Inherited deficiency may affect alpha granules containing the vWF, fibronectin and thrombospondin. These proteins are needed for normal adhesion and aggregation of thrombocytes.

2.4.1.4   Acquired disorders of thrombocyte function

Acetylsalicylic acid (Aspirin) and other non-steroid antiphlogistic drugs inhibit the **cyclooxygenase** the key enzyme in arachidonic acid transformation into cyclic endoperoxides and thromboxan A2. These compounds induce the thrombocyte aggregation and mediate the reaction of thrombocytes to agonists like ADP, adrenaline and collagen. The contact of thrombocytes with acetylosalicylic acid impairs the platelet aggregation. The cyclooxygenase inhibition following one dosis of acetylosalicylic acid lasts at least a week. The effect of other non-steroid antiphlogistic drug lasts about 24 hrs. Acetylosalicylic acid (Aspirin) given during haemophilia, thrombocytopenia or anticoagulant treatment may induce severe bleeding.

In uraemia is a tendency to bleeding. Ecchymoses and gastrointestinal bleeding appear. The thrombocyte aggregation and the bleeding time are prolonged. The thrombocyte dysfunction and thromboxane synthesis can be due to the accumulation of toxic metabolites in plasma. Haemodialysis improves the function of thrombocytes. In neoplastic diseases can the paraproteins (formed in this condition) induce the haemorrhage. The underlying cause can be the binding of monoclonal immunoglobulin with the surface of thrombocytes. This binding inhibits the binding of thrombocytes to each other. Disorders of thrombocytes occur frequently in leukaemia and DIC.

2.4.2   Vascular purpurae

In these conditions insufficiency of haemostatic mechanisms is involved due to vascular disturbance. Typical finding in vascular purpurae is the positive tourniquet test (Rumpel-Leede test). It is a proof of increased capillary fragility at normal count of normal thrombocytes and physiologic spectrum range of coagulation factors. Congenital vascular purpura occurs in inborn disturbances of connective tissues.

In children occurs the acquired Hennoch-Schönlein purpura – a type of vasculitis of allergic genesis

manifested by abdominal pain, haematuria, glomerulonephritis, haemorrhagic urticaria, arthralgia. Another type is the hereditary haemorrhagic teleangiectasia (morbus Osler-Weber-Randu). The bleeding arises from mucosal teleangiectasia as a autosomal dominant trait. Manifestations of vascular abnormalities are more outstanding in puberty. The bleeding can affect all mucosae. The nasal bleeding (epistaxis) is frequently observed and bleeding in mucosae of respiratory system, GIT, urogenital tract are present. Fistulae use to be present in pulmonary vessel bed. Teleangiectasia may be present also in the skin.

2.5   Disorders of haemocoagulation

The haemocoagulation is initiated by generation of a strong serine protease – the thrombin. Thrombin splits the soluble plasmatic protein – fibrinogen. A non-soluble fibrin network is formed "traping" the erythrocytes and thrombocytes. This complicated process is triggered by blood vessel or tissue injury. Strictly controled interaction between more than 20 various proteins is rapidly developing, potentiating the initial activity of some molecules to create an adequately large clot. The damaged vessel and the thrombocyte aggregation form the specialized base where these molecules are localized catalyzing the coagulation reactions.

The coagulation proteins circulate in form of inactive zymogens in amount farly exceeding the needs of blood coagulation. The process can begin with two mechanisms – the intrinsic and the extrinsic. The extrinsic mechanism requires the lipoprotein activity of damaged tissue in form of tissue factor. Factor X becomes active at the thrombocyte surface. The plasmatic coagulation proteins are mostly proteinases of serine type some of them operate like cofactors being without the enzymic activity (factor V and factor VIII).

