Coagulation Glossary
This test consists in timing the appearance in fresh drawn blood of a clot in presence of silicious earth as contact activating agent. The ACT is a rapid test used especially in Intensive Care units and for monitoring heparin therapy.

A venom purified fraction of Agkistrodon Contortrix Contortrix (Southern Copperhead snake) which has a procoagulant activity on citrated plasma or on purified solutions of fibrinogen, even in absence of Calcium ions. Its enzymatic action is carried out releasing the Fibrinopeptide B (FPB) of the β chain of the fibrinogen. This action occurs only if previously there has been a sufficient release of Fibrinopeptide A (FPA) or with a long incubation time or by adding Batroxobin (Venom of Bothrops Atrox).

Coagulation time (expressed in seconds and decimals) of platelet poor plasma after the addition of an activator (Ellagic Acid, Kaolin, Silica) of the contact phase, of phospholipids as platelet substitute and calcium ions. This test screens the intrinsic pathway of coagulation and is prolonged in cases of deficiencies of Kallikrein, High Molecular Weight Kininogen, Factors XII, XI, IX, VIII, X, V, II and Fibrinogen. It is also sensitive to the presence of endogenous Lupus-like (LAC) inhibitors (auto-antibodies), to the presence of Heparin and other oral anticoagulants.

Substance with an enzymatic and catalytic activity able to promote the start of the clotting mechanisms (inhibitor or fibrinolytic). The clotting factors, after their partial proteolysis, are potential enzymatic activators. They are usually not present in blood, with the exception of the Factor VIIa. Some, negatively charged, natural and artificial substances are catalytic activators of the coagulation system. The binding of these surfaces and the Factor XII activation starts (through the kinin/prekallikrein system) an autocatalytic loop with the activation of the intrinsic pathway.

Adenilic phosphorilated nucleotide contained in many cells, including the platelets. In the platelets, the ADP is present in two pools: a metabolic pool, which assures the energetic metabolism and is constantly consumed, and a storage pool which is located in dense body granules. The ADP of the storage pool is released into the extracellular medium during the platelet activation.

This substance induces aggregation at a very low concentration (10⁻⁶ M).

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**Coagulation Glossary**

**ACT**

*(Activated Coagulation Time)*

This test consists in timing the appearance in fresh drawn blood of a clot in presence of silicious earth as contact activating agent. The ACT is a rapid test used especially in Intensive Care units and for monitoring heparin therapy.

**A.C.T.E.**

*(Agkistrodon Contortrix Thrombin-like Enzyme)*

A venom purified fraction of Agkistrodon Contortrix Contortrix (Southern Copperhead snake) which has a procoagulant activity on citrated plasma or on purified solutions of fibrinogen, even in absence of Calcium ions. Its enzymatic action is carried out releasing the Fibrinopeptide B (FPB) of the β chain of the fibrinogen. This action occurs only if previously there has been a sufficient release of Fibrinopeptide A (FPA) or with a long incubation time or by adding Batroxobin (Venom of Bothrops Atrox).

**Activated Partial Thromboplastin Time**

Coagulation time (expressed in seconds and decimals) of platelet poor plasma after the addition of an activator (Ellagic Acid, Kaolin, Silica) of the contact phase, of phospholipids as platelet substitute and calcium ions. This test screens the intrinsic pathway of coagulation and is prolonged in cases of deficiencies of Kallikrein, High Molecular Weight Kininogen, Factors XII, XI, IX, VIII, X, V, II and Fibrinogen. It is also sensitive to the presence of endogenous Lupus-like (LAC) inhibitors (auto-antibodies), to the presence of Heparin and other oral anticoagulants.

**Activator**

Substance with an enzymatic and catalytic activity able to promote the start of the clotting mechanisms (inhibitor or fibrinolytic). The clotting factors, after their partial proteolysis, are potential enzymatic activators. They are usually not present in blood, with the exception of the Factor VIIa. Some, negatively charged, natural and artificial substances are catalytic activators of the coagulation system. The binding of these surfaces and the Factor XII activation starts (through the kinin/prekallikrein system) an autocatalytic loop with the activation of the intrinsic pathway.

**ADP**

*(Adenosin-5’ diphosphate)*

Adenilic phosphorilated nucleotide contained in many cells, including the platelets. In the platelets, the ADP is present in two pools: a metabolic pool, which assures the energetic metabolism and is constantly consumed, and a storage pool which is located in dense body granules. The ADP of the storage pool is released into the extracellular medium during the platelet activation. This substance induces aggregation at a very low concentration (10⁻⁶ M).

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**Fig. 1 - ADP (adenosin-5’ diphosphate).**
A fibrinogenemia

The congenital afibrinogenemia derives from a lack of the specific protein synthesis. The inheritance is autosomal and recessive. In the plasma of patients with this disease, the fibrinogen, with a protein concentration of less than 5 mg/dL, is not detectable with electrophoresis, precipitation with thrombin or heat precipitation, but only and seldom with immunological methods.

Agregometer

A photometer which measures the changes of the light transmission through a platelet suspension in an agitated cuvette, thermal controlled. The light transmittance increases as the platelets aggregate and the signal is graphically recorded. With this instrument, the aggregation measurement is semi-quantitative.

Alpha 1-Antitrypsin

A glycoprotein with a molecular weight of 54 KD. It inhibits not only trypsin but other proteases. Until few years ago, it was considered a plasmin inhibitor of secondary importance. Recently, it has been identified as the main inhibitor of activated Protein C.

Alpha 2-Antiplasmin

A fibrinolysis inhibitor that focuses its activity against the plasmin. This protein has a molecular weight of between 60 and 70 KD, migrates as an α2-globulin on electrophoresis gel and is a single chain glycoprotein. The α2-antiplasmin binds to and inhibits plasmin very rapidly and irreversibly: therefore, it is almost impossible to demonstrate the presence of the circulating plasmin with diagnostic tests.

Alpha 2-Macroglobulin

A glycoprotein with a relatively high molecular weight (725 KD) able to form inactive complexes with a large number of proteases including plasmin, thrombin and kallikrein. It acts on the plasmin only when the entire α2-antiplasmin has been saturated.

Antiaggregating drugs

Substances able to inhibit platelet aggregation. According to their mechanism, they can be divided into four groups:

a) Calcium-antagonists that directly interfere with the Calcium ions transport and / or with its effects.
b) Inhibitors that act through the cyclic AMP (when increased, it inhibits the aggregation).
c) Inhibitors that modify the metabolism of the arachidonic acid (inhibiting its synthesis or its release). The inhibitors of Cyclooxygenase belong to this group. The best known inhibitor is acetylsalicylic acid.d) Substances that act in a non-specific way or in non-detectable concentrations in vivo.

