral blood can be performed very easily. To the patient's blood sample sodium metabisulphid is added desoxygenating the erythrocytes. These erythrocytes become typically shaped – the haemoglobin is localized excentrically in form of a stripe.

There are also mild forms termed sickle cell syndromes where the participation of HbS in the entire haemoglobin content is less than 80 per cent. The symptoms worsen especially during the pregnancy.

The pathogenesis of sickle cell anaemia is unknown and the treatment can be only symptomatic. During the crisis the acidosis may be corrigated, oxygen and analgetics applied. The microinfarctions cause the fever. The blood transfusion is dangerous because of the iron content elevation above the limit with all consequences. Oxygenation and avoiding the cold are recommended. During the pregnancy the placental infarctions with following placental dysfunction are the most severe complications.

### 2.2.6.2 Unstable haemoglobins

They represent autosomal dominant mutants with impaired binding of the haem to the globin. This mutation results in intracellular haemoglobin precipitation in form of Heinz bodies binding with the membrane. These cells are rapidly taken up by the mononuclearphagocytic system. Erythrocytes with this type of haemoglobin have a shorter life-span. Impairments of these conditions appear in form of

crises manifested by acute haemolysis and jaundice.

### 2.2.6.3 Haemoglobins with impaired oxygen affinity

The capacity of haemoglobin to saturation by oxygen is an appropriate indicator of oxygen affinity. Under physiological circumstances is the HbA saturated by oxygen to 50 per cent, if the partial pressure is about 25 mm Hg. The enhancement as well as the fall of the affinity is unfavorable. The haemoglobins having a higher affinity to oxygen are usually associated with erythrocytosis and with tendency to thrombotic disorders. The haemoglobins with low affinity to oxygen use to be connected with anaemias.

### 2.2.6.4 Methaemoglobinaemia

**Methaemoglobin is haemoglobin with oxidated iron in the haem part of haemoglobin molecule. Instead of  $Fe^{2+}$  it contains  $Fe^{3+}$ .** Methaemoglobin is unable to transfer the oxygen and thus completely useless for respiration, (resp. for oxygen transport). The underlying cause can be the enzyme defficiency (e.g. NADH-methaemoglobin reductase) or the effect of some chemical substances and drugs. The nitro- and amino- derivates of benzene or nitrates are frequently involved. These substances cause the haemoglobin oxidation by molecular oxygen during which the iron remains in form of  $Fe^{3+}$ . Hence, it can not bind reversibly the oxygen and transport it. Especially the nitrates are very dangerous in children. The methaemoglobin presence changes the colour of blood into dark brown. If the methaemoglobin amount in peripheral blood reaches 1,5 g/100 ml the cyanosis appears. Under physiological circumstances is about 1 per cent of methaemoglobin present in healthy subject.

### 2.2.6.5 Thalassaemia

**It comprises a group of inherited haemoglobinopathies characterized by quantitative fall of production or by complete absence of normal globin chain.** Under normal circumstances haemoglobin contains  $\alpha$  or non  $\alpha$  ( $\alpha 2$ ,  $\beta 2$ , or  $\alpha 2$ ,  $\chi 2$ ) chains. Clinically important are cases with impaired  $\alpha$  or  $\beta$  chain production. According to this is distinguished  $\alpha$  thalassaemia or  $\beta$  thalassaemia. The defect in haemoglobin synthesis can be of various degree. Therefore the hidden carriers exist (heterozygotes for  $\alpha$  thalassaemia) up to

very severe anaemia – thalassaemia major (homozygotes for  $\beta$  thalassaemia).

In  $\beta$  thalassaemia there is a deficiency or complete absence of  $\beta$  chain. It is classified according to the  $\beta$  chain suppression (thalassaemia major, minor, intermedia,  $\delta$ - $\beta$ ,  $\chi$  and Lepore thalassaemia).