The intrinsic mechanism begins with the factor XII activation on the damaged vascular surface (endothelium, subendothelium) or on another surface

INTRINSIC PATHWAY

Activating factors

EXTRINSIC PATHWAY

Tissue factor ← Tissue injury

XII → XIIa

XI → XIa

IX → IXa   VIIa ← VII

X → Xa

V

Protrombin → Trombin

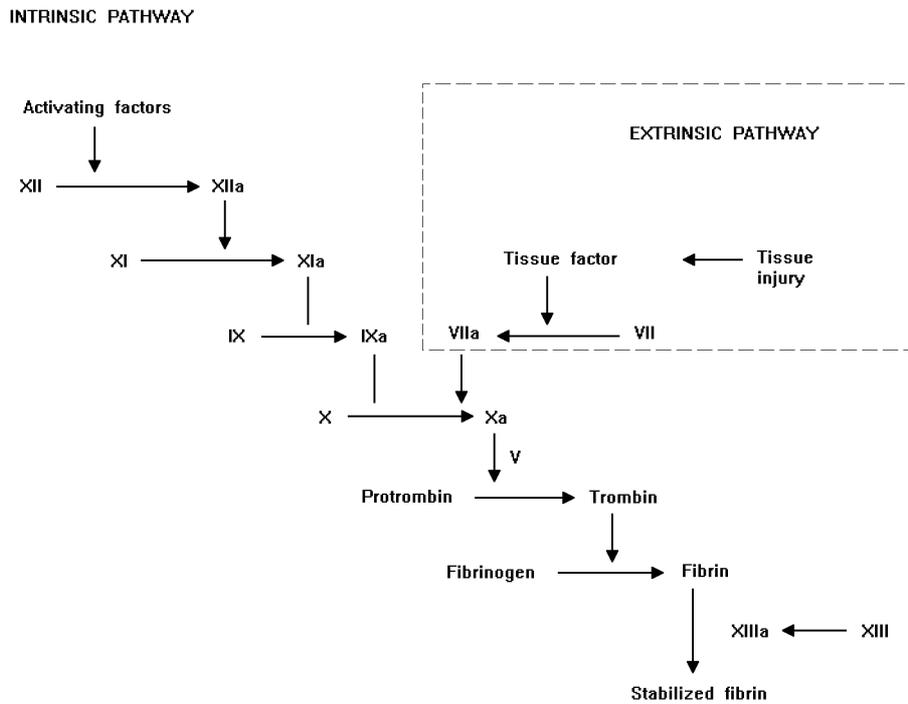Fibrinogen → Fibrin

XIIIa ← XIII

Stabilized fibrin

Figure 2.4: Simplified scheme of blood clotting

with negative charge (e.g. glass). Intact cell surface is positively charged. The cofactors and promoters of factor XII are the prekallikrein, kininogen with high molecular weight – HMWK (high molecular weight kininogen) and factor XI. On a suitable surface this complex activates the factor XII. Factor XIIa converts then factor XI into its active form – XIa and prekallikrein into the kallikrein. Kallikrein splits HMWK into bradykinin. Factor XIa activates factor IX and can activate factor VII too; as well as the plasminogen into the plasmin. By this way is the fibrinolysis as well as the haemocoagulation initiated. In presence of Ca^{2+} and of phospholipids factor IXa activates factor X into Xa. This activation usually occurs at the plasmatic membrane of stimulated thrombocytes or on vascular endothelium, but it can occur also without them (in vitro) (see picture 2.4, page 73).

Extrinsic mechanism. During the extrinsic mechanism released tissue factor from damaged tissue directly activates factor X into Xa in presence of Ca^{2+}.

