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**Abstract**

The authors give an account of the important developments in blood coagulation knowledge from the times of Malpighi and Moravitz to data. The article is followed by original tables providing a general and comprehensive view on blood coagulation, hemorrhagic syndromes and fibrinolysis.

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# BLOOD COAGULATION FROM THE BEGINNING UNTIL TO-DAY*

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Blood coagulation research constitutes an important chapter of hematology and strives to resolve problems of lively interest in medicine. Blood coagulation physiology plays a predominating role in many medical fields. During the last three decades blood coagulation physiology has been disclosed with significant progress. So, it is not surprising to see that the practising physician, who is not directly interested in blood coagulation, has difficulties in following all the rich literature; and learning the terminology concerning this progressing field. (Table 1). This article, which has been written with this goal in view, contains our old[1, 51] and many new[150, 154, 155, 159, ~164] works on this subject.

## HISTORICAL SURVEY

From the time of Hippocrates, it was known that blood flowing out of an injured vessel coagulates in a short time. The mechanism of this event remained unkown for a very long time. By means of the literature which is at our disposal, we know that MALPIGHI[1] was the first to study the problem of blood coagulation. He was the investigator who succeeded to show in 1683 that when coagulated blood is washed, one can obtain a fibrous whitish mass (the fibrin). However, the name "fibrin" was not created by Malpighi but by CHAPTAL[2] a century later. This French investigator reported that the part that coagulates is not the formed elements of the blood, but the fluid part, — that part called "fluid lymph" by HEWSON[1] in 1770. The mechanism of fibrin formation was described first in 1844 by an English investigator BUCHANAN[2]. This author thought that coagulation occurred not spontaneously with fibrin, but through the interaction of some other factors (1845) or ferments which became active outside the body. MORAWITZ considers[3] BUCHANAN[2] as the founder of modern coagulation research. The so-called Buchanan theory can be summarized by the following formula:

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* To my previous teacher and Nobel Prize winner Prof. A. SZENT-GYÖRGYI

Table 1

The Clotting Factors (Names According to International Blood Clotting Nomenclature Committee) and the Hemorrhagic Disorders in Relation with the Factors.

| I Defect of: | Clot promoting factors | II Hemorrhagic syndrome | Congenital | III Acquired |
|---|---|---|---|---|
| Coagulopathy: | | | | |
| Fact. I | Fibrinogen | Afibrinogenaemia, Hypofibrinogenaemia, Fibrinogenopenia | + | Liver disease, Medullary tumors, Fibrinolysin defibrination |
| Fact. II | Prothrombin | Hypothrombinaemia | + | Liver disease after cumarine |
| Fact. III. | Thrombokinase-Thromboplastin | see Fact. V-XII | | |
| Fact. IV | Calcium | | | |
| Fact. V | Proacelelerin (Ac-Globulin, SPCA) | Parahaemophilia (Owren) | + | Liver parenchymal damages; Fibrinolysis: Liver disease |
| Fact. VI | Accelerin | | | |
| Fact. VII | Proconvertin | Prooenvertinaemia | + | Liver parenchymal damages (diseases) after cumarine treatment |
| Fact. VIII | Antihaemophilic Globulin A | Haemophilia A Fibrinolysis | + | several liver diseases |
| Fact. IX | (Antihaemophilic Globulin B) Plasma-thromboplastin Component-PTC | Haemophilia B Christmas disease | + + | Liver parenchymal damages (diseases) |
| Fact. X | Stuart-Prower-Factor | Stuart-Prower-Fact. Defect | + | after anticoagulants cumarine |
| Fact. XI | Plasmathromboplastin-antecedent-PTA | PTA-defect | + | several liver cirrhosis |
| Fact. XII | Hageman-Factor | Hageman-Factor-Defect | + | ? |
| Fact. XIII (Laki-Lôránd) | Fibrin establishing Factor (Fibrinase) | | + | ? |

Coagulation Inhibitors

Inhibitor of the first phase (Antithrombin ? Antithrombokinase ?) ..... So-called antibody hemophilia

Inhibitor of the 2nd phase (Antithrombin) ..... Liver disease, allergic states purpura fulminans, purpura abdominalis, heparin action.

and others (see the schema of fibrinolysis)

Buchanan's Ferment (Thrombin, according to Schmidt, later on)

Blood ——————————→ Fibrin

In 1832, Johannes MÜLLER[2] described one of the important coagulation factors, namely, the fibrinogen. The name "fibrinogen" was created by VIRCHOW[2] who postulated the existence of a relation between blood coagulation and oxygen. The successful isolation of fibrinogen from sulfate-plasma through precipitation by sodium chloride led the French investigator DENIS[2] to use the name "plasmin" in 1856. One year later, in 1857, the physiologist Ernst BRUCKE[2] (1819～1892) pointed out the important role of the vascular wall in blood coagulation.

De BLAINVILLE[2] showed in 1834 that the intravenous injection of cytoplastic substances such as brain extracts produced massive intravascular coagulation in experimental animals. However, a long period of time passed before we were able to understand that the cytoplastic substances of Blainville, and the ferment of Buchanan, were not similar. The great physiologist Alexander SCHMIDT[4] (1841～1914), the pupil of Felix Hoppe-Seilers, discovered (1892～1895) that these two substances were different from each other. According to SCHMIDT[54], during the blood clotting proccss, the ferment of Buchanan was formed out of a supposed precursor, under the influence of "Protozym" (SCHMIDT and RAUSCHENBACH) or "cytoplastic substance" (SCHMIDT) or "thrombokinase"+ (MORAWITZ). The ferment of Buchanan was called "thrombin" by SCHMIDT and its precursor "prothrombin". Accordingly, the blood coagulation theory of Alexander SCHMITD was as follows:

Cytoplastic substances

Prothrombin ——————————→ Thrombin

Thrombin

Fibrinogen ——————————→ Fibrin

It is interesting to note that Schmidt did not take into consideration the role of the last factor, namely, calcium in coagulation; although ARTHUS and PAGES had shown, as early as 1891, that coagulation is impossible without calcium. Two Swedish chemists from the University of Uppsala, PECKELHARING[2] (1891) and HAMMERSTEN[2] (1941～1932) demonstrated that calcium is necessary for the actio nof Protozym. HAMMERSTEN[2] had also demonstrated that, although calcium was necessary for the first phase of blood coagulation, it was not needed in the second phase.

