result of activation of inhibitory system through protein C (a vitamin K – dependent plasmonic protein activated by serine proteinases). Thrombin forms a complex with thrombomodulin (a protein localized on the surface of endothelial cells having a strong affinity for thrombin). The thrombin-thrombomodulin complex activates the C protein. In the presence of phospholipids and of S protein (a further cofactor) the activated C protein inactivates the factor Va and VIIIa. C protein is involved also in fibrinolysis initiation.

3. The third control mechanism are the original inhibitors: especially the alpha 2 macroglobulin (a component of serum proteins), α-1-antitrypsin (a glycoprotein with inhibitory action in serum), antithrombin III (the main regulator of blood coagulation; it neutralizes the thrombin serine proteinase), α-2-antiplasmin (one single-chained glycoprotein forming a complex with plasmin) and other factors. In coagulation cascade regulation antithrombin III plays the key role. It inactivates the serine proteases. Heparin and the contact with damaged endothelium accelerates this effect.

The fibrin deposition and its removal from circulation is regulated by fibrinolytic system – a multicomponent system composed of circulating plasminogen proenzyme, of activators, cofactors and inhibitors. The human plasminogen is a single-chained globulin easily adaptable by proteolysis. Plasminogen (lys-plasminogen) has higher affinity for fibrin and alpha 2-antiplasmin. It is easily activated also by urokinase. The plasminogen molecule contains two parts – one of them has an active site and the other has a binding site for the binding with fibrin and alpha-2-antiplasmin. The plasminogen activation occurs in the case when the activator cleaves the peptide binding and two chains of plasmin enzyme arise. The plasmin activation may occur in three ways:

1. Intrinsic mode represents the proactivator activation through the contact system
2. In the extrinsic system are the activators released into the blood flow from damaged tissue of blood vessel wall or of thrombocytes
3. During the treatment the streptokinase or urokinase (fibrinolytic substances) are applied into the blood flow.

Plasmin hydrolysis many fibrin bindings causing thus fibrinolysis. The fibrin rests are named fibrin degradation products (FDP). Besides fibrin, plasmin involved in the factor V and VIII degradation. Massive fibrinolysis is simultaneously inhibited by a potent inhibitor – the alpha-2-antiplasmin and by a less strong alpha-2-macroglobulin.

2.4 Disorders of primary haemostasis

The spontaneous stopping of bleeding from a smaller damaged vessel is performed with essentially important life saving mechanisms. In the process of bleeding stopping from a vessel a complex of reactions between three participating systems is involved:

1. the vessel wall,
2. the thrombocytes,
3. the plasmatic coagulation factors.

If all occurs in framework of physiological usefulness the result is the normal haemostasis. In case that certain limits are exceeded, the reactions may lead to pathologic bleeding or to undesired thrombosis. The haemostasis initiation occurs during few seconds following vessel injury. Its termination may last even an hour. In the temporary consequence three successive plases can be distinguished:

The first phase – the primary haemostasis includes:

- the constriction of the injured vessel
- the subendothelial collagen exposure
- the adhesion and aggregation of thrombocytes on the demaged surface

During these processes the primary haemostatic (thrombocytic) plug is formed in about 3 to 7 minutes. In this process the von Willebrand’s factor
2.4 Disorders of primary haemostasis

(vWF) is involved mediating the adhesion of thrombocytes, the release of vasoactive substances and the intensification of thrombocyte aggregation. The primary haemostasis may be investigated in clinical practice e.g. by the bleeding time determination.

The second phase – the secondary haemostasis represents the fibrin clot formation at the site where before the primary haemostatic (thrombocytic) plug has been formed. The surface of activated thrombocytes catalyzes the thrombin formation by its ability to involve coagulation factors in presence of membrane phospholipids into the action. The thrombin activation results in several important effects:

1. Thrombin catalyses the conversion of fibrinogen into fibrin. Fibrin forms a network in which the erythrocytes are caught, thus the definitive clot and vessels plug arise.