**Thalassaemia major** (homozygous) is termed Cooley's anaemia. The absence or an considerably reduced number of  $\beta$  chains is associated with overproduction of  $\alpha$  or  $\chi$  chains. In peripheral blood abnormal findings are present: microcytosis, extreme poikilocytosis, target cells and ovalocytosis, Cubot's ring bodies, Howell-Jolly bodies, nuclear fragments, siderocytes, anisochromia and anisocytosis. These anomalies reflect a very severe anaemia associated with ineffective erythropoiesis. There is usually outstanding bone marrow-, spleen- and liverhypertrophy. With the alterations of organs are abnormalities of bones, fractures and mongoloid face associated. There has been a hope to save the patient by repeated blood transfusions, however this treatment is accompanied with troubles induced by action of accumulated iron.

**Thalassaemia intermedia** is a quantitative less severe disturbance than the previous type. The haemoglobin concentration reaches 6 to 10 g/100 ml. Without being stressed the organism can prosper during long time without the crisis and inevitable transfusion.

**Thalassaemia minor** with mutation responsible for the globulin  $\beta$  chain synthesis occurs in heterozygotes. Microcytic, moderately hypochromic anaemia with elliptocytosis prevails. The bone marrow is lightly hyperplastic. The HbF content reaches 5 to 20 per cent of the total haemoglobin content.

$\delta$ - $\beta$  thalassaemia is a variant of  $\beta$  thalassaemia. There is a combination of  $\beta$  and  $\kappa$  chain synthesis.

$\kappa$  thalassaemia is manifested by hyperchromic microcytic anaemia. Decrease or complete absence of chain is associated with  $\beta$  chain hyperproduction. This condition in homozygotes is incompatible with life.

Lepore thalassaemia is a variant of  $\beta$  thalassaemia. Three haemoglobin variants, being a combination of  $\delta$  and  $\beta$  chain disorders, has been described in this syndrome.

**$\alpha$  thalassaemia.** In this type of thalassaemia there is a molecular defect concerning  $\alpha$  globin gene deletion. Several alleles from two ones for  $\beta$  chain pro-

duction and four alleles responsible for its regulation may be absent. The clinical picture reflects these disturbances.

Silent  $\alpha$  thalassaemia is usually an asymptomatic form. The heterozygous  $\alpha$  thalassaemia is manifested by microcytic hypochromic anaemia of medium degree. HbH disease is a severe haemolytic anaemia compatible with life. It is characterized by hypochromic microcytic anaemia with fragmentation of erythrocytes. Haemoglobin H contains four  $\beta$  chains and is detectable by electrophoresis. It represents 5 to 30 per cent of the global haemoglobin. The  $\alpha$  chain synthesis is depressed, the haemoglobin not effective in oxygen transport. In the homozygous  $\alpha$  thalassaemia only  $\chi$  chains are formed. No HbA is produced. The  $\chi$  chains are the cause of haemoglobin incapability to transport oxygen. The fetus dies before the birth.

### 2.2.7 Normochromic normocytic anaemia

The mean volume of erythrocytes and the mean haemoglobin content in erythrocytes (HbEry) is normal in normocytic normochromic anaemias. This type of anaemia **occurs during many diseases**, especially in systemic diseases. Anaemia is frequently the first finding in correct enddiagnosis together with revealing the underlying causes of anaemia itself. The causes are usually considerably various. In spite of this the fundamental question is the fact if the bone marrow is or is not responsible for anaemia. Normal bone marrow is capable to enhance the erythropoiesis eightfold. This erythropoiesis enhancement is always associated with the finding of reticulocytosis in the peripheral blood. Providing that the reticulocytosis is outstanding the mean volume of erythrocytes may be larger owing to the accelerated erythropoiesis. It is due to the presence of young erythrocytes having larger proportions. The reticulocytosis is manifestation of bone marrow response to acut haemorrhagia or haemolysis.