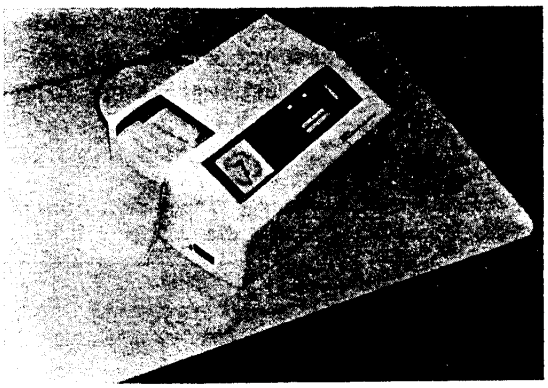
MORAWITZ who also insisted on the importance of calcium in coagulation, proposed in 1905 the following scheme which became classical:

+ Thrombokinase (Europ. literature)=Thromboplastin (America literature).

1st phase : Prothrombin → Thrombokinase (Thromboplastin)
Ca ↑ ↓
2nd phase : Fibrinogen + Thrombin ⟶ Fibrin

The works of Morawitz have cleared up many contradictions which existed on this subject in the earlier publications. The activation of prothrombin, discovered by SCHMIDT[4], which occurs through alkali-reactivation of blood serum and was shown not to be the normal pathway. MORAWITZ and WOHLISCH[5] discovered that the alkali-reactivation of blood serum proposed by SCHMIDT was not the real activation of prothrombin, but it was rather a reaction which reversed the inactivation of thrombin that occurs in the serum shortly after coagulation. This substance in the serum which can be activated in this manner and which is, according to our knowledge today an association of thrombin with antithrombin, was called Metathrombin hy MORAWITZ[3] (WOHLISCH[5]). The theory of Morawitz given above may be called the "theory of 4 factors". Accordiug to MORAWITZ, four factors were necessary for blood coagulation, namely : prothrombin, thrombokinase, ionized calcium and fibrinogen. Some other theories appeared soon after that of MORAWITZ. For example, NOLF[3] proposed in 1908 his theory on blood coagulation with five factors and three phases. The five factors are : calcium and four other factors of proteinic nature in plasma, namely, thrombokinase, thrombozyme, thrombogen and fihrinogen. According to NOLF[6], plasmakinase is a tissue-kinase of lipoproteinic nature, which accelerates the reaction of thrombozyme and thrombogen in the presence of calcium. He thinks that thrombin results from the association of these two factors. In 1912 DELANGE[2] and the great Belgian serologist and Nobel prize-winner BORDET[2] presented a similar theory with five factors. BORDET[2] and the American physiologist HOWELL[4] worked independently and reported that the theory of SCHMIDT was acceptable. Both authors came to the conclusion that the active component of Morawitz's thrombokinase was a lipoidic substance, considered by HOWELL to be a cephalin. MCLEAN[9], a student working in Howell's laboratory[7] in 1916 discovered heparin. It is an inhibitor occurring in the body and active *in vivo* and *in vitro*. According to the research of MCLEAN, heparin works as an antithrombin which inhibits the formation of thrombin from prothrombin. The modern era in blood coagulation research began in 1934. This era is characterized by the fact that, theoretical knowledge was put gradually and progressiyely into practical therapeutic use. To begin this new era of blood coagulation research, we have to mention first of all, the distinguished discovery, honored with the Nobel prize, of SZETGYRÖGY (Vit. C+P) (Vasal Wall)* and

* Under Prof. Szent-Györgyi's influence developed particulary the author (SZIRMAI) several years ago the instruments besides registering the muscle and nerve functions, also for the mesurement of capillary-fragility



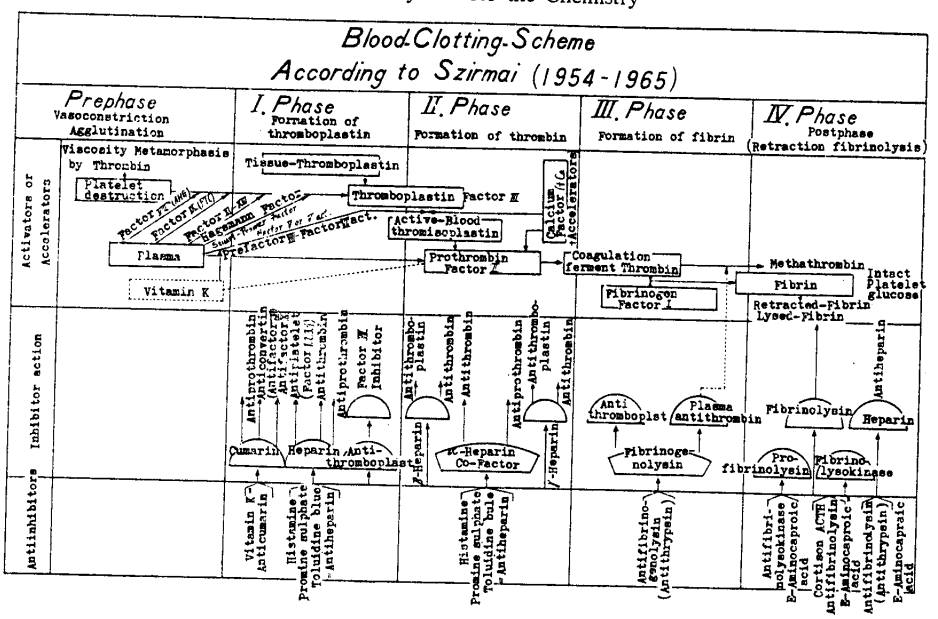
Eig. 1 Angio-Myograph by SZIRMAI for the determination of capillary resistance and fragility also by haemorrhagic diatheses *etc.* but also for the determination for vasal, muscular and other conditions in pathologic and physiological cases. (New prototype fact. Mr. Franz PRINZ (MSG) Dagersheim Production: Dr. E. HENSCHEN, Fabrik Technische Messinstrumente, Sindelfingen, F. R. of Germany)

First instrument in this direction was developed by Dr. SZIRMAI 18 years ago particularly under the influence of his previous Professor, Nobel prizewinner A. SZENT-GYÖRGYI.