Anticoagulant drugs

Substances able to inhibit the coagulation cascade both in vivo and in vitro, acting according to a specific action mechanism. The most widely used anticoagulants in therapy are the following:

- vitamin K antagonists (cumarolics and inandionics)
- heparin (unfractionated)
The vitamin K antagonists are indirect anticoagulants because they do not act directly on the coagulation mechanism, but they decrease the liver synthesis of Factors II, VII, IX, X, Protein C and Protein S. Heparin, on the other hand, acts directly on the clotting mechanism increasing the anti-proteases activity of the antithrombin-III. The most widely used anticoagulant for the sample preparation for the clotting tests is the Sodium Citrate at a concentration of 3.8 g/dL (129 mmol/L) or 3.2 g/dL (109 mmol/L).
Antithrombin III (AT-III)  Protein of 65 KD present in plasma at an average concentration of 20 mg/dL. The AT-III neutralizes the thrombin, the Factor Xa and other factors (IXa, XIIa and XIa). It forms a stoichiometric complex with the enzymes. The complex results from an interaction between an active serine site on thrombin and a reactive arginine site on the AT-III. This reaction is slow and needs to be accelerated by heparin and dermatan sulphate. Quantitative deficiencies and structural alteration of the molecule of this protein are associated with venous thrombosis.

Antithrombotic drugs  These drugs are divided into three classes:
1) platelet antiaggregant drugs
2) anticoagulant therapy drugs
3) fibrinolytic drugs

Fig. 2 - AT-III mechanism and Coagulation Cascade.
**Arachidonic Acid**

A 20-carbon fatty acid, essential precursor in the biosynthesis of the prostaglandines; it is able to induce platelet aggregation, by metabolic products, as the endoperoxides of the prostaglandines and the thromboxane $A_2$. It is also present in lipoproteins, in membrane phospholipids and in the vessel wall. It is used in vitro as an aggregation factor for the study of platelet aggregation, while in vivo, during the platelets activation, it is metabolized by thromboxanes.

**Batroxobin**

Thrombin-like enzyme extracted from the venom of Bothrops Atrox. In vitro, it acts on citrated plasma or on purified solutions of Fibrinogen, in the absence of Calcium ions, releasing the fibrinopeptide A (FPA) of the fibrinogen $\alpha$ chain. It is the active ingredient used in the reagent to measure the reptilase time.

**Beta-Thromboglobulin**

Protein with a molecular weight of about 36 KD. It is specific for platelets, stored in the $\alpha$-granules and released during the activation. In vivo, plasma levels of $\beta$-thromboglobulin (radioimmunoassay) can adequately reflect platelet release.

**Bleeding Time**

It is the time (minutes and seconds) necessary to stop the bleeding caused by a skin cut of defined length and depth done under standardized conditions on the flexor surface of the patients forearm. This test reflects the platelet functionality and the capillary integrity. It is a test of the hemostatic functionality and it is prolonged in thrombocytosis, thrombocytopenia, hypo- and a-fibrinogenemia and in von Willebrand disease; usually it is normal in the coagulation defects. The Bleeding Time is influenced by many analytical variables, for this reason it must be performed by skilled people and standardized in the procedure.

**C4b-BP**

*(C4b-Binding Protein)*

It is a large glycoprotein with a molecular weight of 570 KD. It is composed by seven long identical segments that radiate from a central core. The mobility of C4b-BP on agarose gel electrophoresis is that of a slow $\beta$-globulin and is important in the regulation of the complement pathway. The normal plasmatic concentration is about 20 mg/dL. It has a regulating function on the free Protein S, binding about 60% of the total. Inflammatory states increase its concentration moving the Protein S equilibrium from the free form to the binding form; as a consequence there is a reduction of the anticoagulant activity of the Protein C + S system.
Cofactors needed in some reactions of the coagulation cascade: during the formation of the activator complexes of Factor X (IXa-PF3-Calcium Ions-VIIIa and VIIa-TF-Calcium Ions), in the formation of the prothrombinase (Xa-Va-PF3-Calcium Ions) and in the stabilization of the fibrin by Factor XIIIa. The presence of these ions makes the platelet aggregation irreversible and is needed to bind factors to phospholipids.

Silicate of organic origin, fragments of various species of diatoms, insoluble in water, which gives a high negative superficial charge. This characteristic makes it a good activator of the intrinsic pathway and is used together with cephalin as a reagent for the APTT test.

Group of phospholipids found in all living organisms. Significant constituent of nervous tissue and brain substance. It is a brain chloroformic extract used as a substitute for the PF3 (Platelet Factor 3). The most important phospholipids of the mixture are: fosfatidic acid, fosfatidilserine, fosfatidylethanolamine, fosfatidilinositol and fosfatidiglycerol. The composition is variable according to the animal species and the extraction method.

It is constituted by a short oligopeptide (46 a.a.) to which is linked, in carboxyterminal part of Arg or Lys, a molecule of para-nitroaniline (pNA) as chromophorous group (able to develop colour) that can be detected with usual spectrophotometers. The protease splits the peptidic bound with a release of pNA (coloured) that is proportional to the proteasic activity. The composition and the aminoacid sequence of the synthetic substrate is similar to the active site of the “natural” substrate of which you are measuring the activity or the concentration. If the dimethyl ester of the 5-aminoisoftalic acid (fluorophorous group) is substituted to the para-nitroaniline (pNA), you will obtain fluorogenic substrates similar to chromogenics, but it is necessary to have a fluorometer to detect them.

Fig. 4 - Chromogenic substrate (S-2160)
Functional test for the fibrinogen determination, based on a modified thrombin time method. In presence of a high concentration of thrombin, the fibrin polymerization time of diluted citrated plasma is correlated to the quantity of clottable protein. This test measures the rate of the proteolysis, operated by thrombin, on the $\alpha$ chain of Fibrinogen. This reaction is influenced by substances with an antithrombinic action (FDP, heparin) or by modifications of the tertiary structure of the fibrinogen protein that causes a steric hindrance. In these cases, there is an underestimation of the protein content but a functional reduction will be evident.

All the molecules participating to the so-called “coagulation cascade” are indicated as Coagulation Factors. Since 1954, they have been identified with Roman numbers and some of them are also named after the first patient discovered with this deficiency. Factor activity in plasma is measured as the capacity to correct the prolonged clotting time of a factor deficient plasma and it is expressed in percent activity or in U/mL compared with a well defined standard.

**Factor I - Fibrinogen:** it is a dimer (MW = 340 KD) of three polypeptide chains ($\alpha$, $\beta$ and $\gamma$) with a carboxylic terminal residue. Plasma concentration is usually about 200 - 400 mg/dL. Thrombin is the physiological agent which converts fibrinogen to fibrin and there are other thrombin-like enzymes able to polymerize it.

**Factor II - Prothrombin:** it is a glycoprotein with a MW of 69 KD, that is doubled compared with thrombin (its active form, 35 KD). It is synthesized in the liver and is Vitamin K dependent. Its concentration in the plasma is 10 - 16 mg/dL.

**Factor V:** It is also known as labile factor or pro-accelerin. It is a glycoprotein (MW 330 KD) synthesized in the liver but not vitamin K dependent. It takes part converting Prothrombin to Thrombin.

**Factor VII:** The stable factor is present in plasma in a concentration of 0.1 mg/dL. Human Factor VII has a MW of 60 KD. It is a liver synthesized glycoprotein that is vitamin K dependent and is involved in the extrinsic pathway together with a Tissue Factor. It is the only factor that can exist “in vivo” in its active form.

**Factor VIII:** Antihemophilic globulin (MW > 200 KD, not yet well defined). The concentration in plasma is 1 mg/dL. According to recent studies, it is a complex of two components: one (low MW) with coagulant action and the other (high MW) with antigenic specificity able to adhere the platelet for aggregation. The first component has an important function in the intrinsic pathway of the coagulation cascade. It is deficient in classical hemophilia; on the other hand, the second component is deficient in the Von Willebrand-Jürgens disease.