In case that in existing anaemia no signs of accelerated erythropoiesis are found the disorder may be directly or indirectly in bone marrow. The suspicion of bone marrow disturbance may be declared when the leukopenia, thrombocytopenia or morphologic alterations of erythrocytes are observed simultaneous. The morphologic abnormalities of erythrocytes are

the erythrocytes with nuclei, poikilocytes, immature granulocytes, fragments of megakaryocytes or large thrombocytes. Renal, hepatic and endocrine diseases may influence the erythropoiesis and reduce the erythropoietin production.

### 2.2.7.1 Acute posthaemorrhagic anaemia

**It occurs following loss of large blood volume.** Clinical signs of anaemia depends on the volume of blood lost, on the bleeding rate and on the bleeding duration.

**The first phase lasts by three days.** In this phase period the blood loss regarding the volume dominates. The clinical picture depends on the fact whether the hypovolaemia induced by the blood loss persists. In this case the anaemia is not detectable by the haematocrit and haemoglobin determination. If the hypovolemia becomes to be compensated anaemia and often also reticulocytosis appear.

If the blood volume is reduced by 20 per cent, no symptoms may appear. In case that the blood loss reaches 30 to 40 per cent of the total blood volume, the haemorrhagic shock develops. During acute blood loss is not possible to estimate its extent by haematocrit – and haemoglobin values. The volume of plasma may be compensated by endogenous mechanisms in 24 hrs. The compensation is attained above all by the shift of electrolytes and water from tissues into the intravascular space. Anaemia appears only as late as **the dilution of plasma by compensation is attained.** The erythropoietin secretion rises immediately with plasma dilution and anaemia appearing. The hyperplasia of bone marrow can be observed later.

**The reticulocytosis** occurs on 3rd or 5th day following the haemorrhagia. Reticulocytosis reaches the highest values on the sixth to eleventh day. The degree of reticulocytosis is in certain relation with the volume of blood loss. Infrequently however, it exceeds 14 per cent. Transient increase in mean volume of erythrocytes can be observed. During some hours following the haemorrhage a dramatical increase in **thrombocyte- and leucocyte- number occurs.** In extreme situation the thrombocytes may rise in 1 to 2 h up to  $1000 \times 10^9/l$  and the leucocytes up to 25 to  $35 \times 10^9/l$ . The number of leucocytes increases during 2 to 5 h after the haemorrhage. In about 3 to 5 days the values of thrombocytes and leucocytes return to the norm.

### 2.2.8 Anaemia in uraemic syndrome

Anaemia occurs frequently during the uraemic syndrome. The haemoglobin level uses to be variable and the degree of anaemia is usually proportional to the degree of azotaemia. The haemoglobin level reaches in severe cases only 40 g/l. In spite of this, the patients tolerate this anaemia rather passably owing the blood redistribution and affinity for the oxygen.

Anaemia in uraemia is usually normochromic and normocytic. The process of erythrocyte forming is usually not impaired and also the morphology of erythrocytes uses to be normal. On blood smear echinocytes – erythrocytes with prominent edged surface are present. The reticulocyte number is decreased. **The basis of anaemia is most probably the reduced erythropoiesis.** Renal damage is the cause of reduced production of erythrocytes and in erythropoietin production. In patients is the erythropoietin level always decreased. Except for this the retained substance in uraemia cause the depression of erythropoiesis. The iron incorporation into the erythrocytes is usually also impaired.

In uremic patients in advanced of disease **haemolytic attack** may occur. Several factors can support the haemolysis. Renal failure can be the consequence of thrombotic thrombocytopenic purpura or of the haemolytic uraemic syndrome. An intense microangiopathic haemolytic anaemia may develop in these cases. In some patients the anaemia may becomes worsen also by water contamination with aluminium. The impairment is manifested in these cases by microcytosis and hypochromia. Renal transplantation causes dramatical reverse and so does the erythropoietin application.

### 2.2.9 Anaemia in hepatic cirrhosis

Liver cirrhosis and other hepatic diseases are frequently accompanied with anaemia, usually of normocytic normochromic type. Macrocytic anaemia occurs infrequently. In liver cirrhosis the underlying cause of anaemia is often the chronic alcoholism influencing the haemopoiesis directly or indirectly. Important factors contributing to anaemia development are: the iron deficiency due to the blood loss in gastritis, peptic ulcer, oesophageal varices, in deficiency of coagulating factors, sequestration of erythrocytes and other blood elements in the enlarged

spleen due to portal hypertension. In cirrhosis often occurs and due to it the lipid content in membrane of erythrocytes increases leading to their premature sequestration in the spleen.