Henrik DAM[29] who showed the absolute necessity of vitamin K for the synthesis of prothrombin. The description in 1935 of prothrombin determination by QUICK[10] constituted a definite advance and provided, in a decisive manner, the progress of the works on Dicumarol and vitamin K. From this time, many authors made contributions to the classical coagulation scheme and enlarged upon it. OWREN[11] showed that besides the four known factors, that is Fibrinogen (I), Prothrombin (II), Thromboplastin (III) and Calcium (IV), another factor existed in coagulation. The active form of this factor, shown as VI was necessary for the conversion of prothrombin into thrombin. KOLLER[12] recommended the use of numbers to replace the names of clotting factors and soon factor VII was described as an accelerator of the prothrombin conversion. The investigations on hemorrhagic syndromes constituted an important section of blood coagulation research. It was shown that platelets were necessary for the formation of blood thromboplastin and to achieve this, they had to react with some plasmatic factors: the antihemophilic globulin A ($^{AHG}$: factor VIII), the antihemophilic globulin B (factor IX), and factors X, XI, XII and XIII. The classic view accepted that the action of prothrombin depended on three factors and that many factors (namely, the platelet factors 1, 2, 3 and plasmatic factors 8, 9, 10) were necessary for the formation of blood thromboplastin. The deficiencies of these factors determine definite diseases. The deficiency of factor VIII is called Hemophilia A, that of factor IX, Hemophilia B and that of factor X Hemophilia C[16]. Blood coagulation is completed in three phases according to the classical

theory. Later on, this theory or scheme was enlarged upon. So, MARBET and WINTERSTEIN[17] and also SZIRMAI[159] have added to the classical scheme a prephase dealing with platelet functions and a terminal phase describing the disintegration of fibrin (Table 2).

Table 2
This Scheme is for the Blood-clotting so Important, as Mendeléeff's Periodical System for the Chemistry




PROPHASE

*1st Phase : The Formation of Thromboplastin :* In order for clotting to occur, active thromboplastin must circulate in the blood. This may happen in two ways :

1. Blood may extravasate and mix with thrombokinase.
2. Thromboplastin may be activated auto-catalytically in blood. This process is not completely understood. Most of the authors (FONIO[18], FEISSLY[19], OWREN[20], HORANYI[21], *etc.*) share the view of MORAWITZ, which states that the foreign surface activation of platelets results in the formation of active thromboplastin. BIZZOZERO[22], the discoverer of platelets, was also the first to recognize the unique role of platelets in physiological hemostasis and their importance in the pathogenesis of thrombosis.

According to MILSTON[23], precursor of thromboplastin activates itself under

the influence of ionized calcium in plasma. LENGGENHAGER[24] thinks that an enzyme named Thrombokatalysine is necessary for this activation. KUDRJASCHEW and UTILINA[25] share the same view, but they call thrombotropin the activating enzyme. Others, among whom one may mention VIRCHOW, WIDENBAUER and REICHEL[16] and some authors[28] think that blood coagulation is in relation with respiration. WIDENBAUER and REICHEL[26] believe that the thrombokinase is activated bceause of the decrease in carbon-dioxide levels of extravasated blood. The investigation of LAKI[30] seemed to confirm the existence of a precursor of thrombokinase. According to QUICK, the inactive precursor of the thrombokinase is activated by means of an enzymatic substance which may originate from decayed platelets.

For a long time, it was assumed that active thrombokinase resulted from the interaction of plasmatic and platelet factors. We also know that thrombokinase is present in great quantities in tissues, such as lungs, brain, placenta, *etc.* We have also used the amniotic fluid of SZIRMAI[32] as thrombokinase (BARON C. *et al.*, Nucl. Hemat., March-May, Vol. III, Nr. 2, 167, 1964). The latter plays important roles in all phases of coagulation, but is primarily active on platelets. The authors believe that all the above-mentioned reactions begin with the alterations taking place in platelets, after agglutination or after contact activation. BRINKHOUS[30] has found that the foreign contact causes an activation of thrombocytolysin which in turn destroys the platelet membrane. According to MAEBET and WINTERSTEIN, the contact with histamine or histamine-like substance causes the disintegration of the platelets. Electron microscope studies reveal that histamine prevents or loosens platelet agglutination. A strong vasoconstrictor, Serotonin (5-oxytryptamin) is liberated from disintegrated platelets. Then the injured vessel is constricted. The quantity of extravasated blood remains minimal and this serves a hemostatic purpose. MARBET and WINTERSTEIN[33] and M. B. ZUCKER[36,37] reported that the vascular contraction begins 15 seconds after the injury to the vessel, and at the end of one minute the lumen may be narrowed up to 80 percent of its initial. STEFANINI[38] suggested that Retraktozym (FONIO[39]) is a definite platelet factor which accomplishes the retraction of the clot. The platelets also contain the antifibrinolysin. According to the works of STEFANINI[38], CREVELD[40], JURGENS[41] and SEEGERS[42], the platelets possess three factors that are active in blood coagulation:

1. Platelet factor 1 accelerates the conversion of prothrombin into thrombin and is probably identical with factor V;
2. Platelet factor 2 supports the influence of thrombin; and
3. Platelet factor 3 is a factor taking part in the formation of thromboplastin, reacting together with factors VIII, IX and X.

Platelet factor 3 also possesses anti-heparin activity. In cases of total or partial

deficit of this factor, as in thrombopathy, antithrombin of the heparin-type will be generally increased because of lack of platelet factor 3 neutralization (SZIRMAI, JÜRGENS and others[43]). Deficit of platelet factors causes various haemorrhagic diatheses. In the table below, we perceive the platelet functions known up to 1955 :

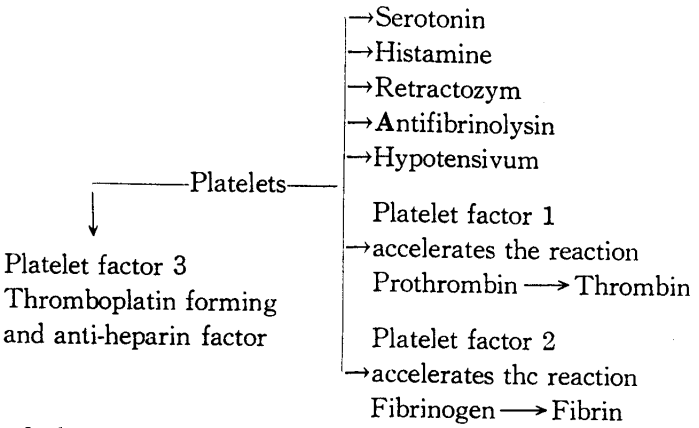



Table 3 also shows platelet factors active in blood coagulation (1961).

