Thrombelastographic Observations on the Characteristics of Hemophilic, Thrombocytopenic and Heparinized Blood

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The significance of thrombelastography in blood coagulation studies was analyzed in previous investigations. Thrombelastographic determinations consist in the graphic measurement of the change in the elastic forces of the clot during the whole coagulation process. Such variations are concerned with structural modifications of the fibrin molecules and are supposed to be significant with respect to the hemostatic function of the thrombus.

In the graphic representation thrombelastogram (TEG), of the tracings obtained by this method, three variables are usually evaluated: the reaction time \( t \), the \( k \)-value, corresponding to the clot formation, and the maximal amplitude or \( ma \), which is related to the maximal clot elasticity. The \( ma \) values vary in direct proportion to the platelet number and function.

Among the coagulation defects, the most typical alterations are observed in the hemophilic syndromes, the syndromes due to circulating anticoagulants, and in thrombocytopenias. In the hemophilic syndromes, the characteristic patterns are represented by a marked prolongation of the reaction time and of the \( k \) value, without significant alterations of \( ma \). Similar modifications are observed in hemophilia-like syndromes, and by injecting or adding, in vitro, heparin and heparin-like substances. In this case, however, a more or less marked decrease of \( ma \) is usually observed. Further details on this behavior will be given in the course of the present paper. In thrombocytopenias and in platelet diseases in general, a normal reaction time is accompanied by a prolongation of \( k \) and a marked decrease of \( ma \).

The purpose of this paper is to analyze the correlations between the various components of the TEG in hemophilic, in thrombocytopenic and in heparinized blood, with particular reference to the variations of \( ma \), in connection with platelet function. The variable response to heparin and synthetic heparin-like substances was compared with the typical behavior in hemophilia and thrombocytopenia. Further suggestions for the evaluation of TEGs were presented on the basis of the obtained results.

Methods and Material

1. Thrombelastographic determinations. The Hellige thrombelastograph was employed as described elsewhere. All determinations were made on recalcified, oxalated plasma, by mixing 0.25 ml of plasma with 0.1 ml of 1.29 per cent calcium chloride.

2. Hemophilic syndromes. Thirteen determinations were made in cases of deficiency of antihemophilic globulin (AHG), in one case of Plasma Thromboplastin Component (PTC) deficiency and in one case of Plasma Thromboplastin Antecedent (PTA) deficiency, when the patients were not under the influence of blood transfusions or other therapy. The differentiation was made by means of the crossed recalcification tests, by employing normal plasma, aged normal serum, and BaSO₄-adsorbed plasma or serum, as previously described.

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3. Thrombocytopenic syndromes. Twelve determinations were made in cases of primary and secondary thrombocytopenias.

4. Heparin and heparin-like substances. Commercial heparin (Liqaemin), $\beta$-heparin (Ro 1.2232/715\textsuperscript{1}),\textsuperscript{11} and synthetic heparin-like substances were used at various concentrations. The heparin-like substances were as follows:

   a. treburon (Ro 2.3053\textsuperscript{1}),\textsuperscript{12}
   b. polyanethol sulphuric acid or liquor\textsuperscript{1}
   c. a mucooitin sulphuric acid derivative (SMA) (Ro 1.7198\textsuperscript{1});\textsuperscript{11}
   d. a polyvinylalcohol derivative (PVA) (Ro 1.5071/15\textsuperscript{1});\textsuperscript{15}
   e. xylan sulphuric acid ester or thrombocid;\textsuperscript{16},\textsuperscript{17}

   The concentrations used for the heparin-like substances were 0.3; 0.6; 0.9; 1.2; 1.5 per thousand. The same concentrations were used for $\beta$-heparin. Heparin, containing 130 units per mg., was used at dilutions ranging between 0.05 and 0.3 per thousand.

   One ml. of oxalated human, normal plasma was mixed with one tenth ml. of the various solutions of heparin and heparin-like substances. In all samples the recalification times were determined as already indicated. Saline was substituted for heparin and heparin-like substances as a control.