**Factor IX:** Christmas Factor. It is a glycoprotein (MW = 72 KD) synthesized in the liver in presence of vitamin K. The deficiency of this factor shows a symptomatology similar to classical hemophilia.
**Factor X:** Stuart - Prower Factor. It is a glycoprotein (MW = 59 KD) synthesized in the liver in presence of vitamin K. Its plasma concentration is 0.8 - 1.2 mg/dL and it is a key enzyme of the prothrombinase complex which activates the prothrombin. It is the focal point at which the intrinsic and extrinsic coagulation system converge.

**Factor XI:** Plasma thromboplastin antecedent. It has a MW of about 124 KD. It is synthesized in the liver and its plasma concentration is 0.4 - 0.7 mg/dL.

**Factor XII:** It is also known as Hageman Factor. The deficiency of this factor is asymptomatic. It is the central enzyme of the “contact phase” because it is present in the early phases of the coagulation and its activation happens by the contact with extraneous surfaces. It is also involved in the activation of fibrinolysis and in the system of plasma kinins. It has a MW of about 80 KD.

**Factor XIII:** It is the Fibrin stabilizing factor and it is a tetramer of two proteins with a MW of 320 KD. It is present in plasma as zymogen and it is activated by thrombin in the presence of calcium ions, it catalyses the formation of cross-links between fibrin monomers.

**Fletcher Factor** - See Prekallikrein.

**Fitzgerald Factor** - See HMWK.

**Coagulometer**
Analyzer dedicated to the in vitro screening of the coagulation reactions. It measures the clotting time of plasma after the activation of the coagulation process. The instruments present in the market have different degrees of automation and different clotting detection systems. Under this system, the instruments can be divided into two classes: photo-optic and mechanical detection. The first group, which uses the clot optical property, measures with a turbidimetric or with a nephelometric technique. The second class uses mechanical or electronic properties (drag resistance of solid bodies or electrical conductivity).

**Collagen**
It is a macromolecule with a helicoidal structure (triple helix), mainly composed by hydroxyproline and hydroxylysine; it is present in large quantities in the vessel wall and it is able to induce “in vitro” platelet aggregation. According to the biochemical configuration, it is possible to identify a different kind of collagen. The molecular structures of the collagen responsible of the platelets adhesion are well known.

**Complement**
The complement system consists of distinct proteins that interact sequentially in order to determine specific effects of an inflammatory response. It also participates in the interaction with other systems: coagulation, fibrinolysis and kinins.
D-Dimer

It is a fragment formed by the proteolytic action of the plasmin on the fibrin. The D fragment is a dimer; it is combined by two units linked by $\gamma$-$\gamma$ bonds formed by Factor XIII action. Recently it becomes possible to test D-Dimer by immunoenzymatic or radioimmunologic techniques which use monoclonal antibodies. The dosage of the D-Dimer has the advantage, if compared to the FDP test, that is performed using citrated plasma. It can be considered as a marker of a past or present coagulation activation.

Dermatan Sulphate

It is an endogenous glycosaminoglycan with a molecular weight of about 30 KD. It is synthesized by the vessel endothelium cells. It catalyses the thrombin inhibition by heparin cofactor II and keeps the lumen of the blood vessels open.

DIC (Disseminated Intravascular Coagulation)

In certain disease states, procoagulant activity may develop in the blood and bring about disseminated intravascular clotting. As a consequence, platelets and blood coagulation factors may be consumed and fibrin formed during the process is deposited in the microcirculation, in particular in brain, kidneys, lungs and skin. In severe cases of DIC there is usually a depletion of platelets, fibrinogen, prothrombin, Factor V, Factor VIII and AT-III. This syndrome has been observed in many diseases.

The most frequent DIC causes are cancer, sepsis, inflammatory states, leukemia, liver diseases, obstetrical complications, autoimmune diseases, traumas and surgery operations. Useful laboratory tests with this syndrome are the platelet count, fibrinogen concentration, the level of fibrinogen-fibrin degradation products (FDP and D-Dimer) and their follow-up.

Fig. 5 - Dermatan Sulphate and Coagulation Cascade.
Deep Vein Thrombosis (DVT)

The deep vein thrombosis is defined as a thrombotic occlusion of one or more veins in the venous system. A venous thrombus is formed in the valvular pockets where physiologically a slow-flow stream of blood is present. Thrombosis has many different origins; the hypothesis of the vascular component (lesion) in the thrombogenesis induction is no longer indicated as the only cause. Damage to the blood vessel endothelium, which occurs together with the stasis, the hypercoagulability and the coagulation inhibitor deficit are some thrombogenetic causes. The vascular damage is present among the thrombogenetic factors in some specific situations such as surgery operations and severe leg trauma.

Dysfibrinogenemia

An inherited functional defect of fibrinogen affecting the final step of the blood coagulation cascade: the conversion of fibrinogen to fibrin. The functional defect is caused by a structural abnormality of a variant molecule. Fibrinogen variants are named after the city where they were found or characterized for the first time.

Ellagic Acid

A substance slightly soluble in water; when the pH is neutral, it is dissociated in two hydroxylates and becomes negatively charged. Because of this characteristic, it can be used as a catalyst of the contact factors activation mechanism, as many other substances which are negatively charged (collagen, kaolin, silica). It is usually used together with phospholipids (cephalin) in the APTT reagent, the global test for the intrinsic pathway factors screening.

Fig. 6 - Ellagic acid

Emboli

Occlusion of one or more vessels caused by emboli. The embolus can be solid, septic (derived from inflammatory states), tissutal, cellular (cancer, platelet aggregates, leucocytes, erythrocytes) or liquid such as the amniotic liquid or adipose tissue (fractures, burns, etc.) and gaseous (air, nitrogen bubbles). The most common form of embolism is the pulmonary embolism.

Fibrin (ogen) Degradation Products (FDP)

The plasmin digests fibrinogen to soluble degradation products. Initial products derived from fibrinogen was denoted as fragment X (a mixture of several molecular species in which the two α chains had been degraded to various extent). Fragment X is further split asymmetrically into Fragment D and Y. Fragment Y is finally digested to core fragments D and E.
Fibrin

Stabilized fibrin is the result of the physiological polymerization of the monomers derived from Fibrinogen after the release of the fibrinopeptides A and B caused by Thrombin. It has a lamellar structure that is obtained by successive links between the domains. It is stabilized by Factor XIIIa which inserts cross-links between oriented fibrin monomers. The plasmin is able to produce FDP and D-Dimer fragments.

Fibrinogen

See Coagulation Factors

Fibrinolysis

Fibrinolysis is the term given to the process dissolving fibrin clots or deposits within the body. The fibrinolysis plays a major role in the repair of injured tissues and in the proteolytic mechanisms of ovulation and embryo implantation. This system results from the action of three components: plasminogen, plasminogen activators that can be of different origin (haematic, vascular and tissutal) and the inhibitors that neutralize the plasmin or interact at the plasminogen activation phase.

Fibronectin

Glycoprotein (MW 420 KD) that is found in many connective tissue matrices of the organism. It is present in plasma as dimer of two very similar subunits joined by disulphide bounds. It seems that Factor XIII is able to link it with a covalent bound to the a-chain of the fibrin. It is considered a platelet adhesive protein and a marker of the endothelium damage.