2. Thrombin stimulates the further activation of thrombocytes, the prothrombin conversion and the \( \text{TXA}_2 \) production.

3. Thrombin activates the coagulation factor XIII – the fibrin stabilizing factor promoting the stability of fibrin.

The state of haemostasis can be considered in clinical practice according the time needed to clot formation of whole blood. It lasts in average 8 to 10 minutes.

The third stage of haemostasis (coagulation) is the coagulum retraction during which the free net of aggregated thrombocytes, the fibrin net and the caught erythrocytes are formed into a solid formation. During this process the thrombostenin is retracted (thrombostenin is a contractile protein present in thrombocytes – similar to muscle acto-myosin) compressing the coagulum. This process lasts about one hour, if observed in vitro.

Clinically important disorders associated with the possibility of undesired bleeding may occur like consequence of vessel, thrombocyte or coagulation protein defects being of various degree, quality or quantity. It is necessary to know the haemostasis status before a surgical intervention, in case of inappropriate bleeding after trauma, or if spontaneous bleeding arises, which can not be stopped by usual methods. It is also necessary to realize the fact, that liver diseases, malignant haematological processes, and uraemia impair the status of haemostasis. Among the drugs and toxins the acetylsalicylic acid, non-steroidal antiphlogistic drugs, antibiotics, anticoagulant drugs and the alcohol also impair the haemostasis. These substances may induce bleeding. The basic information on haemostatic integrity can be obtained by prothrombin time, bleeding time, partial thromboplastin time, and thrombocyte count determination.

2.4.1 Disorders of thrombocytes

The normal function of thrombocytes is necessary for the maintenance of the primary haemostasis. In addition to their normal function, certain number of thrombocytes is inevitable for optimal function of haemostasis. Under physiological circumstances the thrombocyte number should be higher than 150 000/\( \mu l \) of peripheral blood. The thrombocyte number reflects their production in bone marrow and their destruction in peripheral blood. The bone marrow can increase the thrombocyte production 8 to 10 times. The life-span of thrombocytes in peripheral blood is 10 days. Younger thrombocytes are more active in their metabolic and haemostatic functions than the older ones. The adhesion of thrombocytes, the procoagulation activity, the aggregation, and release of vasoactive substances have to be normal in order that the haemostatic effectivity may be functioning. Impairment of any of these thrombocyte functions may be the underlying cause of bleeding. Petechial bleeding into the mucous membranes and skin are typical in these cases. This condition if generalized is named purpura.

2.4.1.1 Thrombocytopenia

Decrease in number of thrombocytes under 100 000 /\( \mu l \) increases the risk of bleeding. If the number is less than 50 000/\( \mu l \) even spontaneous bleeding may occasionally occur. Bleeding arises more frequently in anaemic patients and in patients with fever. In these case the bleeding is an evidence of long-lasting thrombocytopenia. A rapid acute fall in thrombocyte number is usually not the cause of bleeding.

The underlying cause of severe thrombocytopenia can be:

1. the decrease in thrombocyte production in bone marrow or an ineffective production

2. increase in peripheral thrombocyte destruction
3. hypersequestration of thrombocytes
4. intravascular thrombocyte dilution

Reduced thrombocyte production observed in systemic infections, nutritive defects (folic acid or vitamin B\textsubscript{12} deficiency), following irradiation, chemotherapy or bone marrow replacement by fibrosis or by tumor. A transient decrease in thrombocyte production occurs frequently in viral infections. Many drugs may inhibit the megakaryocytes. The most important ones are the anti-convulsive drugs and thiazides. The bone marrow hypoplasia in aplastic anaemia or in Fanconi’s syndrome is usually associated with thrombocytopenia.

The thrombocyte destruction is caused by various drugs: digitalis, quinidine, thiazides, imipramin, phenotiazines, sulfonamides, antibiotics (especially penicillins, cephalosporins) and the chrysotherapy. These drugs cause thrombocytopenia by immune mechanism. The antibodies against the drugs or the complexes of plasmatic proteins with drug are passively adsorbed to the Fc receptor on the surface of thrombocytes (the thrombocytes are, in fact, innocent victims of these processes). In this way marked thrombocytes are quickly sequestrated into the spleen from circulation. Several substances may be adsorbed on the thrombocyte surface. A neoantigen arises stimulating the production of antibodies against the thrombocytes.