The accumulated prothrombinase complex on the surface of thrombocytes accelerates in presence of factor V the conversion of prothrombin into the thrombin. Thrombin splits the soluble fibrinogen (m.w. 340 000 daltons). Fibrinogen is composed of three pairs of polypeptide chains. Thrombin splits first the small peptides from fibrinogen chain A alpha. By this is its polymerization started end to end. Fibrin I is formed. Thrombin splits than the small peptides from B beta chain. By side to side polymerization fibrin II is created. By the action of plasmatic glutaminase (factor XIII) the become crossed fibrin fibres – a nonsoluble fibrin clot is definitively formed. Thrombin plays the main role by converting the fibrinogen into fibrin. Besides this it has further important effects. It activates the thrombocytes by opening the binding sites for prothrombokinase complex. Thrombin induces the release of thrombocytic coagulation substances like thromboxan, Ca^{2+}, ADP, von Willebrand's factor, fibronectin and thrombospondin. Thrombin activates

the factor VIII and factor V, potentiates the coagulation by its action on factor XIII. Thrombin influences the endothelium by activating the C protein which binds to the surface protein – the thrombomodulin. C protein inactivates the factor Va and factor VIIIa, and stimulates the fibrinolysis. Thrombin elicits the endothelial cell contraction. Tending to a balance the endothelium can bind and inactivate the thrombin, and sometimes it may produce vasodilating prostacyclines in response to the thrombin effect.

The terminal phase of coagulation is the **fibrinolysis**. The fibrinolysis process is initiated in fact already during the coagulation. Besides the fibrin formation thrombin namely activates the C protein and releases the plasminogen activators from the vessel wall. C protein in combination with protein S (the C protein cofactor) inhibits the coagulations activities of factor Va and VIIIa. The circulating plasminogen is converted into an active protease – the plasmin. The splitting is provided by plasminogen activators. Then plasmin solubilizes the fibrin. The activity of plasminogen tissue activators is potentiated by their binding with fibrin, therefore is the plasmin generation localized just at the clot (coagulum). Moreover, the plasmatic inhibitors of proteases – alpha1-antitrypsin, alpha2-antiplasmin inhibitor, and alpha2-macroglobulin rapidly inactivate the serine proteases including thrombin and plasmin. Antithrombin III binds with procoagulation proteins (proteases) (factor Xa and thrombin). The antithrombin activity is enhanced by heparin and the heparin-like substances. The antithrombin III – proteases formed complexes are rapidly eliminated from the blood flow by liver and the mononuclear phagocyte system.

Liver is the site of synthesis of a great number of coagulation proteins. Factors II, VII, IX and X and the C and S proteins requires vitamin K presence in order to be synthesized. In K vitamin absence molecules are produced lacking the carboxylglutamate binding sites for Ca^{2+}. This abnormal molecules are ineffective in coagulation. Fibrinogen, factor V and inhibitors of proteases are also produced in the liver and their production may be reduced in hepatic diseases. Factor VIII is probably also synthesized in liver and spleen, nevertheless the right site of its production is not strictly known. Von Willebrand's factor and the tissue plasminogen activator are produced by endothelium of vessels. Von Willebrand's factor and the factor VIII circulate in the peripheral blood together in form of a macromolecular complex, although they are produced on different sites. Their production is regulated by different genes. Their structures and functions are also different.

2.5.1   Haemorrhagic diatheses

Severe bleeding after injury, during epistaxis, or surgical intervention, bleeding into the muscles or joints are suspect to be the manifestation of **coagulation disorder**. If in family history is the statement of bleeding in males, it should be considered a possibility of occurence of haemophilia.

2.5.1.1   Haemophilia A

The classical haemophilia is the most common severe inherited disorder of blood coagulation. **In haemophilia A is a quantitative decrease of factor VIII accompanied with its qualitative alteration – molecular dysfunction of this factor**. The gen of factor VIII has been recently identified. In 70 per cent of cases is a positive family history of haemophilia occurence in male population stated. Spontanneous mutations occur in 30 per cent of haemophilia. Random inactivation on X chromosome in women usually causes, that the mother is the carrier of haemophilia, her level of factor VIII is only 50 per cent of the normal value. In patients with severe haemophilia A is the factor VIII activity below 1 per cent of the norme leading to spontaneous or posttraumatic bleeding epizodes from the birth. The level of factor VIII above 5 per cent represents the medium severe form of haemophilia. The motor activity of children usually leads to the bleeding into the muscles and joints. The bleeding into the the joints may result in severe deformations of extremities. Bleeding can occur also in usual stress situations. Haemorrhage can occur in every organ. Vitally important structures can be involved where by the life can be threatened.