FPA
(Fibrinopeptide A)

It is the peptidic fragment of the α chain of fibrinogen that is released by thrombin. When the peptides (A and B) are removed, the fibrinogen is transformed in fibrin monomers. The dosage of this peptide is a clear index of the “real” activation state of the coagulation system because of its short life (3 - 5 minutes).

FPB
(Fibrinopeptide B)

It is the peptidic fragment which is released by splitting Arg-Gly bounds of the β chain of the central domain of the Fibrinogen molecule. This cleavage is caused by thrombin.

Fragment F 1 + 2

It is the amino-terminal fragment released from Prothrombin when the prothrombinase (complex Xa - Va - PF3 - Calcium Ions) converts it to thrombin. It has a half-life of 90 minutes and it is possible to dose it as a marker of activation of coagulation (RIA or Immunoenzymatic method).

Fragment X

See FDP

Fragment Bß 1-42 and 15-42

See Plasmin

Glycosaminoglicans

See Heparin

They are polysaccharides containing glucuronic acid and glucosamine.
**Hemophilia**

This term is sometimes used in a general sense referring to all inherited coagulation defects but more often and specifically to deficiencies of Factor VIII (Hemophilia A) and Factor IX (Hemophilia B). Hemophilia A, also identified as “classical hemophilia”, is a Factor VIII deficiency that is inherited through sex-linked recessive gene.

In hemophilia A, the clotting activity of Factor VIII (Factor VIII : C) is absent or reduced, while the carrier protein (VIIIIR : Ag) is apparently normal. It is almost exclusively in male subjects because they are holders of only one x chromosome. It is possible to identify at least four kinds of hemophilia A: “serious”, when the Factor VIII is present in a quantity lower than 2%, “moderate” between 2% to 5%, “light” between 5% to 25% and “subhemophilia” between 25% to 50% which is considered a subclinical case. The clinical features of the disease are strictly related to the degree; the serious hemophilia shows repeated and severe hemorrhatosis, while in the moderate deficiency these aspects are less frequent and in the light deficiency they are totally absent or only following traumatic injury. In the hemophilia A, normally the APTT and the thrombin generation time are prolonged (in time).

In the serious haemophilic, beside the Factor VIII deficiency, it is found also an anti-Factor VIII antibody, probably due to an immuno reaction after the supply of cryoprecipitates. The presence of this inhibitor makes the total clinical picture very serious.

The clinical feature of hemophilia B is indistinguishable from hemophilia A, but it presents a quantitative decrease of the Factor IX coagulant activity.

**Hemostasis**

It is a complex sequence of biological processes that have the aim to protect the blood from possible losses and to restore the vascular integrity following injuries. The process can be divided conveniently in three stages: vascular-platelet, coagulation and repair. Each one of these phenomena is the result of a complex interaction between blood (plasma and cellular elements) and vessel wall. In the first stage there is the formation of the platelet plug; in the second phase it will be consolidated by Fibrin. The coagulative process must be localized; this happens because it presents a physiological inhibitor system (AT-III, PC, PS, HC II) and because the reactions happen on the receptors sited on the cellular membranes.

The Fibrinolysis is the final stage of hemostasis. The Fibrinolysis removes the fibrin deposits and restores the integrity of the injured vessels.

**Heparin**

Heparin is composed by a family of polysaccharide chains mainly constituted by regular sequence of a trisulphate disaccharide (L-iduronic acid-2-sulphate D-glucosamine-N,6-disulphate) interrupted by irregular regions constituted by various combinations of disaccharides di and mono sulphates that contain also glucuronic acid and N-acetylated D-glucosamine.

The proportion between regular and irregular sequences, the length of the single sequences and the average length of the chains are related to the animal species, to the tissutal source and in some cases also to the extraction and purification procedures. The different sequences of heparin are to be considered functional domains with a different affinity for the plasma proteins and different contribute to the biological activities and their pharmacological expression.

The present knowledges about structure-activity relations of the heparins can be summarized in the following way:
- over 70% of anticoagulant activity of the most used heparins is related to a specific sequence that is the active site for the Antithrombin. 

- the anticoagulant properties that have different mechanisms not mediated by AT (direct interaction with the Thrombin and other factors and indirect action mediated by HC II) and also the lipase activity, are related to the regular sequences of trisulphate disaccharides. 

The capability to inhibit Factor Xa reflects only partially the in vivo antithrombotic properties of the heparin species. Heparin acts as a catalyst of the reaction between AT-III and serine-protease, increasing 1000 times the inhibition speed. 

The use of heparin is indicated for concomitlated thrombosis and for short-term prophylaxis of situations with high thromboembolic risk.

![Heparin structure](image)

Fig. 7 - Heparin structure  
$I_{2S}$ = L-iduronic acid 2-sulphate; $A_{NS,6S}$ = D-glucosamine N, 6-disulphate; $I$ = L-iduronic acid; $G$ = D-glucuronic acid; $A_{NA}$ = N-acetylated aminosaccharide

**Heparin Anticoagulant Therapy**

It consists in a parenteral administration or in a continuous infusion of heparin.  

In the first case the anticoagulant action has a short duration (4-5 hours) because it is quickly metabolized and eliminated.  

The aim of the anticoagulant therapy is fundamentally prophylactic, it prevents the extension of thrombus already formed and avoids the formation of new thrombi.  

Clinical studies have demonstrated that the risk of recurrent venous thromboembolisms is low if the activated partial thromboplastin time (APTT) is maintained higher than 1.5 times and lower than 2.5 times control value.  

Test used to monitor therapy include APTT and tests that measure the specific interaction of heparin with activated coagulation factors.  

The heparin rebound is referred to the heparin re-appearance in circle after its neutralization by protamine.
Heparin Cofactor II is a member of the family of anticoagulant proteins that inactivates thrombin (but no other serine-protease) by forming an equimolar complex. The rate of thrombin inactivation by Heparin Cofactor II is greatly potentiated by heparin as with antithrombin III and by the glycosaminoglycan dermatan sulphate, the latter having no potentiating effect on the thrombin-inactivating activity of antithrombin III. Heparin Cofactor II is a minor inhibitor of thrombin formation in vitro, accounting for a small proportion of the total antithrombin activity of plasma. The role of Heparin Cofactor II deficiency as a cause of inherited thrombophilia requires confirmation.

Hirudin

It is a potent inhibitor of thrombin, originally obtained from the medicinal leech (Hirudo medicinalis) and currently made through recombinant technology.

Recombinant hirudin (MW= 6.5 KD) has been investigated for its potential clinical applications as an anticoagulant. Studies have shown that the mechanism of the anticoagulant action of this agent is markedly different from that of heparin.

Plasma kininogens are large proteins that contain potent vasoactive peptides, the kinins.

Human plasma contains at least two distinct kininogens, HMW kininogen and low molecular weight (LMW) kininogen. HMW kininogen contains approximately one-fifth of the kinin content of plasma and exists as a single polypeptide chain of approximately 105 KD MW. LMW kininogen contains approximately four-fifths of the kinin content of plasma and consists of a single polypeptide chain of approximately 60 KD MW.