### 2.2.10 Anaemias in endocrine disorders

It is generally known that the thyroxin, glucocorticoids, testosterone and growth hormon influence the proliferation of erythroid cells in vitro. It is not surprising therefore that in endocrine diseases moderate to severe normocytic normochromic anaemia occur. Anaemia occurs most frequently in hypothyroidism, Addison's disease, hypogonadism and in panhypopituitarism. It is understandable, that the anaemias associated with hypothyroidism and panhypopituitarism are in relation with reduced oxygen consumption due to the  $T_3$  and  $T_4$  deficiency, or to the absence of growth hormone.

The anaemia in myxedema is usually normocytic. In some patients anaemia is due to the folic acid – or  $B_{12}$  vitamin deficiency. In patients with hypothyroidism and especially in women with metrorrhagia the microcytic anaemia and iron deficiency may develop. In hypothyroidism hidden anaemia can occur owing to the decreased volume of plasma of these patients causing an increase in erythrocytic mass and in the erythrocyte number in volume unit. The symptoms of myxedem may be inaparent and to make the correct diagnosis is not easy.

In Addison's disease is anaemia also masked by reduced volume of plasma. Even untreated patients have the haemoglobin level 130 g/l. The anaemias become revealed at the beginning of hormonal treatment because the volume of plasma increases. Anaemia, however recovers later spontaneously.

In adolescence the testosterone effect is manifested by increase in haemoglobin values. In eunuchs the haemoglobin level is lower. The hypophysis dysfunction is usually associated with normochromic, normocytic anaemia, occasionally accompanied with leukopenia.

### 2.2.11 Anaemias in chronic inflammatory processes

In patients with chronic systemic inflammatory diseases anaemia can develop in several months. The

severity of anaemia is proportional to the severity of inflammatory process. Anaemia occurs usually in chronic infections like endocarditis, osteomyelitis, pulmonary abscess, tuberculosis and pyelonephritis. The occurrence of anaemia associated with non infectious diseases appears in chronic inflammatory process due to rheumatic arthritis, lupus erythematosus, vasculitis, sarcoidosis, regional enteritis and in tissue damage by injury like the fractures of long bones.

This type of anaemia occurs frequently **in neoplastic processes** e.g. Hodgkin's disease, lung carcinoma and breast carcinoma. More severe anaemia develops in patients with cancer of gastrointestinal or urogenital system, where the blood loss contributes to the anaemia appearance. Iron deficiency is developing thus anaemia may progress rapidly. When metastases in bones arise invading the bone marrow a type of anaemia with very rapid progression develops.

The anaemias mentioned above are characterized by decrease in haemoglobin values, moderate microcytosis and normochromia. In bone marrow examination normal maturation of erythroids is found. During chronic infections the **myeloid hypoplasia** develops. The number of reticulocytes in peripheral blood is usually decreased. Extracorporeal mechanisms are probably involved in the etiopathogenesis of anaemia. It is known that enhanced haemolysis is present in mononuclear phagocytic system. In some diseases is the life-span of erythrocytes evidently shortened e.g. in chronic infections, especially in infective endocarditis, miliary tuberculosis, and in splenomegaly. Spherocytosis is observed frequently in these cases. The iron and ferritin levels in serum are usually lowered. Values of other plasmatic proteins are elevated in inflammatory processes probably owing to stimulations with interleukins being released from activated macrophages. The picture of this inflammatory process is completed by increase in gammaglobulin level, C3 component of complement, haptoglobin,  $\alpha$  1 antitrypsin, orosomucoid and fibrinogen. This increase of proteins is the underlying cause of elevated sedimentation of erythrocytes used in practice as indicator of inflammatory process.