In supplementary treatment the concentrated factor VIII is applied. The factor VIII application improves the condition acutely, but the problem is not solved by this approach. The (biological) half-life of factor VIII is namely very short, 8 to 12 h.

Factor VIII has to be given before dental and surgical interventions and in stress situations. Repeated factor VIII administration is the cause of high inci-

dence of hepatitis B and of AIDS in these patients. New methods of factor VIII preparation considerably reduced the risk of hepatitis and AIDS viruses transfer. Repeated administration leads to forming of factor VIII inhibitors and its rapid destruction after application. The patients should not receive acetylsalicylic acid, or nonsteroid antiphlogistics. These substances impair the functions of thrombocytes and in this way they impair the bleeding. Longstanding treatment with factor VIII leads to several complications:

1. generation of factor VIII inhibitors

2. hepatitis

3. hypertension

4. AIDS

2.5.1.2 Von Willebrand's disease

Under physiological circumstance the thrombocyte adhesion to the vascular endothelium may be performed only in presence of a plasmatic glycoprotein termed von Willebrand's factor (vWF). VWF circulates in peripheral blood with antihaemophilic factor (factor VIII) in form of multimer. **The vWF level is in von Willebrand's disease reduced or is lacking at all**. VWF can be present in sufficient amount, is however functionally impaired. The antihaemophilic factor (VIII) level may be moderately or considerably decreased.

This inherited condition occurs frequently. Both sexes are affected. **A double disturbance** also exists with **abnormal thrombocyte adhesion in combination with decreased factor VIII activity**. The clinical state of patients with von Willebrand's disease is usually variable. Frequent symptoms are mucosal bleeding, ecchymoses, epistaxis, gastrointestinal bleeding and menorrhagia. In some patients may haemarthrosis occur like in haemophilics. In about 75 per cent of patients are the vWF and factor VIII levels decreased. This condition is designated as type I. In some cases is the vWF level reduced only moderately, but the bleeding time is considerably prolonged (type IIa). In type IIb the vWF is lacking at all. In long lasting bleeding the transfusion of normal plasma or cryoprecipitate is given, being rich in vWF. In moderate bleeding desmopressin (aminoarginine vasopressin) is applied elevating the vWF level. During the gravidity the condition may improve owing to the raised vWF and antihaemophilic factor levels. Acetylsalicylic acid and the nonsteroid antiphlogistic drugs are dangerous for these patients.

2.5.1.3 Defects of contact factors

These defects occur infrequently. They are recognized in laboratory examinations. They are not clinically manifest because they do not enhance the bleeding tendency. In factor XII (Hageman's factor) are the patients asymptomatic. Absence of prekallikrein and of high molecular kininogen is uncommon. The factor XI deficiency occurs in Japones and Jews.

Defects of vitamin K-dependent coagulation factors are accompanied with a strong tendency to bleeding.

Factor IX deficiency is in clinical manifestations similar to haemophilia. It is an inherited disorder manifested by haemarthrosis, bleeding into the GIT and CNS. It is termed **haemophilia B**. Application of factor IX, with biological half-life of 20 h has a favorable effect except the fact, that it could be connected with thromboembolic complications.

The factor VII deficiency is a rare autosomal recessive defect. The bleeding is usually considerably variable. Treatment is performed with application of plasma or with concentrates of prothrombin complex factors. Factor VII has a short half-time, 2 to 6 h only. It is necessary to repeat its application.

The factor X deficiency is an infrequent autosomal recessive defect with bleeding. There exist two types of this condition. In the first quantitative alteration of factor X (decrease) and in the second the dysfunction of factor X are involved. The bleeding is similar to the bleeding in haemophilia. Bleeding and menorrhagia post partum may be very severe.