Idiopathic thrombocytopenic purpura (autoimmune). This type of thrombocytopenia (thrombocytopenic purpura) appears without toxic or drug influence. Increase in thrombocyte bound IgG and complement is found. Large thrombocytes in peripheral blood and increased number of megakaryocytes in bone marrow are present. The life-span of thrombocytes is shortened. The acute form of idiopathic thrombocytopenic purpura (ITP) occurs preferentially in children during viral diseases. The recovery is usually spontaneous. In adults the acute form does not occur. In some cases the autoimmune haemolytic anaemia accompanies the lupus erythematosus, lymphoproliferative disorders and AIDS. Homosexual patients with ITP are nearly all HIV positive. In pregnant women with ITP the antithrombocytic antibodies, usually the IgG1 and IgG2 passes through the placenta. Splenectomy has usually a favorable effect in severe cases, even if the spleen is not enlarged.

The posttransfusion purpura is uncommon syndrome. It occurs following transfusion of blood with thrombocytic antigen PL\textsubscript{A1} to a patient in whom this antigen is not present. The recipient response according this principle to the received antigen is the production of Anti PL\textsubscript{A1} antibodies leading usually to thrombocytopenia in 5 to 8 days following the transfusion. The thrombocytopenia may persist for several weeks. The above mentioned principle may function also between the mother and fetus.

Thrombotic thrombocytopenic purpura – Moschcowitz’s syndrome is an acute repeated condition where the microcirculation is affected. It is characterized by thrombocytopenia, microangiopathic haemolytic anaemia, neurologic disorders, renal dysfunction and fever. It occurs most frequently in young women. A viral disease precedes almost always its occurrence. The most typical finding is the presence of hyaline thrombi containing fibrin and aggregation of thrombocytes. These thrombi occlude small arterioles and capillaries. Except for this, endothelial proliferation is observed, but a typical vasculitis is not present. The blood smears show schistocytes, increased reticulocyte number and the presence of normoblasts – a picture of microangiopathic haemolytic anaemia. Thrombocytopenia is outstanding the values of LDH and bilirubin are elevated. This condition is usually lethal.

Haemolytic uraemic syndrome occurs commonly in children. Characteristic signs are the microangiopathic haemolytic anaemia, thrombocytopenia, diarrhea and acute renal failure. Neurologic symptoms are not present in this condition. The most important finding are the hyaline thrombi in renal microcirculation.

Thrombocytopenia in splenomegaly. Under physiological circumstances the spleen contains about 1/3 of all thrombocytes. Enhanced sequestration of thrombocytes in the spleen occurs in splenomegaly. The recovery is usually spontaneous. In adults the acute form does not occur. In some cases the autoimmune haemolytic anaemia accompanies the lupus erythematosus, lymphoproliferative disorders and AIDS. Homosexual patients with ITP are nearly all HIV positive. In pregnant women with ITP the antithrombocytic antibodies, usually the IgG1 and IgG2 passes through the placenta. Splenectomy has usually a favorable effect in severe cases, even if the spleen is not enlarged.

Dilution thrombocytopenia occurs following a massive blood transfusion and can last for some days.

Thrombocytopenia due to extracorporeal circulation. The extracorporeal circulation is used in surgical interventions on open heart. The thrombocytes may adhere to membrane surface of oxygena-
2.4. Disorders of primary haemostasis

to the dialysis membrane or other parts of equipment for extracorporeal circulation. The thrombocytic dysfunction and thrombocytopenia in association with the extracorporeal circulation may lead to postoperative bleeding.