The primary cause of anaemias in chronic inflammatory processes is **the defective production of erythrocytes and their reduced life-span**. There is a discrete disturbance of iron transfer in erythrocyte production, manifested finally by smaller erythrocytes and impaired haem synthesis. Hyperplasia

sia of mononuclear phagocytes is responsible for the shorter life-span of erythrocytes. The macrocytes catch the iron from haemoglobin, hence it cannot be transported into the bone marrow. The aggressivity of macrophages towards the iron is caused by interleukin 1. The interleukin releases lactoferrin from neutrophils. Lactoferrin is an iron binding protein taking up the free iron and transports quickly it into the macrophages.

Anaemias in chronic inflammatory processes are usually not curable by iron, folic acid or vitamin B<sub>12</sub>.

### 2.2.12 Anaemias due to impaired haematopoiesis

The primary bone marrow disturbance represents the underlying cause of an important group of anaemias. **There is an impairment of erythropoietic precursor forming.** The term aplastic anaemia should be used for conditions with bone marrow hypocellularity and pancytopenia. Thus, in peripheral blood anaemia, neutropenia and thrombocytopenia are present. Selective aplasia of erythroid cells prevails sometimes. The myelophthisic anaemia with erythropoiesis suppression owing to the bone marrow infiltration by tumor or fibrosis represents an other type of anaemia. The anaemias due to bone marrow impairment are frequently associated with various degree of neutropenia and thrombocytopenia due to pluripotent stem cell disturbances.

#### 2.2.12.1 Aplastic anaemia

The aplastic anaemia is actually **the consequence of pluripotent stem cell impairment and of successive population of cells impairment and destruction.** Frequently various toxins, especially benzene, an endogenous or inborn disturbance may be involved. The congenital underlying causes are more outstanding in Fanconi's syndrome, a constitutional aplastic anaemia being an autosomal, recessive inherited condition. It appears soon in childhood and is usually associated with somatic abnormalities, e.g. with renal or cardiac deformations, skin hyperpigmentation, malformations of bones, hypoplastic thumb or absence of radius. The chromosomal abnormalities involve DNA defects. A progressively impairing bone marrow function dominates in these patients, being concomitantly risk of leukaemia development.

In many cases of aplastic anaemia immune mechanisms are involved. A possible damage caused by antibodies or by autoimmune process are supposed in some patients with aplastic anaemia.

**The bone marrow aplasia** can develop as an adverse **consequence of cytostatic or immunosuppressive treatment or of ionising radiation.** A severe aplastic anaemia can be induced by folic acid antagonists, anthracyclines, nitrosourea and further substances. This type of aplastic anaemia can be at the beginning reversible. After the treatment omission the bone marrow function recovers spontaneously. Long lasting combined treatment may lead to irreversible bone marrow aplasia.

**The broad spectrum antibiotic agent – chloramphenicol** can induce aplastic anaemia, if taken in lower doses it may lead to reversible suppression of erythrocytes and sometimes also the precursors of granulocytes and megakaryocytes. Vacuoles appear in these cells, like in chronic alcoholism. After the chloramphenicol application develops in some cases a severe pancytopenia with irreversible fatal bone marrow aplasia. The appearance of this condition does not depend of the chloramphenicol dosis and can not be predicted.

Aplastic anaemia occurs rather often in **infectious hepatitis**, more frequently in non-A, non-B hepatitis (NANB) but also in other types of hepatitis. Aplasia may reach a threatening degree and may result in lethal end. The aplastic anaemia may develop also during viral diseases, especially during viral infections of respiratory system. It is named idiopathic aplastic anaemia.

Aplastic anaemia is manifested at the beginning by **progressive weakness, petechiae, ecchymoses in skin** and gingivae, nosebleeding, gingival, vaginal or gastrointestinal bleeding. Retinal and cerebral haemorrhage occur. Thrombocytopenia and neutropenia are usually present. Fever can occur present. The degree of aplastic anaemia is substantial. Advanced aplastic anaemia is characterized by the fall of granulocytes below 500/ $\mu$ l, of thrombocytes below 20 000/ $\mu$ l, and of reticulocytes below 1 per cent. The bone marrow is hypoplastic. The patient with aplastic anaemia is always at risk of death from fatal bleeding or from infection. The patients with the moderate degree of aplastic anaemia may survive even several years.