Kinins are released from the internal amino acid sequences of kininogens by limited proteolysis by kallikrein that may be derived from plasma or from tissue sources. The tissue enzyme is equally potent in releasing kinin from both types of kininogen whereas plasma kallikrein is 40 times more active in liberating bradykinin from HMW kininogen than from LMW kininogen. HMW kininogen is a non enzymatic cofactor that is central to contact activation reactions. The carboxyl terminal region of the molecule contains a most unusual aminoacidic sequence with a high positive charge. This sequence is essential for the contact activation reactions, probably because it creates a link to negatively charged surfaces.

Howell Time

A very basic protein (MW 60 KD) that shows big affinity for acid substances, in particular for Heparin and Glycosaminoglycans.

It has a regulatory function (also of fibrinolytic system) because binds the Plasminogen, leaving only the free form available for the Fibrinolysis.

INR (International Normalized Ratio)

It is calculated raising the ratio value (PT patient in seconds/PT normal reference value in seconds) to the ISI value of thromboplastin.

ISI (International Sensitivity Index) is the index of sensitivity of a thromboplastin in comparison to a primary standard.

ISI value is strictly related to each sales batch and also to the technique used.

\[
\text{INR} = \frac{\text{Ratio}}{\text{ISI}}
\]
Protein composed by two polypeptidic chains linked by disulphide bonds, one heavy chain of 43 KD and one light chain of MW between 33 and 36 KD. It is a serine-protease present in plasma as zymogen (prekallikrein) which is activated by limited proteolysis of Factor XIIa. The enzymatic action of the kallikrein is carried out releasing kinins from the kininogens, activating the Factor VII, the plasminogen and the Factor IX. The kallikrein inactivation in plasma occurs by the plasma protease inhibitors. The main inhibitor is the C1 while AT-III and α2-antiplasmin are less effective.

Fig. 8 - A proposed mechanism for the surface activation of blood coagulation in the presence of an anionic surface.

Kaolin

It is an aluminium silicate, insoluble in water, which presents a clear superficial negative charge. This characteristic makes it suitable as an activator of the intrinsic pathway and it is used together with the cephalin phospholipids as an activator for the APTT test. This substance (in absence of phospholipids) is sensitive to the antiphospholipids antibodies present in patients with Lupus Anticoagulant and it is used in the KCT test (Kaolin Clotting Time) in order to diagnose this disease.

KCT
(Kaolin Clotting Time)

Coagulation time (expressed in seconds) of a platelet poor plasma after addition of a kaolin suspension (20 g/L) and of calcium ions. It is a test used to confirm the presence of LAC inhibitors.

LAC
(Lupus Anticoagulant)

Inhibitor often present in the plasma of subjects with frequent thrombotic events and spontaneous abortions, together with thrombocytophaenia. LAC is G or M immunoglobulin against the phospholipids which are essential for intrinsic and extrinsic coagulation pathways. It is an antibody not always present in cases of Systemic Lupus Erythematosus (SLE).
The LAC-inhibitor presence determines a prolongation of APTT or PT. The sensitivity of these tests is related to the reagent and it must be used only platelet free plasma. The presence of an inhibitor makes unable Normal Pool to correct the Coagulation Time, otherwise Normal Pool is able to correct it when there is a factor deficiency.

**Latex**

They are polystyrene particles with variable dimensions (0.1 - 10 micron) on which are coated specific immunoglobulin or purified antigens. The reaction between antigen and antibody forms large macroscopic or microscopic aggregates. This technique is used to dose the concentration of FDP in serum and D-Dimer in plasma.

**L.M.W.H. (Low Molecular Weight Heparin)**

This acronym is referred to a family of Heparins with a MW of 5 KD. It has been proven that, being equal the contents of active site for the AT and sequences of trisulphate disaccharides, the anticoagulant activity (expressed by Thrombin Inhibition Time) is strictly related to the MW. It decreases with the shortening of the heparin chains to become insignificant for MW below 3 KD. A well defined sequence is a requirement in order to form a ternary complex among heparin, antithrombin and thrombin.

On the other hand it must be considered that the chains and the pentasaccharide of the active site for the AT-III, are enough for the inhibition (mediated by AT-III) of the Factor Xa, even if their MW is lower than 5 KD. These heparins seem to have a better bioavailability and a better antithrombotic activity.

The bioavailability is determined by the competition with plasma components as lipoproteins and calcium ions and by the absorption of the vessel wall.

The L.M.W.H. can be obtained by fractionation methods, but more frequently they are obtained by controlled depolymerization.

**Fig. 9 - Hypothetical model of interaction among Heparin (HEP), Antithrombin III (AT-III) and Thrombin (F IIa).**

The model shows the formation of a ternary complex involving an octadecasaccharidic sequence of the Heparin chain.
Nephelometry

It is an analytical technique used to study non homogeneous solutions. The colorimetry measures the quantity of the incident light which is absorbed or transmitted by the solute molecules, the nephelometry measures the quantity of light scattered (reflected or refracted) by the particles in suspension. The quantity of light refracted depends on the dimension of particles, on the wavelength of incident light (Rayleigh law), on the angle between the source of light and the detector and on specific parameters of the analyzed system (temperature, pH, ionic strength).

Oral Anticoagulant Therapy

It consists in an oral administration of vitamin K antagonists drugs, in order to inhibit (in vivo) the liver synthesis of Factor II, VII, IX and X and of the anticoagulant proteins known as PC and PS. The dose and duration of the therapy are related with the severity of the disease. The most commonly used coagulation tests to monitor this therapy should be sensitive to the reduction of activity of all the factors of the prothrombin complex. The most simple and used is PT. All tests that use a bovine thromboplastin, in presence of Fibrinogen and bovine Factor V are more specific because sensitive also to PIVKA. The ideal therapeutical range is between 2.0 and 4.0 (INR).

PA-I (Plasminogen Activator Inhibitor)

It has been demonstrated the existence of at least three specific inhibitors of t-PA:

- PA-I 1 (endothelial) - it is produced or associated in the endothelium cells that produce t-PA and to the platelets. It is a glycoprotein that represents 60% of all the circulating PA-I;
- PA-I 2 (binding protein) - it inhibits the t-PA and the urokinase and is almost 40% of the total PA-I;
- PA-I 3 (placentar) - it is produced by placenta monocytes and its level increases during pregnancy. These inhibitors act quickly and they are more specific than other t-PA inhibitors. It is supposed that high levels of these inhibitors (congenital or acquired) induce thromboembolic complications.

PAF (Platelet Activating Factor)

This term describes the biological activity of a substance released by several cells (e.g. basophils, neutrophils, macrofages). This substance is a phospholipid that appears to be a mediator of inflammation and allergic reactions. It is a strong inducer of platelet aggregation and its action is independent on ADP or arachidonate metabolism.

Pathways

Common pathway - The common pathway includes distinct reactions that, starting from Factor Xa, lead to the formation of Fibrin. Factor X, whatever has been formed (Extrinsic or Intrinsic Pathway) reacts with Factor V, calcium ions and phospholipids of the activated platelets (PF3) forming a macromolecular complex (prothrombinase) able to convert Prothrombin into Thrombin. Thrombin is a proteolytic enzyme that converts fibrinogen into fibrin, splitting the fibrinopeptides A and B from the molecule of fibrinogen. The residual part of the molecule is the Fibrin monomer; several monomers polymerize by themselves to form an unstable polymer. Afterwards, Factor XIIIa and calcium ions form covalent links between the monomers and stabilize the fibrin clot.