2.4.1.2 Thrombocytosis

Increase in thrombocyte number above 500 000/µl can appear on physiological basis. It can occur as response to the bleeding. Thrombocytosis may appear commonly in infections, trauma or can be due to the primary bone marrow disease. Transient and mild thrombocytosis can appear after physical overexertion or other stress. The underlying mechanism is the thrombocyte release from spleen and lungs mediated by adrenaline effect.

Secondary or reactive thrombocytoses are due to increased thrombocyte production after the bleeding, during haemolysis, infections (TBC), inflammatory processes or malignancies. Thrombocytosis also appears following splenectomy, its duration is short, some weeks only. The secondary thrombocytosis does not lead to thrombotic complications.

The primary thrombocytosis is named essential thrombocytosis. Increased thrombocyte number is due to their enhanced production. The thrombocytes are of bizzare shape and the signs of their dysfunction are often present (reduced number of adrenaline receptors and abnormal reaction to adrenaline). The thrombocytes may occur in clusters.

2.4.1.3 Qualitative disorders of platelets

Inborn defects of thrombocytic functions are uncommon. If occurring they are associated with mucosal bleeding. Acquired defects of thrombocyte functions are more frequent, especially during acetylsalicylic acid taking and in haematologic diseases. They become clinically important if a haemostatic effect occurs simultaneously (e.g. thrombocytopenia, anticoagulant therapy).

Thrombasthenia (Glanzmann’s syndrome). This condition is an inborn disorder characterized by prolonged bleeding time, absence of thrombocyte aggregation and their adhesion to collagen, therefore mucosal bleeding occurs. In thrombasthenia there is absence of two surface glycoproteins (IIb and IIIa), necessary for binding of fibrinogen with thrombocyte’s surface and for thrombocyte aggregation. The bleeding can be life-threatening in this condition.

Release defects of thrombocytes resulting in impaired platelet aggregation comprise several syndromes. It can be a defect similar to the acetylsalicylic acid effect, where the thrombocyte structure is normal but the thrombocyte activity is impaired. Defect of phospholipase activity or enzyme deficiency in prostaglandin formation, like cyclooxygenase or thromboxane synthetase, may be involved. In other cases a defect of the content of thrombocytic granules may be present, especially of ADP or serotonin content. In Hermanovsky–Pudlak syndrome is the disorder of thrombocytes associated with albinism. The ADP release is needed for normal function thrombocytes, thus this defect becomes manifest in trauma, birth or another stress.

Disorders of thrombocyte adhesion. This condition occurs in two inherited haemorrhagic diatheses:

1. in von Willebrand’s disease and
2. in Bernard-Soulier syndrome.

In von Willebrand’s disease occurring in both men and women is the thrombocyte count normal. Owing to the plasmatic factor deficiency needed for interaction of thrombocytes with collagen is the adhesivity of thrombocytes to subendothelium impaired. Von Willebrand’s factor (vWF) is under physiological circumstances syntetised in endothelial cells and in megakaryocytes. The vWF formed circulates in blood in a makromolecular complex with coagulation factor VIII. Patients with von Willebrand’s disease exhibit except for vWF missing also decrease in the factor VIII. In von Willebrand’s disease the laboratory tests show prolonged bleeding time, reduced adhesion of thrombocytes, normal aggregation, but the agglutination of thrombocytes is not present. It is manifested by mucosal bleeding and menorrhagia. The condition can improve after application of vWF. In Bernard-Soulier’s syndrome giant thrombocytes are found in peripheral blood, the bleeding time is prolonged and mucosal bleeding is present. Glycoprotein Ib mediating the binding of vWF is not present in platelet membrane. The level of vWF is nevertheless normal, but the defective adhesion of thrombocytes is not corrigeable by vWF owing to the absence of thrombocytic receptors. This hereditary condition occurs infrequently.
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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 thromboxane 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 24hrs. 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 teleangectasia (morbus Osler-Weber-Randu). The bleeding arises from mucosal teleangectasia 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 mucosa of respiratory system, GIT, urogenital tract are present. Fistulae use to be present in pulmonary vessel bed. Teleangectasia 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