The diagnosis is based on the pancytopenia pres-

ence in peripheral blood. The erythrocytes are normochromic and moderately macrocytic. Marked reticulocytopenia is present. The thrombocytopenia and neutropenia must be confirmed through repeated examinations. The bone marrow examination reveals the hypocellularity and replacement of bone marrow tissue by adipose tissue.

**Patients with severe aplastic anaemia may be treated by bone marrow transplantation** requiring multidisciplinary approach. The blood transfusion represents rather a risk than a method of treatment. Except for hepatitis and haemosiderosis, the blood transfusion can cause an undesirable sensibilisation before the bone marrow transplantation. The bone marrow responses sometimes favorably to the androgen application. GM-CSF (granulocyte-macrophage colony stimulating factor) is effective in pancytopenia developing during AIDS, during myelodysplasia, or under the influence of myelotoxic substances.

#### 2.2.12.2 Primary bone marrow disturbances

**The aplasia of erythrocyte line** represents a selective disorder in production of erythroid cells. Granulopoiesis and megakaryopoieses remain intact. Patients have normochromic normocytic anaemia, but with associated reticulocytopenia. The underlying causes of this condition are not known. A very similar clinical picture of aplasia is observed with frequent findings of thymoma and of myasthenia gravis. In these case the erythropoiesis may be inhibited by IgG affecting selectively the erythroblasts in bone marrow. Erythropoietin inhibitors have been observed in some patients.

The myelodysplastic syndrome is considered to be an independent clinical unit. It is the refractory anaemia associated with neutropenia and thrombocytopenia.

#### 2.2.12.3 Myelophthisic anaemia

**Infiltration of bone marrow by tumors**, fibrosis and granulomas may lead to anaemia development. Among the solid tumors prevail the metastatic deposits from breast carcinoma, stomach, prostata, lung and thyroid gland.

**The bone marrow fibrosis** is usually associated with myeloid metaplasia. In tuberculosis, but also in metabolic disturbances e.g. osteoporosis, anaemia may develop.

**Myelophthisis** results in normochromic normocytic anaemia. In the peripheral blood normoblasts may occur. At the beginning the number of reticulocytes moderately elevated.

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## 2.3 Haemostasis and haemocoagulation

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**Normal haemostasis** is the result of complicated relations between the vessel wall, thrombocytes and coagulation and fibrinolytic system. If the mutual proportions are not impaired the whole system is balanced. The balance is continuously maintained between the systems supporting the local haemostasis and those inhibiting the disseminated thrombosis.

**The inner surface of vessels – endothelium – is a nonthrombogenic barrier** inhibiting the interaction of blood components with subendothelial structures. If these substances have been in contact the thrombus formation would be initiated. This inhibitory function of endothelium is its primary function and is called the non-thrombogenicity.

**The thrombocytes circulate in an inactive form.** They do not contain nucleus and have irregular discoid shape. Their surface is covered with phospholipid membrane and the contractile filaments are situated below it. They contain three types of granules (dense, alpha and lysosomal) and the system of minute channels through which the content of granules is released into the plasma.

**If the endothelium is damaged, the thrombus is formed immediately.** First the thrombocytes adhere to the subendothelial components. The thrombocyte adhesion is an interaction between the membranous thrombocytic receptor (glycoprotein Ib) and the subendothelial collagen with participation of the plasmatic cofactor (von Willebrand's factor – vWF). To this reaction contributes a further plasma protein – fibronectin (glycoprotein present in cell membranes and is a component of plasmatic proteins, it is produced in fibroblasts, in endothelial cells and in macrophages). To the impaired site adhere several thrombocytes simultaneously. Such a thrombo-