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5. INTERNATIONAL CONGRESS OF BIORHEOLOGY

POISEUILLE AWARD LECTURE

BIORHEOLOGY IN THE PRACTICE OF MEDICINE: RESONANCE THROMBOGRAPHY

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The former heading of my invited lecture today did implicate a more general character of performance. This at least was the leading idea, when I prepared the provisional abstract some months ago.

The president of our society, Professor Siegfried Witte, later asked me to understand the title more in the sense of my own contributions to this topic. So I beg your pardon if I am recovering some aspects in my lecture which not have been announced in the abstract. Of course I am very grateful for this kind of concession.

Rheology is not only a natural science, it is including also some kind of philosophy. The famous sentence "Corpora non agunt nisi fluida" (Paracelsus) is referring living nature to actions, to happenings. A standstill this way seems to be the opposite of life. Actually processes of living in the organism of vertebrates are extending from the comparably fast flowing blood to regions where action is extremely scarce, but comparatively important. In Rheology we are used to keep up with these extreme contrasts. They all have their wide range of particular mechanisms.

There is now one unparalleled spot in the physiological structure of organism. This spot is producing a rather fast transition of a really fluid matter into a rather solid one. It is the coagulation of blood. The extremely fast action partners of this process are not only life saving in certain situations. Some of them even are imperative for the function of cell division by moving the chromosomes.

The mechanism of blood clotting has evelved in countless genera-

KEYWORDS: Resonance thrombography, Retractography

tions of living organisms. They always retained the ability to transform the blood or blood-like fluid into a material resistant against the stripping forces of passing blood flow.
The general scientific interest in blood clotting for a long time mainly applied to the change of fluidity by increase of viscosity. It was explained by interference with the clumpy state of clot, finally cutting short the blood flow in a vessel or in a wound.

My interest primarily was the mode of formation and structural order of these flow stopping brake blocks with their admirable stability.

In the first order it seemed obvious that in a material which inevitabily would be destroyed by conventional methods of viscosimetry, a measurement of viscosity perhaps was not the proper parameter to find out its physiological stability. So I tried to find out a method which was able to measure some qualities of a clot - or better coagulum - without destroying its structure.

This intention (1947, 1) lead to the surprise, that the blood coagulum is a highly elastic structure due to an up to that time unknown elastic quality of fibrin. I called the method I had constructed between 1943 and 1947 for this purpose thrombelastography (TEGraphy, 1, 2, 3, 4), because it mainly showed the elastic qualities of a blood clot.

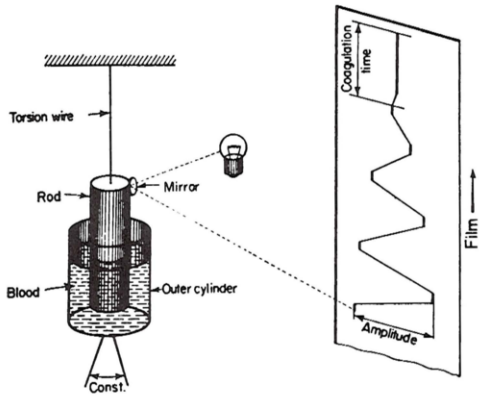

FIG. 1
Method of thrombelastography schematically

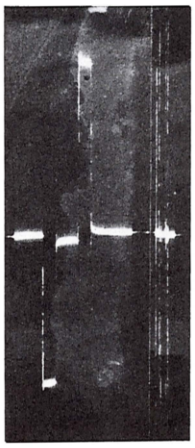

FIG. 2
Hysteresis of a platelet rich plasma. Left: release after 1 min constant deviation. Right: bending back and forth, release after 1 sec.

FIG. 1 shows the principle of the TEGraph well known to its users in clinical medicine. Some of them, who did not believe in this uncommon quality of fibrin had proposed to rename the new method into thrombodynamography (5), because it seemed unbe-

lievable to them, that fibrin is marked by such an extraordinary elasticity. Yet in the same year Ferry and Morrison (1947, 6) in USA had similar results investigating fibrin by another procedure. They compared the structure and elasticity of fibrin with that of rubber.