Table 3
Platelet Factors Active in Coagulation

| Names | Function and Properties |
|---|---|
| 1. Platelet factor 1 | Factor V-like activity. Probably identical |
| 2. Thrombin-accelerator | Accelerates thrombin's action and the conversion of fibrinogen into fibrin |
| 3. Platelet factor 3 | Takes active part in the formation of thromboplastin |
| 4. Antiheparin factor | Nentralizes the inhibitory action of heparin |
| 5. Clottable factor | Identical with fibrinogen |
| 6. Platelet's co-thromboplastin factor (viper-venom factor) | Action sinilar to that of factor VII. Accelerates the conversion of prothrombin into thrombin through the action of viper venom |
| 7. Thrombosthenin (Retractozyme) | Actomyocin-like factor. Takes part in clot retraction |
| 8. Antifibbinolysin | Inactivates the fibrinolysin |
| 9. Fibrin stabilizing factor (Fibrinase) | Inhibits the lysis of fibrin clot in urea |
| 10. 5-Hydroxytryptamin (Serotonin) | Vasoactive (constrictor) factor |

The investigators believed in the beginning (MORAWITZ, NOLF[3,6]) that thrombokinase was a compound which could be defined chemically, containing one part of phosphatide and another of protein nature. Today this concept is accepted only for tissue thrombokinase (extracts of brain, lung, placenta,

amniotic fluid and others) but not for blood thrombokinase. BIGGS[47] advanced that the product of the reaction of platelet factors with plasmatic factors (VIII, XIV and X) is the equivalent of tissue thrombokinase. Tissue thrombokinase is a lipoprotein (FEISSLY, CHARGAFF[91,45]) relatively stable. STUDER[46] divided this lipoprotein by means of ether into two parts: 1. a factor of lipidic nature, which is thermostable and of cephalin type; and 2. the proteinic part which is thermolabile. When they are combined, these two parts assume their full acti-vity.

Blood thromboplastin is the end-product of the reaction between pletelet factor 3, antihemophilic globulin, factor IX and factor X.

Factor VIII or antihemophilic globulin is a very labile plasmatic factor of proteinic nature. It is found with fibrinogen and in Cohn's fraction number one. Antihemophilic globulin activity disappears rapidly in stored blood. Coagulation of blood also consumes it.

Factor IX or Christmas factor of Biggs and MACFARLANE[44] is probably the same factor called plasma thromboplastin component (PTC). It is stable in stored blood and can be shown in serum after blood clotting. Therefore the treatment of hemophilia B, the disease due to the deficiency of factor IX, is possible with stored blood.

Factor X (Stuart-Prower factor) is decreased during cumarin treatment and its normalization after the withdral of the medicament is slower than that of stable factor (factor VII).

The thromboplastin generation test (BIGGS[47]), DUCKERT[48]) gives us valuable information about the mechanism of blood thromboplastin formation. Other interested factors are factors XI, XII and XIII (see Tables 1 and 6).

*2nd phase: The Conversion of Prothrombin into Thrombin:* The study of the literature which we summarized above shows clearly that authors agree with the existence of two separate thrombokinase systems leading to blood coagulation (SCHWICK[150], SZIRMAI[151]). According to this agreement, DEUTSCH[152] proposed to differentiate two types of mechanisms in blood coagulation, namely, the exogenous (extrinsic) and the endogenous (intrinsic) systems.

As we have mentioned above, during intrinsic blood coagulation, the prothrombin is converted into thrombin through the action of blood thromboplastin. As it can be seen from our coagulation schemes in 1955 and in 1960[155], blood thromboplastin formation starts with contact activation, and progresses through the intermediary of various active products. Factor XII (Hageman factor), activated by foreign surface contact activation reacts with factor XI (PTA) to form a labile product, which enters into reaction with factors VIII, IX, X, XIII and calcium. Thus intermediary propuct I is formed. The intermediary product I and platelet factor 3 act together to prepare the intermediary product

2 which, under the influence of active factor V, forms blood thromboplastin. The most severe coagulation disorders in hemorrhagic diatheses are encountered in the formation of blood thromboplastin. Besides cases with one factor-defect, there are cases with multiple defects of thromboplastinic factors. For example, KOLLER[153] reported hemophilia A case deficient also in factor VII. In Owren's parahemophilia[155] and in carcinoid syndrome (SZIRMAI[151]) deficiencies of factors V and VIII are associated. In thrombopathy of Willebrand-Jürgens type, platelet defect is associated with deficiency of factor VIII (Table 1).

SCHWICK[150] thinks, together with other authors, that a continuous latent coagulation takes place in the vessels which probably causes minimal deposits of fibrin on the endothelial surface. Some authors suppose[153] that factor VII arises from prothrombin in peripheral blood and probably on its return to the liver is again transformed to prothrombin. This concept is in concord with the works of SEEGERS and collaborators[150] who have shown, by chemical methods that factor VII (Autoprothrombin I) and factor IX (autoprothrombin II) derive from prothrombin.

The works on latent intravascular blood clotting are especially important in learning the relative changes in coagulation dynamics during extra-corporal circulation.

Recently, many new factors have been added to the above-mentioned three platelet factors active in coagulation[150,156] (Table 3). GROSS and collaborators[156] have shown a deficiency of "glyceraldehyde phosphatedehydrogenase" and of "pyruvate kinase" activities in thrombasthenia of Glanzmann. Upon adding these enzymes to platelets, the defective coagulation activity is corrected.

We investigated[157] the activity of "succinildehydrase" activity of the platelets. LÜSCHER and collaborators have shown the presence, in the platelets of a contractile protein, the "retractozyme", of a structure similar to that of actomyosin, probably interested in the retraction of the clot.

Now we shall take a look on the process of the conversion of prothrombin into thrombin.