Extrinsic pathway - The extrinsic pathway may be considered as a series of reactions which follow tissutal damage caused by an injury or by a thrombogenic surface (atheroma); the pathway is activated by the release of Thromboplastin or Tissue Factor which forms a complex with Factor VII in presence of calcium ions that converts Factor X to Factor Xa and is capable of activating Factor IX.
**Intrinsic pathway** - It is the mechanism able to show a procoagulant activity after the contact with particular surfaces; in vitro, these surfaces are materials with a molecular or a crystalline structure with a negative charge such as Ellagic Acid, glass, Kaolin, some preparations of connective tissue or collagen; in vivo, the real nature of the surface is still unknown.

At least four plasma proteins are involved in the contact phase: Factor XII, Factor XI, Prekallikrein and the High Molecular Weight Kininogen. The complicated interaction among these four factors on the contact surface transforms Factor XII in Factor XII activated (Factor XIIa), this Factor converts the Factor XI in Factor Xla.

The Factor XIIa is able to activate the prekallikrein in kallikrein and the kallikrein is able to convert Factor XII. This reciprocal activation of Factor XII and prekallikrein is a positive feedback mechanism.

Factor Xla activates Factor IX; Factor IXa, Factor VIIIa, calcium ions and PF3 form a complex which activates Factor X to Factor Xa. Factor Xa has also the capability to activate Factor VII.

The concept of two separate systems for blood coagulation is no longer valid, but from a diagnostic and didactical view point the concept still remains useful.

*Fig. 10 - Coagulation pathways.*
The PF3 is a membrane phospholipid which has the capability to enhance prothrombin activation. When the platelets are stimulated, a marked increase of this phospholipid on the membrane external surface occurs.

PF4
(Platelet Factor 4)

A 30 KD protein, contained in the α granules of the platelets, released during the activation phase and able to neutralize heparin. It can be tested in plasma with the immunoenzymatic technique as a platelet marker.

PIVKA
(Protein Induced by Vitamin K Absence or Antagonists)

These substances are vitamin K-dependent proteins (Factors II, X, VII, IX, PC and PS) without γ-carboxyglutamic acid residues. The lack of these residues inhibits the binding with calcium and the protein becomes functionally inactive and a competitive inhibitor of the active factor.

Plasmin

It is a double chain serine-protease derived by a limited lysis of Plasminogen. The active site is placed in the light chain. The heavy chain contains the binding sites which could be blocked by various fibrinolytic inhibitors. Plasmin is able to digest several proteins including Fibrin, Fibrinogen, Factor V and Factor VIII. The plasmin acts always over the Bβ chain. If the substrate is the fibrinogen or the fibrin I, the detached peptide is the fragment 1-42. On the other hand, if the substrate is fibrin II (produced by the lysis of thrombin with release of FPB), the fragment 15 - 42 is detached. When plasmin acts on stabilized fibrin, some degradation products (with crossed links) are formed. The prototype of these products is known as D-Dimer.

Fig. 11 - Schematic representation of markers of thrombin- and plasmin-mediated proteolysis of fibrinogen.
**Plasminogen**

It is a glycoprotein (MW 80-90 KD) synthesized in the liver and present in plasma in its native form (Glu-Plasminogen). The electrophoresis method recognizes it as a β-globulin with a heterogeneous pattern. The plasma concentration of plasminogen is about 200 mg/L (in normal adults). From a functional point of view, it takes a position in the fibrinolytic pathway similar to that of Prothrombin in the coagulation cascade. Traces of plasmin transform the Glu-Plasminogen to Lys-Plasminogen and this form is more susceptible to plasminogen activators so it is quickly converted to plasmin.

**Platelet Activation**

Phenomenon that occurs by the action of stimulating substances that includes morphological and biochemical changes of the platelet. The biochemical modifications include the exposure on the platelet wall of specific membrane receptors. The activation phase is a preparation of the platelet aggregation.

**Platelet Adhesion**

Platelet capability to adhere to artificial (glass, biopolymers,...) or natural (subendothelium) surfaces. Adhesion of platelets to subendothelium surfaces is dependent upon the Von Willebrand factor that interacts with a specific platelet membrane receptor (glycoprotein I b) and a component on the blood vessel wall.

**Platelet Aggregation**

The platelet aggregation describes the formation of a cell to cell contact between adjacent platelets. In vitro, the aggregation can be reversible or not. It is necessary to change the shape in order to let the aggregation start. The discoid platelets change into irregular spheres with numerous pseudopods. This is necessary but not a specific requirement; the presence of specific platelet membrane glycoproteins are needed for the platelet aggregation that is determined by the release of substances (ADP and Thromboxane A2) which activates the circulating platelets. In vitro the aggregation can be induced by several different substances. In vivo, in order to start the aggregation, two fundamental requirements are needed: the endothelium injury and the coagulation activation. The main aggregation activators (in vivo) are the collagen and thrombin.

**Platelets**

Anucleated blood cells with a diameter of 2 - 5 µ. They have in the cytoplasm different types of organelle, among them there are α and δ granules. The δ-granules include ADP-ATP, serotonin and calcium; the α-granules include many components (e.g. platelet fibrinogen, platelet Willebrand, PF4 etc.). Platelets play a key role in hemostasis because they release vasoconstrictor substances (i.e.: serotonin and catecholamine) and aggregate in order to form the platelet plug. Their cellular surface (beside the presence of PF3) is important in the coagulative phase as a major supplier of phospholipids.

**PPP (Platelet Poor Plasma)**

This plasma is obtained after the centrifugation of the blood sample when spun at 1000 - 2000 g for at least 10 minutes. In order to obtain a platelet free plasma, it is necessary to centrifuge twice the blood for 20 minutes.

**Prekallikrein**

It is a single polypeptidic chain glycoprotein (MW 85-87 KD). It is a serine-protease zymogen activated by a partial lysis by Factor XIIa. The plasma levels of prekallikrein are between 2.5 and 4 mg/dL.
Protac

It is a protein isolated from the Southern Copperhead snake venom (Agkistrodon C. Contortrix); it is composed by a single polypeptidic chain (MW 39 - 42 KD).
It does not have any proteinase activity and is a fast-acting activator of Protein C.

Protamine

It is a basic protein with the capability to induce non enzymatic polymerization of soluble complexes of Fibrin monomers that are formed in plasma in the presence of Thrombin.
This phenomenon is known as paracoagulant and it consists in the formation of fibrin filaments in presence of the protamine sulphate.
It does not occur in normal plasma.
The protamine is used in therapy as a specific antidote against heparin effects, because its positive charge forms a stable salt with heparin.