FIG. 2 is representing a special hysteresis examination of a coagulum from normal platelet rich plasma (PRP) in the circular gap of the measuring chamber of the TEGraph. A small turn of the inner cylinder against the unmoved outer cylinder was the shearing action. It was released after 1 min of arrest. A distinct but for a physiological substance rather low hysteresis is to be seen, testifying the nearly technical quality of fibrin.

The clinical adoption of TEGraphy was asking for the possibility of an extensive differentiation of the structural assembly of coagulum on the one hand. On the other hand it had to be clarified, which of these assembling processes could be recognized in the thrombelastogram (TEG).

One of the most complicated things in coagulum structure is the part played by platelets. In the thrombelastogram there is no primary sign which would allow effects caused by fibrin activity to seperate from effects caused by platelet activity.

The seeking for a method to record the mechanical activity of platelets during coagulation led to a special construction which is able to demonstrate the condensating (retractive) activity of platelets. The recording is beginning during the production of the first coherent traces of fibrin structure.

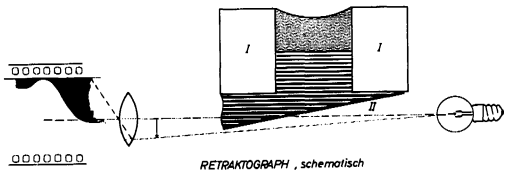

FIG. 3
Recording Retractograph schematically. I = ring, II = surface tension suspended disc. In between PRP with overlayed saline.

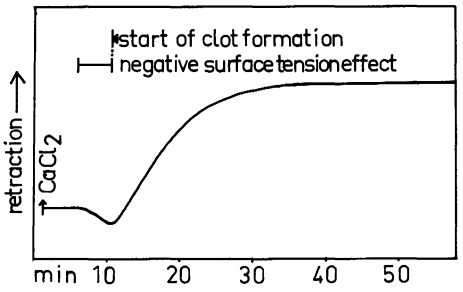

FIG. 4
Recorded silhouette of contracting platelet rich plasma. See text.

FIG. 3 is showing the method, which I published 1958 (7). A small metal ring (I) is put on the thin disc of the same outer diameter. Two drops of coagulating but still fluid plasma or blood is filled into a ring. If the ring is lifted the little disc by the surface tension of the fluid always will as shown take an inclined position. In addition the plasma is overlayed by saline.

It does prevent the plasma from interposing a special surface layer. This layer normally would retain the emerging serum from passing out of the coagulation drop. To escape drying up of the drop the whole set is put into silicone oil. This all sounds a little trouble-some but really is simple to perform. The only purpose of this little machine was to find out, what platelets are doing mechanically during the process of coagulation.

Now if the rim of the disc is projected on a recorder film, one can see that the disc is raised during coagulation of PRP, that on the other hand it will move not at all, if there are no platelets in the plasma (PPP).

FIG. 4 is such a recorded silhouette of a platelet rich plasma, showing contraction. The main thing yet is the following: We see the contracting movement. But where is the answer for the main question, namely which correlation in time may exist between start of contraction and the start of structure producing coagulation? The record in FIG. 4 begins about 5 min before the rising line indicates clotting resp. contraction. For the answer to our question the short part of the record before contraction is important. It does not show a horizontal line of quietness as one would expect. It indeed for a short while shows a decline of the little disc, indicating a lowering of surface of the plasma. These substances are released in the beginning of clotting process and this way lower surface tension (8). This lowering of surface tension only can cause a lowering of the disc, if the disc is still free from attachment of coherent fibrin structure, because any fibrin structure would tie up the disc to the overlaying ring. With other words: as long as the line left hand on the slide is going down, there can be no coherent coagulation structure. But in the moment the graph is bending up, contraction by platelets is in action. This implicates the conclusion, that platelets are exerting their contractive activity already with the first traces of coherent fibrin structure, a fact of which up to now not many coagulationists are aware.