In the classical theory of MORAVITZ[3], we find the conversion of prothrombin into thrombin. For this conversion, it has been shown that calcium and thromboplastin are necessary, and recently factors V and VII. According to chemical investigations, especially by SEEGERS, LOOMIS and VANDERBELT[49], pure prothrombin is a glucoproteide, soluble in water and containing sulphur. The isoelectric point is about pH 4.8. It is found in Cohn's fraction III/2. In comparison with other factors, prothrombin is relatively stable. Storing of the blood diminishes its activity only slightly. It can be stored in frozen plasma for very long time. Methods of adsorption, such as barium sulfate or calcium phosphate adsorption and Seitz filtration are able to take it off the plasma. According to HOWELL and

HOLT[4], prothrombin circulates in blood, in a bound form with heparin; and thromboplastin neutbalizes heparin, thus liberating prothrombin. According to DUCKERHOF and MARY[50], prothrombin does not exist as such in the blood, but circulates in the form of thrombin bound to natural anticoagulants. Thromboplastin is supposed to neutralize the anticoagulants and set free the thrombin. COPLEY also is accepting this view[51]. OWREN[58] showed that storage of plasma shortens the prothrombin time. The investigations of QUICK and HUSSEY[52,53] suggest that prothrombin is partly bound with heparin. Only that part which is free can take part in thrombin formation. The decrease in the amount of bound prothrombin or the increase in free prothrombin increases also antithrombin activity. One-step prothrombin time test measures only free prothrombin. Besides the above-mentioned authors, many others have studied the problem of free and bound prothrombin (VÉSZI, KOVACS and GESZTI[54]). The existence of factor V was first forwarded by NOLF[6] under the name of Thrombogen. Factor V is called frequently Proaccelerin and it has many other names as will be seen in Table II at the end of this article. Factor V is a hydrosoluble globulin; its activity decreases on storage in room temperature and when heated to 56°C, it is inactivated spontaneously. MARBET and WINTERSTEIN[55] have seen that the activity of factor V decreases in oxalated blood, after storage of some hours. This fact leads to prolongation of prothrombin time and the control of dicumarol therapy becomes falsified. Deficiency of factor V is called parahemophilic or Owren's disease. SZIRMAI[81] reported factor V deficiency in cases of genital carcinoma in women. Factor V is not adsorbed by barium sulfate. Thrombin activates factor V and accelerin (factor VI) is formed.

Factor VII has many synonyms (see end of the article). Its existence was supposed by BORDET and DELANGE in 1912[56]. However, these authors had mistaken it for prothrombin. QUICK[57] thinks even now that factor VII is an inactive precursor of prothrombin (prothrombinogen). He thinks that factor VII is present in plasma, although its activity is higher in serum[61]. Therefore, some authors[58] think that factor VII is found in plasma in the form of an inactive precursor.

Prothrombin, factors V and VII constitute together the so-called prothrombin-complex. Thromboplastin and the accelerator factors catalyze coagulation, but do not take part in it. Therefore, thromboplastin will not be consumed but will be found in serum as residual thromboplastin. The residual prothrombin of the serum is under the influence of serum thromboplastin. Serum prothrombin is consumed in approximately 24～36 hours (KOVÁCS[60]). QUICK and FAVRE-GILLY[61] also have studied this problem.

Thrombin has an autocatalytic action, that means it accelerates and increases its own quantitative formation. FISCHER[62] was the first author to describe this

property. According to LAKI[30], ASTRUP[63] and recently QUICK[65], thrombin accelerates the activation of Prothrombokinase. But OWREN[58] thinks that thrombin accelerates the conversion of factor V in factor VI and not the formation of thromboplastin. QUICK[57] believes that at the beginning of blood coagulation a small quantity of thromboplastin is formed and it converts a small amount (0.1 percent) of prothrombin into thrombin. This thrombin acts upon the platelets which yield 8～10 times more platelet factor 3 than the first time. This causes a greater activation of thromboplastin which in turn transforms more prothrombin into thrombin. This process goes so far that no thrombin or very little can be found after it. Even this minimal thrombin, however, results in the secretion of fibrinogen.

Thrombin is found in the albumin fraction of the plasma (ASTRUP and DARLING[90]). Heating above 40°C inactivates it rapidly. The activity of thrombin is nowadays mostly measured in NIH units. One NIH-unit corresponds to the amount of thrombin that clots 1 ml of a standard fibrinogen solution in 15 seconds at the temperature of 28°C.

*3rd phase : Formation of Fibrin :* Thrombin exerisces an enzymatic influence on fibrinogen (EAGLE[76], FERGUSON[68], FREDERICQ[69], WOHISCH[70]). It can cause clotting of fibrinogen even in the proportion of 1/100,000. Fibrinogen is a globulin of a molecular weight of 400,000, and it is found in Cohn's fractions I and II. Fibrinogen solutions are not stable in room temperature and coagulate when heated above 50°C.

The authors differ on the mechanism of fibrin formation out of fibrinogen. FERRY and MORRISON[71] think that thrombin causes the polymerization of fibrinogen in a three-dimensional manner and thus fibrin is formed. CHARGAFF[72] believes that thrombin produces fibrin through oxidation of the amino-acid groups of fibrinogen. According to LORAND[73], thrombin separates a peptide molecule of low molecular weight (fibrinopeptide) out of fibrinogen and then thrombin is formed. JENEY, VALYI-NAGEY and VACZY[74] and LYONS[75] share the view of CHARGAFF. LAKI[76] thinks that amino-acid groups play an important role in fibrin formation and that thrombin acts on fibrinogen with the result of setting free the amino-acid groups of the latter. Some authors (APITZ[77]) reported that there is an intermediary substance, the profibrin, which is formed between fibrinogen and fibrin.

## THE LAST PHASE

*Retraction and Fibrinolysis :* The formed clot contracts on standing and serum is squeezed out of its own and slowly. This event is called "retraction of the clot". The optimal temperature of retraction is about 40°C. Platelets enhance clot retraction through their enzyme called "retractozyme". In cases of

thrombocytopathy and thrombocytopeny the retraction occurs late or not at all. This abnormality can be measured quantitatively by means of thrombelastogram (HARTERT[78], MARBET and WINTERSTEIN[17], SZIRMAI and JÜRGENS[43]).