5. TEGs were analyzed by calculating in mm the reaction times $r$, the $k$ values and the maximal amplitude $ma$, and by plotting them in a coordinate system. The normal values are indicated by the shadowed areas in the graphs of figures 2-4. The various components of the TEG are represented in figure 1. The reaction time corresponds to the initial straight line. The $k$ values are measured between the end of the reaction time and a point on the horizontal line, corresponding to a distance of 20 mm. between the two curves. The maximal amplitude corresponds to the maximal distance between the two curves of the TEG.

**RESULTS**

The results obtained in hemophilic and thrombocytopenic blood are given in the graphs referring to the correlations between $r$ and $ma$, $r$ and $k$, $k$ and $ma$ (fig. 2-4). The reproduction of the concerned TEGs is given in figures 5 and 6, including the TEGs of hemophilic and thrombocytopenic patients.

The difference between the two groups of diseases is clearly visualized by the comparison of the groups of values ($r$, $k$, and $ma$), which is located in distinct ranges of the graphs, particularly when $r$ is plotted against $ma$. This characteristic behavior can be differentiated by a straight line, dividing the two groups of results and indicating the limits between their typical patterns. If the $ma$ and $r$ values are compared, the thrombelastographic characteristics of hemophilic

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* Kindly supplied by Hoffmann-La Roche, Basle, Switzerland.
blood are consistent with a definite increase of the $r$ values, without significant variations of $ma$. The opposite findings are true for thrombocytopenic conditions, where the occasionally slight prolongation of $r$ is always accompanied by a more or less marked decrease of $ma$.

The comparison between $k$ and $ma$ allows us only to distinguish the two groups of syndromes on the basis of the different $ma$ values, insofar as the $k$ value might be only moderately prolonged in hemophilic syndromes, owing to the long time required for clot formation in thrombelastograph.

The same is true in the correlation between $r$ and $k$, in which the most sig-
significant pattern for the differentiation is represented by the prolonged $r$ values.

The thrombelastograms of figures 5 and 6 reproduce the coagulation process as a whole in hemophilic and thrombocytopenic blood. Although the typical spindle-shaped thrombelastogram is not found in all hemophilic syndromes and the thin, cylindrical thrombelastogram is not the rule in all platelet defects, the difference between the two groups can be recognized by simply looking at the general characteristics of the tracings.

The results concerning the effect of heparin and heparin-like substances are reproduced in the lower portions of figures 2–4, and are briefly referred to as heparinized blood. In figure 7 are included the most typical TEGs of the performed experiments. The characteristics of heparinized blood can be rather clearly evaluated by comparing the thrombelastographic data in such conditions with the TEGs of hemophilic and thrombocytopenic blood. The action of heparin and heparin-like substances is revealed in the TEGs by typical alterations of $r$, $k$ and $ma$. The prolongation of $r$ is always accompanied by a prolongation
of $k$, as indicated by comparing the two groups of values (fig. 3). Such a behavior is similar to that of hemophilic blood. In fact the values of $r$ versus $k$ are located on the same line in hemophilic and in heparinized blood, which is clearly distinguishable from the behavior in platelet defects.

When $r$ and $ma$ values are compared (fig. 2) the correlation is different. In this case, by considering the line which divides the hemophilic and the thrombocytopenic cases, the results obtained with heparinized blood are to a great extent on the thrombocytopenic side. Some considerations can be made in this connection. The thrombocytopenic-like effect varies according to the employed substance. It is very marked when liquid is used, and is very moderate when using SMA and thrombocid. The behavior for the other compounds is intermediate. Similar results are obtained by plotting the $k$ values against the $ma$ values (fig. 4). This is particularly evident in the concerned TEGs (fig. 7). The most typical anticoagulant effect is characterized by a TEG, which is spindle-shaped, as in hemophilia, and thin, as in thrombocytopenias. When the anticoagulant effect is less marked, such characteristics are not clearly visualized.
Discussions and Conclusions

The experiments presented make it possible to:

1) establish a further differentiation, on the basis of thrombelastography, between hemophilic, thrombocytopenic and heparinized blood;

2) suggest the correlations between the components of the TEGs and identify their most typical variations.

As to the first point, the analysis of a group of TEGs in hemophilic and thrombocytopenic syndromes indicated a more complete way for distinguishing the two syndromes. This applies to the cases of hemophilic syndromes, in which the clotting times are not typically prolonged, and to the cases of thrombocytopenia.
with occasionally prolonged clotting times. Such a differentiation can be useful in forms of mild hemophilia when compared with cases of thrombocytopenic disorders, without modifications of platelet count. Although today there are other ways for making a diagnosis in such cases, thrombelastography is able to give directly an answer to the alterations of the platelet function, as revealed by \( \text{ma} \).