In the permanently increasing number of laboratory tests a critical survey is not existing, which are best for which clinical problem. Regarding the structural mechanics of platelets in combination with fibrin we just depend on testing this structure from its very first traces, if this could be made possible somehow. An adequate method to measure this process should on the other hand work under as physiological conditions as possible. One condition of the first order is a comparatively natural flow up to the moment the turnover of fluid plasma in a coagulum is stopping the flow by itself.

The endeavour to combine these different demands in a method to follow up the proper coagulation process finally brought about an new method. At first it was called Rheosimulator (11) because it simulates a flow of physiological speed in the prephase of clotting. Later I changed its name to Resonance Thrombography +) (12, 13) because the more important feature is the measurement of resonance of the elastic fibrin structure.

+) Produced by Fresenius KG, 6380 Bad Homburg v.d.H. P.O.B. 1480

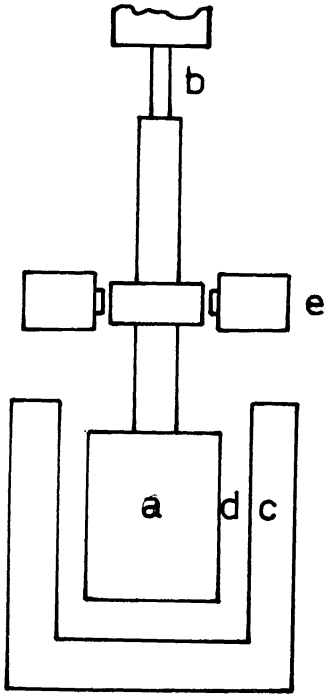

FIG. 5

Measuring device of Resonance Thrombograph. Rod (a), elastic suspension (b), container (c), gap for coagulation substrate (d), electronic drive (e).

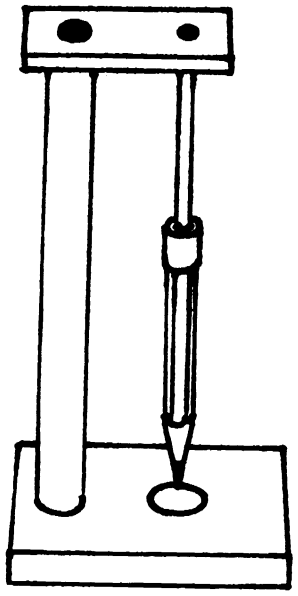

FIG. 6

Swinging pencil, elastically suspended, as an example for orbital movement.

FIG. 5 shows the principle of the measuring device. It schematically consists of a container (c) and a rod (a) with a circular gap for coagulation substrate-as I had shown in FIG. 1 with thrombelastography. Yet as much alike these parts are looking as different is there function. The container (c) - outer cylinder - is fixed and motionless but heated to 37°. The rod (inner cylinder) is suspended from an elastic bar (b) and is driven by a constant electronic stimulus (e) to move around like the pencil shown in the next FIG. 6, similar to a stirring action. The radius of this movement is about 0,03 mm if the gap is filled with not clotting blood. The type of movement is a so called orbital movement, resembling a rolling eye in its eye socket. This simply means, that the rod of our machine does not at all turn around its own axis, like an eye looking around.

The electronically driven rod has a constant orbital frequency of 45 Hz whereas its elastical suspension would give him a natu-

ral frequency of 38 Hz. This means that there can be no perceptible resonance between the two. Yet the growing fibrin structure of a coagulum adds with its elasticity to the elasticity module of the rod suspension, this way increasing the natural frequency of the oscillating set consisting of the elastically suspended rod and the elastic fibrin structure.

The increasing natural elasticity of the set is approaching this way its factual driving frequency up to a coincidence of the two. Yet with further increasing natural frequency there is vice versa a going asunder of the two frequencies. The changing connection between natural and driving frequency is implying the degree of resonance, this way becoming measurable.

The expression of this process is the Resonance Thrombogram (RTG), FIG. 7. It simply is a recording of the changing width of the radius of orbital movement by the effect of resonance. The schematic RTG is showing the reaction time on basic line on the left of the graph. During these about 9 min normally nothing is happening, i.e. the basic radius of the orbital movement remains unchanged as long as the coagulation substrate is fluid. Defects in the plasmatic phase of coagulation, as therapy with heparin or dicumarol, on the other hand congenital defects as hemophilia, of course will proportionally prolong this r-time.