Afibrinogenaemia or congenital dysfibrinogenaemia is a disorder with decreased fibrinogen plasmatic level, or abnormal fibrinogens are formed. Abnormal fibrinogens may cause the bleeding, but also thromboses. About 80 types of dysfibrinogenaemia have been described till now. The treatment implies the application of plasma rich in fibrinogen or of fibrinogen concentrates. The half-time of fibrinogen is 4 days.

The factor XIII deficiency is a seldom autosomal recessive defect. The deficiency of fibrin stabilizing factor may be manifested by difficult wound healing.

The factor XIII level can be lower than 1 per cent of normal values.

2.5.1.4   Acquired disorders of haemocoagulation

Acquired haemocoagulation disorders are the consequences of several causes. Insufficient production or enhanced consumption of coagulation factors may be involved. Functionally defective molecules, selective inhibitors may be produced sometime, or the coagulation factors can be absorbed. The absorption of factors e.g. on vascular amyloid is possible, however seldom.

Vitamin K – dependent haemocoagulation factors are dependent on the protein synthesis in the liver. Prothrombin (II), factor VII, IX, X, C and S proteins are involved. Vitamin K is a compound soluble in lipids. It is absorbed in small intestine and store in the liver. It is partially synthesized by saprophytic microbial flora in small intestine and in colon. Following absorption is vitamin K converted into its active form. Its main site of action are the hepatocytes where it contributes to the definitive forming of coagulation factors II, VII, IX, X, of C and S proteins. It operates like cofactor in glutamic acid rests carboxylation of above mentioned coagulation factors. During this process binding sites for calcium are formed on these factors. The serine proteases can not be activated and the haemocoagulation can not run without calcium binding. Vitamin K deficiency will be first manifested on factor VII, than on C protein because these compounds have a short biological half time.

K vitamin deficiency can appear during malabsorption if there is deficiency of biliar salts, in reduced intake and after "sterilization" of GIT by antibiotic treatment and finaly in peroral anticoagulant treatment. Vitamin K deficiency appears most frequently in inmature newborns, in whom the liver functional capacity is not developed yet and the K vitamin synthesis is lacking in the intestine without bacterial colonization.

The coumarin anticoagulans inhibit competitively the K vitamin effect on carboxylation. The K vitamin dependent coagulation factors become therefore ineffective. It is manifested by the prothrombin time prolongation, above all. Several drugs posses the coumarin like anticoagulant action. In overdoses may these drugs induce severe gastrointestinal bleeding or bleeding into CNS.

In hepatic diseases disorders of K vitamin-dependent factors synthesis appear. Impaired fibrinogen and factor V production is usually involved in terminal stage. In severe bleeding is the K vitamin application insuficient. Application of plasma should be considered.

In uraemia bleeding into mucosae, skin and GIT frequently occurs due to complex causes. In nephrotic syndrome with proteinuria the factor IX deficiency can appear. Finaly, in dialyzed patients (with long termed dialyzing program) repeated heparinization (heparin application) may contribute to the bleeding. In addition to mentioned alterations thrombocytopenia due to bone marrow inhibition is in uraemia usually present. There is an important thrombocyte dysfunction. The thrombocyte aggregation and adhesion are impaired.

Antibodies with anticoagulant effect. In haemophilics with substitution therapy and in immune diseases like lupus erythematosus, lymphoproliferative diseases, in post partum period and in aged persons antibodies – inhibitors of factor VIII and of further factors may be created leading to haemorrhagia and also the thrombosis. Antibodies may be formed against the von Willebrand's factor. It is observed mainly in lymphomas.