As is known, during blood coagulation much thrombin is formed. The organism has to neutralize it and there are two possibilities for this: 1. Thrombin is adsorbed on fibrin and thus it is bound; 2. Antithrombin inactivates thrombin. Antithrombin is a natural anticogulant in the albumine fraction, relatively thermostable and of lipoieic nature. Antithrombin binds thrombin and forms an inactive complex, called "metathrombin". According to the investigations of GERENDAS, CSEFKÓ and UDVARDY[79], the reaction between thrombin and antithrombin is of a monomolecular type. Heparin accelerates the inactivation of thrombin (HORN, GERENDAS and BORSODI[86], SZIRMAI[81]).

Table 4
Synonyms of Coagulation Factors

| Names Xr. | Synonyms | Found in plasma in serum |
|---|---|---|
| Factor I | Fibrinogen (DENIS) | + − |
| | Plasmin (DENIS) | |
| Factor II | Prothrombin (SCHMIDT) | + − |
| | Thrombogen (MORAWITZ) | |
| | Thrombozyme (NOLF) | |
| | Proserocyme (BORDET) | |
| | Prothrombin B (QUICK) | |
| | Plasmozyme (FULD) | |
| Factor III | Thromboplastin (NOLF) | in tissue |
| | Thrombokinase (MORAWITZ) | |
| | Zymoplastin (SCHMIDT) | |
| | Cytocym (BORDET) | |
| | Thrombokinin (LENGCENHAGER[121]) | |
| Factor IV | Calcium (coagulation function: ARTHUS and PAGES 1890) | |
| Factor V | Factor V (OWREN). Ac. globulin | + − |
| | Proaccelerin (OWREN[129]) | |
| | Labile factor (QUICK[122]) | |
| | Plasma Ac-globulin (WARE and SEEGERS[428]) | |
| | Thrombogéne (NOLF) | |
| | Prothrombinase (OWREN) | |
| | Prothrombinogenase (OWREN) | |
| | Prothrombinokinase (MILSTON) | |
| | Plasma-prothrombin-conversion factor (PPCF) (STEFANINI) | |
| | Component A of prothrombin (QUICK[124]) | |
| | Prothrombin accelerator (FANTL and NANCE[125]) | |
| | Co-factor of thromboplastin (HONORATO[126]) | |
| | Carcinophil-Factor (SZIRMAI) | |
| Factor VI | Factor VI (OWREN[127]) | − + |

| | | |
|---|---|---|
| | Accelerin (OWREN[127]) | |
| | Serum-Ac-globulin (WARE and SEEGERS) | |
| | Prothrombinase (OWREN) | |
| | Thrombinogenase (OWREN) | |
| | Serum accelerator (STEFANINI) (see factor VII!) | |
| Factor VII | Factor VII (KOLLER[131]) | + + |
| | Proconvertin (OWREN[129]) | |
| | Serum prothrombin conversion accelerator (SPCA) (De VRIES, ALEXANDER[132]) | |
| | Convertin (133) activated form) | |
| | Stable factor (STEFANINI[130]) | |
| | Serozyme (BORDET) | |
| | Kappa factor (SRBYE and DAM) | |
| | Prothrombinogen ? (QUICK) | |
| | Co-Thromboplastin (MANN and HURN[135]) | |
| | Serum accelerator (JACOX) | |
| | Prothombin accelelator (MAC MILLAN[134]) | |
| | Prothrombin conversion factor (OWREN) | |
| | Prothrombin convertin factor (JACOX) | |
| | Complements B Prothrombin (QUICK[124]) | |
| Factor VIII | Factor VIII (KOLLER) | + − |
| | Antibemophilie Globulin (HG) PATEK and TAYLOR) | |
| | Antihemophilic Globulin A (CRAMER[137]) | |
| | Antihemophilic factor (AHF) (BRINKHOUS and other[144]) | |
| | Plasma thromboplastic factor (PTF) (RATNOFF[138]) | |
| | Plasma thromboplastic factor A (AGGELER) | |
| | Thromboplastic plasma component (TPC)(SHINOWARA[142]) | |
| | Factor Antihémophilique A (SOULIER) | |
| | Thromboplastinogen (QUICK) | |
| | Prothrombokinase (FEISSLY[140]) | |
| | Platelet co-factor (JOHNSON[141]) | |
| | Plasmakinin (LAKI[143]) | |
| | Thrombokatalysin (LENGGENHAGBR[121]) | |
| Factor IX | Plasma thromboplastin component (PTC) (AGGELER) | + + |
| | Christmas-factor (BIGGS and MAC FARLANE[145]) | |
| | Antihämophiles Globulin B (CRAMER[137]) | |
| | Plasma thromboplastic factor B (AGGELER[146]) | |
| | Plasma factor X (SCHULMAN) | |
| | Factor antihémophilique B (SOULIER) | |
| | Moena-Factor ? (147) | |
| Factor X | Stuart-Prower-Factor | + + |
| | Plasma thromboplastin factor (AGGELER[12, 13, 15]) | |
| Factor XI | Plasma thromboplastin antecedent (PTA) (ROSENTHAL) | + + |
| Factor XII | Hagemann Factor | + + |
| Factor XIII | Fibrin stabilizing factor (FSF) | + + |
| | Fibrinase-Factor (FSF) | |
| | Laki-Lôráand-Factor | |
| | Hungarian Factor (name after SZIRMAI for Laki-Lôráad-factor) | |

The last phase has a third step, namely "fibrinolysis" (Table 4). That means the lysis or dissolution of retracted fibrin clot. Many inhibitors take part in this process. For this reason, we shall study it together with inhibitors.

*Inhibitors of Blood Coagulation :* The inhibitors of blood coagulation may be divided in two groups[150]; the physiologic ones and others. In the latter group, cumarin derivatives and heparin, which are useful therapeutic tools in thrombo-embolic conditions may be mentioned. Plasma antithrombin, antithromboplastin and fibrinolysin play an important role, that of maintaining the balance between the forces activating blood coagulation and those inhibiting it. The physiologic inhibitors show their action in three manners :

1. Inhibition of prothrombin activity
2. Inhibition of thrombin
3. Complex inhibition.

Examples to the first group of anticoagulants are heparin and antithromboplastin. Heparin also has other complex actions. The investigations of LANCHANTIN and WARE[82] show that plasma and serum possess a thromboplastin inhibitor. It can neutralize tissue thromboplastin in the presence of calcium. There is another known factor, antiprothromboplastin, that is increased in some pathologic states (hemophilia due to inhibitors). It inhibits the conversion of Prothromboplastin into thromboplastin.