For clinical use we only work with citrated blood to make the practical application as simple and time-saving as possible. Depending on the special diagnostic situation, the blood is recalcified or coagulated by thrombin resp. in addition neutralized by protaminchlorid, if heparin is added in high amounts. These measures if applicated in due form do not falsify the results, of course except the part of r-time.

During the fluid phase before clotting the blood is stirred by orbital movement with a radius of 0,03 mm and with an average speed of flow of about 5 mm/sec, comparable to a flow in a medium sized vein. Investigations by electron microscope (14, 15) have shown, that this flow has a specific influence on the structure of the succeeding coagulum.

The upright angle of the RTG, the so called FP-complex indicates its ascent with the F-side up to the summit and its decline with the P-side. In this up and down of the course natural frequency of the set permanently is going upward. At the F-side its resonance with driving frequency is increasing and this way causing the ascent of the angle. The peak of the angle is representing the culmination point of resonance. Later the still increasing natural frequence is withdrawing upward from driving frequency, this wav reducing resonance. The expression of diminishing resonance is the descending P-side or more figurative: P-leg.

The tracing of the P-side is crossing basic line downward. It indicates that this side of FP-complex is reducing the orbital radius beneath its starting line, often into vicinity of zero line. This damping of movement is caused by platelet activity. Its condensating effect is producing some kind of a tenacity, the confi-

guration of which has been shown in the electron microscope (14).

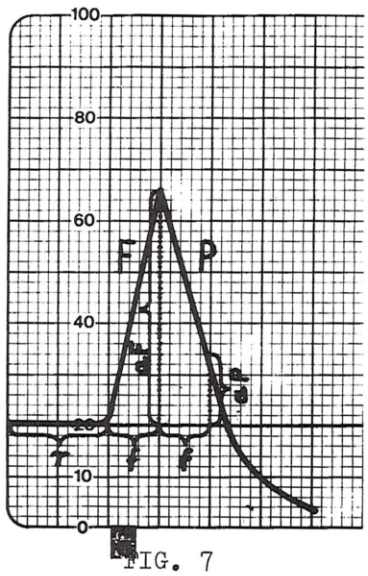

FIG. 7
Resonance-Thrombogram, standard measures. Reaction time (r), FP-complex with amplitude aF. Deviation of P from symmetry = aP. See text.

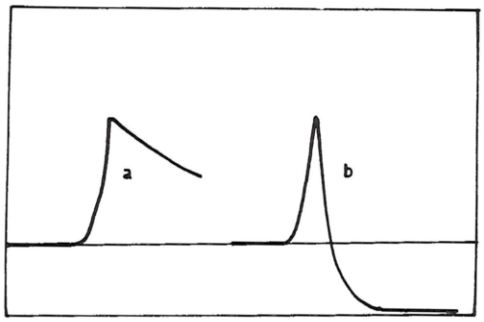

FIG. 8
RTG of platelet poor plasma, PPP, (a) and of normal platelet rich plasma, PRP, (b).

In FIG. 8b there is an RTG with normal platelet rich plasma (PRP), in FIG. 8a is an RTG with platelet poor plasma (PPP, 12.000/mm^3). You see that here only the P-side is affected by elemination of plate lets. The letter F stands for fibrin structure. The (fibrin-) F-leg of the FP-complex is not affected by reduction of platelets in number.

As we saw in the experiment with the clot retraction machine, platelets apparently are taking part in fibrin structure production from the very first moment. This means that in some way also the F-leg must be influenced by platelets. The answer for this question is seen in FIG. 9. It is the RTG of a case of thrombocytopenia - yet also of thrombopathy, because the number of impotent platelets in this clinically severe situation was 16.000, some more than the 12.000 normal functioning platelets of the centrifugated PPP in FIG. 8a. We often see this discrepancy in the rather heterogenous cases of thrombopathies, showing that function and not number is decisive.