2.5.2   Disseminated intravascular coagulation

Acute disseminated intravascular coagulation with **life threatening bleeding and intravascular coagulation** is occuring more frequently in its chronic form. This condition can appear in many diseases. It occurs very often in obstetric catastrophes, in metastatique malignant processes, in extended trauma and bacterial sepsis. In each of these condition the underlying triggerin mechanism of disorder can be defined. On tumors, trauma, tissue necrosis and tissue factor initiating the haemocoagulation is release into circulation. In sepsis the endotoxin of gram–negative bacteria activates some steps of coagulation cascade. Endotoxin activates directly the Hageman's factor (XII) and the binding of tissue factor with the monocyte and endothelial surface wich accelerates the coagulation reactions. All triggering alterations result in **small thrombi and emboli formation** in microcir-

culation. This is characteristic for the early phase of disseminated intravascular coagulation. System of coagulation inhibitors and the fibrinolytic system are activated. During the thrombi formation the coagulation factors and the thrombocytes are consumed.

The plasmatic coagulation factor and thrombocyte consumption, the fibrinolysis activation and haemocoagulation inhibition lead to a severe hypocoagulation condition with haemorrhagia. It is always a very severe state. Haemorrhagia can appear on many sites. Bleeding into the skin and mucosae are observed in patients. Bleeding from wounds, at site of injections or cathethers can occur. Sometimes acrocyanosis, thrombosis and gangrene of fingers, genitalia and nose are present. The microthrombi presence elicit vasospasm. In patients with malignant processes may the disseminated intravascular coagulation persist as chronic state.

In laboratory examination in consuption coagulopathy the thrombocytopenia, presence of schizocytes or of fragmented erythrocytes are found in blood smears. The fibrinogen level is decreased. These are the signs of coagulation factors depletion. The content of fibrin degradation products is increased. The fibrinogen level corresponds with the bleeding stage.

Treatment of this condition is very complicated. If there are signs of bleeding it is necessary to apply fresh plasma to compensate the deficient coagulation factors and the thrombocytes. But if there is acrocyanosis and incipient gangrene or thrombose present anticoagulants are to be administered, heparin above all intravenously. The prophylactic heparin application is most frequently used in patients with tumors, mainly in the period of cytostatic therapy.

2.5.3 Thromboembolic diseases

Disorders of haemostatic mechanisms may appear easily in various diseases. It is important to know the mechanisms which may lead to the haemostasis impairment. The tendency to thrombosis is observed in surgical interventions, inflamatory processes, during gravidity, in vessel diseases and further conditions. Inherited disorders characterized with tendency to thrombosis concern the C and S proteins, antithrombin III deficiency, disfibrinogenaemia and disorders of fibrinolytic system.

The hypercoagulation condition development is facilitated by predisposing diseases, long lasting immo-

bilization, gravidity, malignant processes, hormonal anticonception (with high estrogen doses), thrombocytopenia, increase in blood viscosity and in plasmatic coagulation factors level. The hypercoagulation condition may lead to formation of fibrin degradation products.

2.5.3.1 Antithrombin III deficiency

Antithrombin III is a serine proteases inhibitor blocking the action of coagulation enzyme thrombin. It inhibits also factor X and potentiates the anticoagulation effect of heparin. Heterozygous antithrombin III deficiency is associated with venous thrombosis in young persons. In a severe deficiency of antithrombin III resistance to heparin is observed. In laboratory determined prothrombin III level need not to be a sign of its intact function. A functionally abnormal protein may be involved nevertheless the deficiency of prothrombin III can be also acquired. It is usual in hepatic diseases, in nephrotic syndrome, in disseminated intravascular coagulation and in cytostatic treatment of malignant processes.

2.5.3.2 C and S protein deficiency

Both these proteins belong to the group of hepatic vitamin K – dependent proteins. C protein is activated by thrombin. This activation is potentiated when thrombin binds with thrombomodulin (endothelial cell receptor). The activated C protein (in presence of S protein) destructs the factor Va and factor VIIIa. The fibrin formation is inhibited by this, and so is the further thrombin generation. The activated C protein stimulates also the fibrinolysis. In patients with this inborn disorder thromboembolic epizodes occur and in neonatal period the purpura fulminans appears. The lack of suppressive system of coagulation can be therapeutically compensated by application of anticoagulants.