In connection with the thrombelastographic differentiation of AHG-, PTC- and PTA-deficiencies, no definite results were obtained, since similar patterns were obtained in the three conditions, as far as the few considered cases are concerned.

The presence of a quantitative and or qualitative platelet defect during the anticoagulant action of heparin and heparin-like substances was already identified by means of other techniques. The significance of the thrombelastographic examination is to define the correlations between the anticoagulant, hemophilia-like effect of such substances and their possible effect on platelets. It was observed that a thrombocytopenic effect almost always accompanies the hemophilia-like effect. A different behavior was observed in the thrombelastographic characteristics of the various anticoagulants. Therefore the combined effect should be taken into consideration when heparin and heparin-like substances are used.

With respect to the second point, the correlation between the components of TEGs, represent a further improvement for the evaluation of TEGs. They allow us to locate the concerned alterations in connection with the zones of the most typical, known variations, such as those of hemophilic, thrombocytopenic and heparinized blood. The rate of clot formation as indicated by \( k \) should always be compared with the reaction time; a long reaction time with a slightly prolonged \( k \) value is consistent with a less marked coagulation defect than in the
Fig. 7. Typical thrombelastograms referring to the anticoagulant action of heparin and heparin-like substances: 1-2, thromboeid; 3-4, treburon; 5-6, SMA; 7-8, heparin; 9-10, liquoid; 11-12, PVA; 13-14, g-heparin.

presence of long values for both $r$ and $k$. The comparison of $r$ and $ma$ demonstrates the existence of a platelet defect when a prolongation of the clotting time is observed. This should be taken into consideration not only in typical platelet defects but also when a secondary platelet defect may arise from a primary plasmatic disease, which is accompanied by a slow, decreased thrombin formation. Such a behavior might also be observed in some cases of hemophilia and is the basis for the old interpretation of a thromboytic pathogenesis of hemophilic syndromes.
Summary

1. Thrombelastographic determinations were made in cases of AHG-, PTC- and PTA-deficiencies, in thrombocytopenias and in normal plasmas after the addition, in vitro, of heparin and synthetic heparin-like substances.

2. The components of the thrombelastogram (TEG) were correlated with respect to the reaction time (r), the rate of clot formation (k), and the maximal amplitude (ma).

3. The differentiation of the hemophilic and thrombocytopenic syndromes was made on the basis of the typical variations of r, k and ma: prolonged r and k in hemophilia, prolonged k and decreased ma in thrombocytopenias.

4. The behavior of heparinized blood was characterized by a hemophilia-like prolongation of r and k and a thrombocytopenic-like decrease of ma, with variations depending on the compound used.

5. The correlations between the r, k and ma values of TEG are suggested for the evaluation of thrombelastography.

Summario in Interlingua

1. Esseva executate determinaciones thrombelastographic in casos de carentia de globulina antihemophilic, del componente de thromboplastina plasmatic, e del antecedente de thromboplastina plasmatic, e in thrombocytopenias e plasmas normal post le addition in vitro de heparina e synthetic substantias heparinoide.

2. Le componentes del thrombelastogramma esseva correlationate in respecto a tempore de reaction, rapiditate del formation de coagulos, e amplitude maximal.

3. Le differentiation del syndromes hemophilic e thrombocytopenic esseva establite super le base de typic variationes de tempore de reaction, rapiditate del formation de coagulos, e amplitude maximal. Le prime e le secunde de iste factores esseva prolongate in hemophilia. In thrombocytopenias solo le secunde esseva prolongate, e le tertie esseva reduceite.

4. Le conducta de sanguine heparinisate esseva characterisate per un prolongation hemophilioide del tempore de reaction e del rapiditate del formation de coagulos e un reduction thrombocytopenioide del amplitude maximal, con variationes secundo le varie compositos usate.

5. Correlationes del tres factores mentionate es recommendate como medios utilisabile in le evalutatiom de thrombelastogrammas.

References


THROMBELASTOGRAPHY

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