In many clinical cases with low platelet counts and with or without bleeding tendency this discrepancy between number and function of platelets has been confirmed.

In the case of FIG. 9 there is not only a typical lift of P(latelet)-leg due to the insufficiency of platelets but also a flattening of F(ibrin)-leg. The influence on F indicates, that something in these ill platelets is missing which does not depend on

their number. In cases of thrombopathy like this one the delay of fibrin formation (resp. coagulation) in its early phase cannot be compensated ex vivo by addition of thrombin. It indicates that it is not the basic clotting process with thrombin as its last but one end product which here has become insufficient. In the contrary it is a special disorder of platelets, possibly a defect of something normally delivered from them. It could be a substance resembling clotting factor XIII. Some probability of such a mechanism comes from clinical experience, that a typical petechial bleeding of this type cannot arise without participation of insufficient platelets. The same mechanism of coagulopathy could cause bleeding with the so-called coated platelets in certain leukemias. Here their dysfunction could be explained by trapping of the hypothetical activity in or on the platelets.

A great number of different types of petechial bleeding resp. of purpura could be explained by pathological defects in Copley's EEFL, the endoendothelial fibrin lining (16-19), in the sense of destruction. However petechial bleeding in case of thrombocytopathy as mentioned primarily could be due to absence of a (platelet-born?) substance normally arranging the molecules of EEFL to some kind of a structural order - not identical with proper coagulation - perhaps the fibrin(ogenin) gel formation which according to Copley is likewise responsible for some specific functions of basement membrane. For their activition platelets apparently do not need thrombin. Shear stress for example may be adequate.

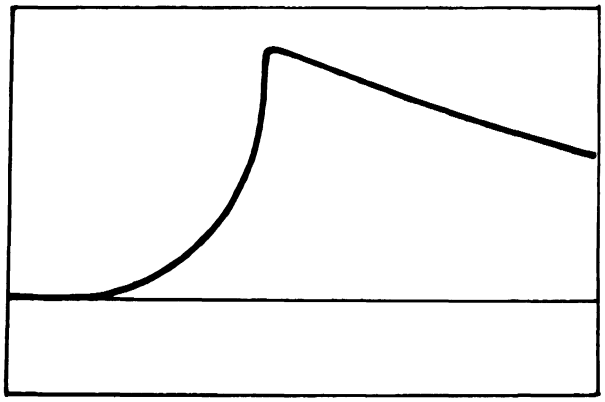

FIG. 9
Severe thrombopathy plus thrombocytopenia with delayed coagulum production.

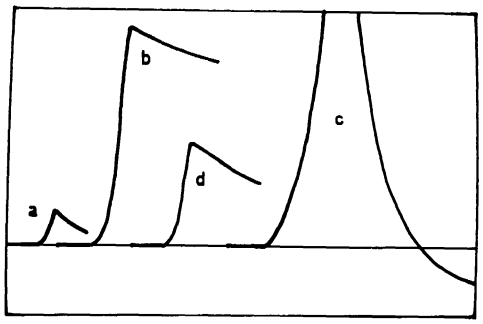

FIG. 10
Platelet poor plasma: with high fibrinogen level (a), normal fibrinogen (d), low fibrinogen (b). Normal PRP, diluted ten times (c).

FIG. 10 is representing the quantitative effects of fibrin on the RTG and likewise its combination with platelet defects. In (d) you see again the platelet poor plasma as in FIG. 8. In (b) there is a plasma with both low platelet count as well as low fibrinogen content.(the same can be found in cases of dysfibrinogenemia yet normal fibrinogen level). Low fibrin content in certain circumstances increases the extent of resonance in an unproportional range because of a weakened tenacity effect in relation to the increasing elasticity module. The opposite is seen in (a), where a high fibrinogen level of 7,5 g/l causes a short F-leg but still a flattend course of P-leg, because of the low platelet count. In (c) there primarily was a normal platelet rich plasma with normal fibrinogen level, but then diluted ten times by saline to a fibrinogen level of only 0, 25 g/l. (For comparison it should be mentioned, that for example the usual Quick-test machines for a common test are requiring at least three times the concentration of fibrinogen designing this RTG). Here you see an extreme reduction of tenacity combined with a proper elasticity.