2.5.3.3 Principles of antithrombotic treatment

Arising of haemostasis mechanisms there exist four basic types of therapy inhibiting the thrombosis:

1. Substances influencing the thrombocytes

2. Heparin

3. K vitamin antagonists

4. Thrombolytic drugs

The first group – **substances influencing the thrombocytes** are used in profylaxis of arterial thrombosis. In the initiation of arterial thrombosis the most important role play the thrombocytes. The acetylsalycilic acid is used the most frequently for this purpose. It inhibits the enzyme cyclooxygenase thus reducing the thromboxan A_2 production in thrombocytes. Activated thrombocytes are the TXA_2 producers. TXA_2 is a potent vasoconstrictive agent. It influences the thrombocyte aggregation and the release of further substances from thrombocytes.

Heparin is used to prevent both the venous and arterial thrombosis. The parenteral administration of heparin inhibits the thrombin formation (heparin is not a homogenous substance, it is a mixture of polysacharides with molecular weight 3000 to 4000 daltons. For the first time it was isolated form the liver therefore it was termed heparin. It activates the antithrombin III).

Coumarin anticoagulants inhibit the synthesis of coagulation factors. This is why their effect appears only in some days. They are administered as prevention of both, arterial and venous thromboses. Following oral application they are absorbed in the intestines. In plasma they are bound with albumin and are transported into the liver, where they are inhibiting the vitamin K – dependent coagulation factors (II,VII,IX,X).

Fibrinolytic substances (the tissue plasminogen activator) and fibrinolytic enzymes (streptokinase and urokinase) activating plasminogen into the plasmin are used in last occurs. Fibrinolytic substances can dilute thrombi in few hours.

The greatest **danger of thrombolytic treatment** is the bleeding. Patients with thrombocytopenia and chronic alcoholism, uraemia, and those taking the acetylsalicylic acid tend to this complication. Except of the drugs mentioned, there exist many substances having the anticoagulation as side effect.

2.6   Disorders of leucocytes

The main function of leucocytes is to defend the organism against diseases, especially against the infection. Mononuclear phagocytes (monocytes and the tissue macrophages) and granulocytes perform this function by swallowing up the microorganism and digesting the tissue detritus. The lymphocytes participate in activating of immune system functions.

The polymorphonuclear leucocytes form the highest percentage of leucocytes. They do not divide themselves or differentiate further in circulating blood. They have the ability to move actively and to phagocytise various microorganisms and niert particles. They release enzymes from cytoplasmatic granules into the phagocytic vacuoles and in certain circumstances into the extracellular space. The neutrophils use their ability of chemotaxis (motion in direction of ascending concentration of the substance of target organism), of phagocytosis (ingestion of the microbe into the phagocytic vacuole) and the capability to scarve the microbe chemically by releasing the content of granules into the phagocytic vacuoles (bactericidal proteins, myeloperoxidase, cathepsins) and by forming of free oxygen radicals as superoxide and hydroxylated anion. In presence of halogens e.g. Cl-toxic substances are formed killing the swallowed up microbes.

Chemotaxis of neutrophiles is extraordinarily important. They are able to force the neutrophils to become attached to the vascular endothelium and therafter to penetrate across the vascular wall (through the intercellular junctions and to migrate into the extravascular space. The migration in sense of concentration gradient may be influenced significantly by bacterial peptides, components of complement system and leucotrien B_4.

The ability of leucocytes to distinguish microorganisms or other particles facilitates the phagocytosis. So do the opsonization or the proteins bound to the surface of microorganisms. Fragments of complement C3b, the plasmatic protein fibronectin and immunoglobulins are involved. These substances adhere to surface of microorganism enhance the binding with the receptors of neutrophil plasmatic membrane.