Protein C

It is a two-chain vitamin K dependent plasma glycoprotein (MW 62 KD) circulating in blood as a zymogen with a concentration of about 0.4 mg/dL. After the thrombin cleavage of a N-terminal peptide from the heavy chain, the zymogen is transformed to the enzymatically active form (Activated Protein C or APC); its anticoagulant function occurs by proteolytic inactivation of Factor Va and Factor VIIIa, non enzymatic co-factors in the coagulation cascade.
Protein C is activated by thrombin (the only physiological activator known).
Thrombin, associated with a cell surface cofactor (thrombomodulin, an endothelial cell protein), is able to enhance the activation of PC. In order to show its anticoagulant properties, it needs also the presence of Protein S (another cofactor).
Protein C stimulates the Fibrinolysis by decreasing the activity of endothelium-derived inhibitor of t-PA.
The PC deficiency is a cause of superficial thrombophlebitis and of juvenile venous thrombosis in general.
The defect is transmitted by autosomal dominant gene.
Two different deficiencies are known: quantitative (Protein C antigen) and functional.
Protein S

It is a single-chain vitamin K dependent protein (MW 64 KD). It is the first known vitamin K dependent protein that is not a serine-protease zymogen. This protein exists in plasma in two forms: free (40%) and bound to complement C4b-binding protein (60%). Free Protein S functions as the cofactor for activated Protein C, but the bound protein is non-functional. The Protein S deficiency is transmitted by autosomical dominant gene. Homozygous protein S deficiency with unmeasurable plasma levels is not associated with dramatic clinical picture typical of homozygous PC deficiency. Individuals with low PS plasma levels are at high risk of developing venous thromboembolic events. Four kinds of deficiency are used for classification: one is antigenic and three are functionals. The plasma levels of PS decrease during pregnancy, during oral contraceptives or vitamin K antagonists therapy and in DIC patients.

Fig. 12 - Protein C/Protein S mechanism and Coagulation Cascade.
**Prothrombin**  
*See Coagulation Factors*

**Prothrombin Complex**  
This definition identifies the factors II, VII, IX and X.

**Prothrombin Time**  
Coagulation time (expressed in seconds and decimals) of a mixture of platelet poor citrated plasma and brain extract of different animal origin (thromboplastin) containing calcium ions. The complex that will be formed by plasma Factor VII and tissue factor, in presence of calcium ions, is able to activate Factor X. This test allows checking the integrity of the extrinsic pathway.

**PRP**  
(Platelet Rich Plasma)

It is a plasma obtained after a centrifugation at 250 - 500 g for 5 - 7 minutes.

**PT**  
*See Prothrombin Time*

**PTT**  
(Partial Thromboplastin Time)

Coagulation time (expressed in seconds and decimals) of a platelet poor plasma after the addition of phospholipids (as substitute of platelets) and calcium ions. As the APTT, it detects the Intrinsic Pathway, but in this test the activation phase is not standardized.  
*See also Activated Partial Thromboplastin Time*

**Quick Time**  
*See Prothrombin Time*

**Recalcification Time**  
It is the coagulation time (expressed in seconds) of a citrated PRP (platelet rich plasma) after the addition of a known concentration of Calcium Chloride. It is a global test to check intrinsic pathway difficult to be standardized.

**Reptilase Coagulation Time**  
Coagulation time (expressed in seconds and decimals) of a mixture of platelet poor citrated plasma in presence of Reptilase. This test is useful to detect the fibrin formation, to quantify the FDP effect and in particular for DIC patients. Unlike the Thrombin Time, this test is not influenced by heparin or antithrombin presence.  
*See also Batroxobin.*

**RT**  
*See Reptilase Time*

**RVV Time**  
*See Stypven Time + Cephalin. In this test, the venom concentration is lower than in the Stypven Time.*

**Silica**  
Silicon dioxide, insoluble in H$_2$O and in acid, except in the hydrofluoric acid. The silica crystallized form is scarcely attacked by alkalis, the amorphous form is a "sol" (especially when finely divided). The second form is used as activator in the tests of the intrinsic pathway because of its superficial electrical negative charge.

**Standard**  
Citrated sample of normal plasma, formed by a pool of at least 10 frozen or lyophilized plasma of normal donors, with an assigned value of 100% for all the factors. The lyophilized plasma does not always comply with this requirement and for this reason it must be titrated against recognized international standards.
Streptokinase

It is a protein (MW 47 KD) produced by ß-haemolytic streptococci. It is a fibrinolysis activator and it is commonly used in thrombolytic therapy of arterial thrombosis. It does not directly activate the plasminogen to plasmin but it forms an equimolecular complex (1:1) with the Plasminogen. This reaction leads to the formation of an active site on the complex that becomes an activator of the plasminogen itself. A ß-hemolytic streptococcus infection or a streptokinase therapy stimulates the production of antistreptokinase antibody (IgG class) and the consequent reaction antigen-antibody could cause fever.

Stypven Time

It is the coagulation time of a citrated plasma, rich in platelets, in presence of Russell Viper venom: this venom is able to activate Factor X in presence of Calcium Ions and in the absence of Factor VII. It is a test to evaluate PF3.

Stypven Time + Cephalin

It is the Stypven Time of a platelet poor plasma plus Cephalin as source of phospholipids. It is a test especially sensitive to identify LAC patients.

t-PA (Tissue-Plasminogen Activator)

The Tissue-Plasminogen Activator present in plasma is a serine-protease (MW about 72 KD) and is composed by a single polypeptidic chain with a two ring-structure similar to plasminogen. The plasmin transforms it, by a limited proteolytic activity, in an activator composed by two chains linked with two disulphide bridges. The activator shows a great affinity for the fibrin. The t-PA plasmatic concentration is 1-8 ng/mL (immunological test) depending both on the liver metabolic removal speed and on the activity of a specific inhibitor. It is thought that the inhibitor forms an equimolecular complex with the activator of consequent hindrance in the active site.

TAT

This acronym means Thrombin-Antithrombin complex, that can be detected in plasma using an immunoenzymatic method. It is a coagulation activation marker.

TC

See Thrombin Coagulase Time

TFPI (Tissue Factor Pathway Inhibitor)

It is a powerful inhibitor of the complex Factor VIIa - Tissutal Thromboplastin. This inhibitor needs Factor Xa as cofactor. It is a lipoprotein with MW of about 40 KD. The main physiological source of TFPI is the vascular endothelium.

TGT

See Thrombin Generation Time

Thrombasthenia

A reduced platelet aggregation induced by ADP with a defective clot retraction. This anomaly brings a formation of a platelet plug insufficient to repair the injury and it is the main factor of hemorrhagic feature of this disease. The clinical manifestations are epistaxis, menorrhagia and purple-like alterations (petechiae). It is a disease inherited by a recessive autosomal gene.
**Thrombin (Factor IIa)**

Thrombin is the physiological enzyme that changes Fibrinogen into Fibrin. Its presence in plasma can be considered as a marker of the activation of the coagulation systems.

Thrombin exists in three forms: \( \alpha \), \( \beta \) and \( \gamma \).

The \( \alpha \)-thrombin (MW 39 KD) is the native form that is generated by a partial proteolysis of Prothrombin and it is transformed by an autolysis in \( \beta \)-thrombin (MW 28 KD). The third form, \( \gamma \)-thrombin, has the same MW of the \( \beta \)-thrombin, but has a very reduced specific activity.

It is quickly inhibited by Antithrombin-III and by HC II in presence of Heparin and/or Dermatan Sulphate. Its complex with thrombomodulin activates the PC and is not able to transform fibrinogen (it may be transformed in \( \gamma \) form). It can be used, in different concentrations, as a coagulant agent for Thrombin Time.