FIG. 11 shows the six basic types of RTG's in its upper part. Beneath there are the corresponding TEG'S with less differences in their physiognomies, a disadvantage if quick diagnosis is required. The TEG primarily cannot differ between platelet activity and fibrin efficiency in the coagulum. In the RTG platelet activity and fibrin efficiency can be differentiated by their appearence in the two different parts of the FP-complex. Here they are also distinguishable by the fact, that platelets act with a condensating and likewise damping effect on the course of RTG whereas fibrin as a primarily elastic material acts more on the dimension of elasticity.

The six RTG's on top of FIG. 11 are from citrated venous blood of

different patients. 0,2 ml of blood was recalcified on the container before immersing the swinging rod - except the last one (f) to which thrombin was added because of the therapeutical admixture of heparin to the blood.

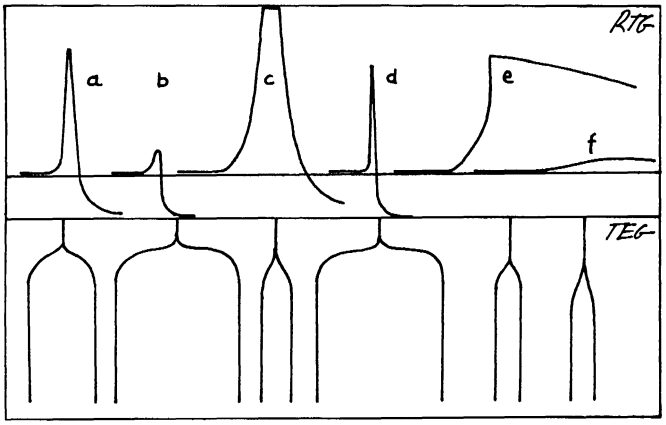

FIG. 11
Upper part: the six basic types of RTG. Normal (a), hyperfibrinogenemia (b), hypofibrinogenemia (c), thrombocytosis (d), thrombocytopenia-thrombopathy (e), DIC plus defect of factor XIII (f).
Lower part: correspending Thrombelastograms.

FIG. 11 is a normal RTG. In (b) there is a case of hyperfibrinogenemia (7,5 g/l). In (c) there is a case of hypofibrinogenemia (0,35 g/l). (d) shows a thrombocytosis of 550.000 Thrombocytes/mm^3; here the P-leg of the FP-complex goes nearly vertical down, the strongly increasing tenacity by platelet condensation leads the line of the graph practically to zero. (e) is a case of M. Werlhof with a relative thrombopenia (47.000/mm^3) but predominant thrombopathy. In (f) there is a case of DIC and an additional defect of factor XIII. The combination of these effects is furnishing an example for the manyfold and often racing variations of RTG's in such cases (regarding DIC and fibrinolysis see later). An extreme flattening of the graph often is caused by an additional defect of factor XIII. The diagnosis simply is given by proper elevation of the graph as well as by improvement of clinical symptoms after administration of factor XIII. A test for the assay of factor XIII on this basis is in preparation (Hartert).

There will be shown now some purely clinical aspects of the RTG-application in diagnostics and in guidance of therapy in two typical examples.

FIG. 12 are three RTG's of a very severe case of thrombocytopathy plus-penia. (In each graph there is for comparison a softlined normal FP-complex). Above is the RTG before splenectomy, the middle RTG was one hour, the low RTG five hours recorded after the operation. A nearly total normalization of RTG already after 5 hours following splenectomy is obvious. The RTG's improved to totally normal measures after some days and remained normal up to

now.