To the second group, heparin and antithrombin constitute two examples.

For the third group, heparin is the unique example. MCLEAN[83] discovered heparin in the liver. It is an ester of mucotinpolysulphuric acid, containing glucosamine, glucuronic acid and sulphuric acid (JORPES[84], WOLFRAM[85], RATHGEB[86]). Contrary to that of the related chondroitin-sulfuric acid, its amino-group is not acetylated, but sulfated. Heparin can be isolated from the liver as well as from the lung, which possesses an activity of 16～280 I. U. per mg of tissue. The international standard preparation of heparin shows 130 I.U. per mg. It is made in Ehrlich's histiocytes. It is a strong acid, binding organic bases and thus forming dissociable complexes. Heparin binds preferably protaminis (clupein and salmin) and teluidine blue. HOLGREEN and WILANDER[88] showed inhibition of heparin with toluidine blue. Heparin does not act as anticoagulant when alone, but only when it is in bound form (MELANDY[89], QUICK[90]). FEISSLY and ENOWICZ[91] reported that, when joined to the so-called cofactor of the plasma (albumin X), heparin exhibits the properties of the polyvalent anticoagulant. HORN and BORSODI[92] think that heparin circulates partly in free form. When the blood stands in a tube, the bound heparin passes progressively into the free form. Protamin and toluidine-blue only bind free or disposable heparin. Heparin is a physiologic shelter against hypercoagulability and compensates the activity of procoagulant substances. The bound heparin holds probably prothrombin in an

inactive complex. Heparin inhibits conversion of prothrombin into thrombin, as well that of fibrinogen into fibrin. On the other hand, it inhibits the activity of platelet factor 3 and consequently delays the formation of thromboplastin out of plasmatic factors. Besides alpha-heparin, McLEAN and beta-heparin (MARBET and WINTERSTEIN), the names gamma-3 and gamma-4 heparin have been given to the inhibitors of inflammation and to menstruation inhibitors (SZIRMAI[93]) (Table 5).

All factors and phases of fibrinolysis are shown in Table IV prepared by SZIRMAI. From the study of this table, it emerges that fibrinolysis may be divided in the following steps:

a) Prephase: Formation or activation of the activators
b) First phase: Activation of profibrinolysin
c) Second phase: Fibrinolysin formation or transformation of profibrinolysin into fibrinolysin
d) Third phase: Thrombolysis.

In the prephase two activators are demonstrable: the tissue activator or tissue fibrinokinase and the blood activator (SZIRMAI[159]).

Dicumarol, synthetised by ANSCHUTZ[4] in 1913 has been shown to cause "sweet clover disease", a hemorrhagic disorder in cattle. The inhibitor action of cumarin on blood coagulation was shown in 1941 by LINK and COLL[95], and attention was drawn to its resemblance with the disease in cattle. Contrarily to heparin, cumarin does not reduce the reaction capacity of clotting factors, but reduces the levels of those factors formed in the liver in presence of vitamin K, namely the prothrombin and factors VII and X together with factor IX. Dicumarin which displays a chemical structure similar to that of vitamin K acts probably by taking its place in coagulation events (competitive inhibition).

On the other hand, we have two antithrombotic substances working against thrombin in different manner. The heparin-type antithrombin (ASTRUP and DARLING[96,119]) also called thrombin inhibitor, behaves like a genuine enzyme; that is, it is composed of a prosthetic group (heparin) and a corresponding coferment, called cofactor (HOWELL and HOLT[7]), heparin complement (CHARGAFF, ZJFF and MOORE[98]) and thrombin co-inhibitor (ASTRUP and DARLING[6]).

A completely different substance is shown by the so-called "serum antithrombin", which was known or assumed as present since the days of classical blood coagulation theory (WÖHLISH[99], GRÜNING[100], SCHMIDT[4], MORAWITZ[3]). Many authors[50,96,101~107] have investigated the properties of serum antithrombin, which is a substance of lipid nature. According to SEEGERS and collaborators[106] there are 4 kinds of antithromin in plasma:

1. Antithrombin I, is identical with fibrinogen, which inactivates thrombin by adsorption

Table 5

Scheme for the Haemorrhagic Ditheses by Dr. Szirmai, 1955, Berlin, Budapest

The Haemorrhahic Diatheses Appertaining to the Different Factors of Blood-Clotting

| | | Caused By | Hereditary Constitutional | Acquired |
|---|---|---|---|---|
| 1. Contents of the Arteries and Veins | 1. Plasma Factors Fluid Part B). Activators Decrease | Factor I. Fibrinogen | Afibrinogenaemy | Liver parenchyma affections worst form |
| | | Factor II. Prothrombin | So-Called idiopathic Hipoprothrombinaemy | Liver parenchyma affections K-avitaminosis Dicumarol-effect newborns |
| | | Factor III. Thromboplastin | See factor Y-X. | |
| | | Factor IV. Calcium | | |
| | | Factor V. (And VI) | Parahaemophilia carcinophilia (SZIRMAI) | Liver parenchyma affections purpura Fulminans |
| | | Factor VII. | Cases of vau Belle Alexander Owren | liver affections K-Avitaminonosis difcumarol effect newborns |
| | | Factor VIII. Antihaemophil globulin | Haemophilia A. | haemophiloid of newborns |
| | | Factor IX. Christmas factor | Haemophilia B. | |
| | | Factor X. Kolles Stuart ? | Haemophilia C. stuart $C_2$ koller | Liver affections K-Avitaminosis dicumarol effect of newborns |
| | | Factos XI ? PTA Factor (ROSENTHAL) | Haemophilia D. | |
| | A) Inhibitors Increase | I. "Hemmkörper" of theiphasis of blood-clotting antithrombin antithrombokinase alfa heparin (Mc LEAN) | So-Called hemmkörper hemophilin (DEUTSCH) Heparinphila A. | |
| | | II. "Hommkörper" of the 2. Phases of blood-clotting antithromb alfaheparin (MCLEAN) | Heparinphilia B. | |
| | | III. Beta heparin marbet and winierstein antithromboplastin antithrombin | Rheumatism heparinophilia C. | |
| | | IV. Gamma 2 Heparin (SZIRMAI) | Menses (norm) menorrhagies-heparinophila D. | |
| | | V. Gamma 4 Heparin (SZIRMAI) | Inflammations inflammable haemorrhages Heparinophilia E. | |
| | | VI. Factor VII. Inhibitor | | Converinopathia |
| | | VII. Fibrinolysin | | Fibrinolytic crisis fibrinolysophilia Fibrinogenolysinephila |
| | | VIII. Fibrinogenolysin | Fibrinogenolysophilia | |
| | Thrombozymes 2.) Compact Part | Serotonin<br>Histamine — Refractozym<br>Antifibrinolysin — Hipotensivum<br>Heparin<br>Platelets<br>Platelets Pactor 1. — Platelets factor 2.<br>Platelet Factor 2. — Platelet factor 4. | A.) Congenital Haemorrhagic Thrombasthenia Glanzmann<br>B.) Constitutional thrombopathy (V. WILLEBRANO, R. JÜRGENS)<br>Essential thrombopenia (Morbns WERLHOF) | |