**Thrombin Coagulase Time**

It is a test similar to Reptilase Time. The Thrombincoagulase reagent is composed by purified Staphilococagulase and by “coagulase reacting factor” that is a plasma factor identical to Prothrombin.

Staphilococagulase is a protein that is synthesized by some branches of Staphilococcus aureus and has a thrombin-like activity.

**Thrombin Generation Time**

It is a global test that check the intrinsic pathway integrity, but it is less standardized than PTT and APTT. A solution of fibrinogen (500 mg/dL) is added to a platelet rich and recalcified plasma. This procedure is repeated every two minutes from the recalcification.

Values over 10 minutes are considered pathological.

**Thrombin Time**

Coagulation time (expressed in seconds and decimals) of a citrated platelet poor plasma, mixed with a known quantity of Thrombin. This test investigates the final phase of the coagulation cascade: the transformation of Fibrinogen in Fibrin and its polymerization.

It is sensitive to fibrinogen concentration and to all the substances with inhibitory activity against thrombin as antithrombins, FDP, heparin and paraproteins. It is useful in fibrinolytic therapy, in diagnosing of congenital and acquired pathologies of fibrinogen. It can be also used to monitor heparin therapy.

**Thrombocytopathia**

It is a functional defect of the Platelets: it may be congenital or acquired. In general, it is related to the functional defect caused by a coagulant activity related to PF3.

**Thrombocytopenia**

It is a quantitative deficiency (< 150,000 / mm\(^3\)) of platelets related to a reduced production or a reduced survival (increased sequestration speed), in a dilution of platelet poor blood (extracorporeal circulation, transfusion).

It may be primary or secondary, acute or chronic. This syndrome is responsible for spontaneous bleeding.
Global method of study of blood coagulation.
A dedicated instrument (thromboelastograph) records the movement of a piston dipped in a system which is going to be coagulated (plasma rich in platelets or whole blood, recalcified) with a spinning alternate movement.
Before the Fibrin formation, the system is balanced and the piston stays still.
During the Fibrin formation, the piston finds a certain resistance and it is dragged. The thromboelastogram is the registration of the mechanical characteristics of the clot. According to the graph, it is possible to extract some parameters: \( r \), \( k \) and \( ma \).
The time of reaction \( r \) is related to the pro-coagulant activity (factor deficiencies, inhibitors presence, Fibrinogen concentration, activity and number of platelets) and it can be considered as expression of Thrombin formation time. The clotting formation speed is indicated as \( k \) (index of fibrin formation speed, clotting organization and fibrinolysis). The maximum graph width (distance between the two branches) is indicated as \( ma \) and is an index of the fibrinogen concentration, activity and number of platelets. A great decrease in \( ma \) and its speed are an index of Fibrinolysis.

![Thromboelastogram](image)

\[ \text{Fig. 13 - Thromboelastogram and related parameters, (r, reaction time; k, clotting formation speed; ma, maximum width).} \]

**Thrombolytic Treatment**
It is the administration of plasminogen activators that act on the fibrinolytic system in order to induce an accelerated and specific lysis on an occlusive thrombus. This kind of therapy does not remove the causes that have led to the thrombus formation, but it acts only on the effect (the thrombus). The most used agents are Streptokinase, Urokinase and t-PA.

**Thrombomodulin**
It is an endothelium membrane glycoprotein (MW 74 KD). It is a receptor of the Thrombin with which forms an equimolecular complex.
In the presence of Calcium ions, the complex increases 20,000 times the speed of Protein C activation in comparison with thrombin only.

**Thrombophilia**
High thrombotic risk related to unknown causes but connected to inhibitor lack, reduced fibrinolysis or coagulation activation.
**Thromboplastin**

It is an aqueous suspension of lipoproteins and phospholipids obtained with various extraction techniques. The extraction is derived from a fresh homogenized tissue or from tissue powder with aqueous solvent. Brain, lung and placenta are the most used sources of lipoproteins and phospholipids. Rabbit, chimpanzee and ox are the animals from which the organs are taken. The use of human organs is not allowed. This reagent is used for all the tests related to the extrinsic pathway.

Bovine and rabbit thromboplastins added with optimal levels of all the coagulation factors, except II, VII, X, IX (Prothrombin Complex Factors) are used for O. A. T. monitoring, for detection of PIVKA inhibitors presence and liver diseases (i.e. IL Pro-IL-Complex and IL Hepatocomplex).

**Thrombosis**

Thrombosis is the formation or presence of a blood clot within a blood vessel during life. The Fibrin formation in circle can produce thrombus that can occlude the vascular system (venous and/or arterious, small or big vessels).

The Thrombosis is not started by a single cause but more frequently is multicause.

**Thromboxanes**

They are substances produced in the platelets by transformation of prostaglandines peroxides. The first thromboxane to be formed is thromboxane A₂ that is able to induce platelet aggregation.

**Thrombus**

Thrombi, arterial or venous, consist of mixtures of fibrin and platelets aggregated with white cells and red cells. The intravascular thrombus generation may happen when there is a damage to the blood vessel endothelium. If the thrombus is sufficiently large enough to occlude the lumen of the blood vessel, it can be present in a tissue ischemia after the obstruction.

**Tissue Factor**

Lipoprotein present in the tissue extracts (Thromboplastin) recently isolated and purified. It forms a complex with the Factor VII which is able to activate the extrinsic and intrinsic pathways.

**TNF (Tumor Necrosis Factor)**

It is a kinin with cytotoxic activity secreted by the macrophages. Some recent studies suggest that the TNF could promote the deposition of Fibrin in blood vessels, stimulating the production of PA-I and inducing a hypofibrinolytic state. It seems also that this factor interferes in the link between Protein C (PC) and Thrombomodulin (TM) and induces the proteolytic removal of TM.

**Trasylol**

Trasylol (commercial name of Aprotinin) is a Kallikrein inhibitor which inhibits Plasmin, Trypsin, Chymotrypsin and other proteases. It is constituted by a single polypeptidic chain of 58 aminoacids of known sequence. It is a drug used in acute pancreatitis when it is necessary to save the organ against proteolytic action and in hyperfibrinolysis haemorrhagic disorders.
See Thrombin Time

Urokinase

It is a kidney synthesized protein and it is present in the urine in two molecular forms (MW 54 KD and MW 31.6 KD).
It is a fibrinolysis activator and it acts on Plasminogen converting it to Plasmin. It could be used for thrombolytic therapy.
It is not antigenic nor pyrogenic (in humans). It is a trypsin-like serine-protease composed of two polypeptide chains (20 KD and 34 KD) linked by a single disulphide bridge.

Willebrand

The Von Willebrand Factor is a High Molecular Weight glycoprotein.
It consists in a series of polymers with a MW between 800 and 1200 KD.
The polymers with high MW have more affinity for the platelets, in comparison with the ones with low MW.
Specific receptors for this Factor are sited on the platelet surface.
The blood of patients with Von Willebrand disease (inherited autosomal deficiency) shows a reduced capability to induce platelet adhesion to the subendothelium and the Bleeding Time is prolonged.
This factor circulates in plasma bound to Factor VIII.

Zymogen

It is a circulating substance without any enzymatic activity.
After the partial proteolysis of its molecule, caused by specific enzymes or by a superficial catalytic action, a new molecule with a procoagulant, inhibitor or fibrinolytic enzymatic activity is generated.

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