Fig. 13 is a case of DIC in a young mother after birth of twins. The first signs of DIC are to be seen (on top) with the so to

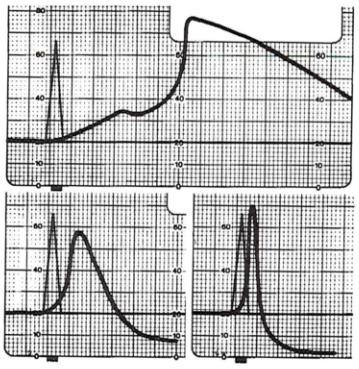

FIG. 12
Above: RTG of severe M. Werlhof. Beneath left: RTG 1 hour after splencetomy, right: RTG 5 hours after splenectomy. Softlined: Normal FP-complexes for comparison.

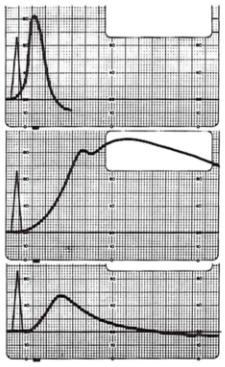

FIG. 13
Above: RTG in the outset phase of DIC. Middle: increasing SFMC's, platelet insuffiency. Below: additional systemic fibrinolysis.

speak "blown up" RTG due to the increase of SFMC's (compare with normal FP-complex). In the middle this condition has increased including an additional insuffiency of platelets. In the RTG beneath a further effect of some systemic fibrinolysis is participating.

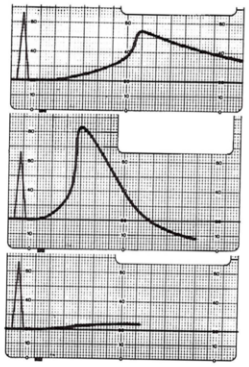

FIG. 14
Above (cf FIG. 13 above): further increase of SFMC. Middle: distinct improvement after relaparotomy. Below: extreme systemic fibrinolysis.

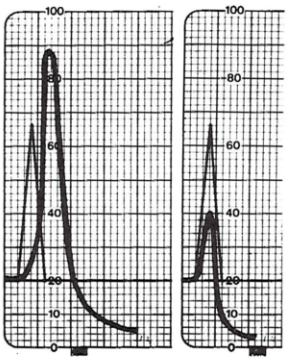

FIG. 15
Left: repeated relaparotomy, decided improvement (compare normal FP-complex). Right: normal RTG with slight hyperfibrinogenemia.

In FIG. 14 (above) this condition is deteriorating with distinct

prolongation of clotting time. In the middle RTG after many therapeutical measures including hysterectomy, two times relaparotomy, infusions of more than 12 l blood, several times high amounts of platelet concentrates and of course after all adequate kinds of corrective actions towards the coagulation system, a significant improving of RTG - still with some platelet insufficiency - is apparent. Beneath is an RTG with intensive systemic fibrinolysis including increase of SFMC. No investigation of factor XIII was made in this case.

FIG. 15, left RTG: After another relaparotomy definite improvement with only a rest of SFMC activity. Some days later a very normal RTG (right) with even a little hyperfibrinogenemia was found.

The two clinical examples and further typical cases recently have been published elsewhere (20) with extensive clinical interpretations.

The clinical employment of RTGraphy includes the sereening of preoperative patients and of diagnostic cases with coagulation defects the classification of which is not yet known. It can be very time sparing if an orientating RTG some minutes after withdrawal of a blood specimen is pointing out the type of coagulation defect in a way that further determinations had to define specific details in only one direction.

The future of diagnostics as well as of control of therapy of coagulation disorders will with more knowledge present more and more methods to differentiate and measure single factors and effects. It will present a situation for the laboratory which cannot permanently continue. The supply of countless results of uncertain validity for practical use is raising the tendency to describe a complex pathological feedback incident only by addition of numbers. This kind of imperfection should give way to more serviceable ideas. One should find methods with a functional evidence, which at times are giving reference to the point.

May I finally give a natural example for my conception: If one tries to estimate the prospective performance of a promising champion runner, one should try to find this out by measurements on his muscles, on his heart, on his psychological intention etc. and then to combine all the respective numbers to learn about the possible effectivity of the champion runner. The result may be interesting. But regarding the type of problem which has to be solved here I have another proposition to judge the runner: Just see how fast he runs!

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