| | | | |
|---|---|---|---|
| II. Walls of Arteries and Veins | Toxic factors<br>(Infection intoxication)<br>Neurovascular factors<br>Diseases of deficiency<br>(Hormon, vitamine) | | 1. Purpura rheumatica (Schönlein henoch)<br>Purpura resp. peliosis Rheumatica (SCHNLEIN)<br>Purpura abdominalis (HENSEH)<br>Anaphilactoid purpura (GLANZMANN)<br>Capillartopicosis (FRANK)<br>Haemorrhagic Hyperergic reactions<br>Purpura senilis<br>Constitutional capillary asthenia (KALTSTEIN) |
| | Deficiency of vitamin C | | II. Scurvy, Möller-Barlow-disease Scurvy of sucklings |
| | | III. The Teleangiectasia Hereditaria haemorrhagica (Morbus OSLER) | |
| Combined Form | | | Purpura fulminans anticoagulantis over-dosing |

2. Antithrombin II represents the plasma co-factor, which is necessary for heparin in its inhibiting action
3. Antithrombin III inactivates thrombin, by forming metathrombin with it.
4. Antithrombin IV interferes with the conversion of prothrombin.

The number of the antithrombins has been augumented recently. Approximately 7 antithrombins have been described, some of which are specific against factors V, VII, VIII or IX and others which non-specifically interfere with blood coagulation. Antithrombin VI among these antithrombins deserves special mention.

There are inhibitors in fibrinolysis, too. TOCANTINS[110] believes that under pathologic conditions, a specific inhihitor of plasmakinase of lipid nature, may appear.

Clotted blood is lysed after some time has elapsed. It has been shown that this fibrinolytic action is an enzymatic process (ASTRUP[111], FERGUSON[112], NOLF[113], ASTRUP[119]). It occurs by the conversion of an inactive substance (profibrinolysin, plasminogen or prolysin) into the active form, fibrinolysin. This activation takes place through the catalyzing influence of fibrinokinase. Fibrinolysin or plasmin can lyse fibrinogen as well as fibrin. Its action is inhibited by antifibrinolysin or antiplasmin. STEFANINI and GENDEL[114] showed that ACTH and cortisone are effective therapeutically in preventing fibrinolytic crisis in clinic. This may especially be useful in premature separation of placenta (KAESER[115], LÓRAND[149], SZIRMAI[132]) and in bases of prostatic cancer (RATNOFF[116], TAGNON *et*

Table 6 Schema of the Fibrinolysis after SZIRMAI (1961)

| | | Prophase<br>Activation of the Proactivators | 1st Phase<br>Activation of Profibr. | 2nd Phase<br>Formation of fibrinolysin | 3rd Phase<br>Thrombolysis |
|---|---|---|---|---|---|
| Positive factors of the fibrinolysis | Therapeutic | Pyrexal, Adrenalin SR+SD, Chloroform Afitidiabetica: Tolbutamide Carbutamid, etc……(indirect Fibrinolysis-activation by pyrexal nicotinic acid, nicotinic acid+heparin) | | Direct activation of fibrinolysin<br>lysokinase<br>streptokinase<br>staphylokinase<br>urokinase<br>polybrenes | |
| | Activators Physiologic | Proactivators<br>I. Tissue-activator ⟶ Plasminogen (Tissue-fibrinokihase)<br>II. Blood-activator Thermolabile = activator<br>Serum Co. proactivator<br>(urokinase from human urine)<br>milk, tears, etc. | Inactive profibrinolysin | Fibrinolysin = Plasmin | Lactoglobulin, Caseine gelatin ↑ Hormones: ↑ ACTH a. STH ↑<br>Thrombolysis<br>⟶fibrinogen<br>⟶fibrin<br>⟶factor V a. VIII<br>⟶prothrombin |
| Negative factors of the fibrinolysis Inhibitors (= Inhibition of the fibrinolysis) | Physiologic Inhibitors | I. Tissue-antifibrinokinase<br>II. Serum-antifibninokinase | | Antifibrinolysin (Antiplasmjn)<br>Pancreas inhibitor (=antifibrinolytic action)<br>Cohn. Fr. IV-1, IV-4, V.<br>ACTH, cortisone (?)<br>prednisolone (?) | |
| | Therapeutic Antidotes | E-aminocaproic acid (=Antiactivator+antiplasmin)<br>trypsin<br>kunitz } Inhibitor of<br>zinc, Cu | | sofainhibitor<br>E-aminocaproic acid<br>(antiactivator+antiplasmin)<br>protaminsulfate, trypsin<br>K 1-vitamine, zinc, Cu | |

*al*[116]).

The activation of profibrinolysine or plasminogen (into the form of fibrinolysine or plasmin) occurs in the following manners:

1. Spontaneous activation under unknown influences
2. *In vitro* activation through chloroform treantment
3. Under the influence of enzymes derived from hemolytic streptococci, Namely streptokinase, and
4. Under the influence of tissue fibrinokinase.

In all cases with increased fibrinolytic activity one may detect a pathologic process that can explain its presence.

All the factors which we quoted in this article have many synonyms that are shown in the tables that follow concerning the factors or the properties of various hemorrhagic states (for example Table 6).

## SUMMARY

The authors give an account of the important developments in blood coagulation knowledge from the times of Malpighi and Moravitz to data. The article is followed by original tables providing a general and comprehensive view on blood coagulation, hemorrhagic syndromes and fibrinolysis.

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