THE VALUE AND THE LIMITATIONS OF THE COAGULATION TIME IN THE STUDY OF THE HEMORRHAGIC DISEASES

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THE DETERMINATION of the coagulation time of the blood is among the most empirical procedures routinely employed in the clinical laboratory, and is one most prone to be misinterpreted. In a critical evaluation of this test, one must consider first the mechanics of the procedure and second the physiological aspects, which require a translation of an *in vitro* observation into a probable *in vivo* behavior that is coordinated with other factors bringing about hemostasis. For this task a short historical survey is helpful since one can thereby acquire a knowledge of the evolution and development of the tests of coagulation time that are now in common use.

Although delayed coagulation of shed blood in various conditions was observed even in antiquity, there appears to have been no formal clinical test until 1878 when Vierordt devised a procedure consisting of drawing a horse hair through blood in a capillary tube and observing first the point when fibrin threads adhered and again when the hair was free. In 1893 Wright determined the coagulation time by filling capillary tubes with blood and noting the time when the contents could no longer be discharged by blowing. This investigator appears to have been the first to state specifically that the coagulation time of hemophilic blood was delayed. Brodie and Russell three years later described a special instrument called a coagulometer in which a hanging drop of blood observed under a microscope is played upon by a current of air and the time determined for arresting the movement of erythrocytes. In 1898, Hayem introduced the simple procedure of putting venous blood in a test tube and noting how much time was required before a sufficient clot was formed to permit tilting without a flow of blood. Fifteen years later Lee and White employing the same principle devised a test which with minor modification has become the most widely used and most acceptable method for estimating the coagulation time. It is this test which is critically studied in this paper.

Several other tests should, however, be mentioned because they have in the past been employed extensively. Two of these methods were described in 1904. The first was Bürker in which a fine glass rod is passed repeatedly through a drop of blood thereby catching the first strands of fibrin formed. The other was devised by Sabrazès who filled capillary tubes with blood and at regular intervals of time broke off a short piece until a fibrin thread appeared between the severed sections. Fuld and Schlesinger, in 1912, introduced another approach; blood was placed in a U-tube and the movement of a metal bead observed as the tube was gently tilted and the moment timed when the density of the clot fixed the bead. A modification of this method by Hedenius is still widely used especially in the Scandinavian
It should be emphasized that the end point selected is arbitrary and does not mark the time of complete coagulation. Unconverted fibrinogen may still be demonstrated. A convenient way to measure incipient coagulation is to insert a glass rod coated with collodion into 1 cc. of blood and then withdrawn gently every 30 seconds. A fine thread of fibrin marks the beginning of coagulation. In normal blood, coagulation usually begins in 3 to 4 minutes and is complete in 10 minutes, whereas in hemophilic blood coagulation may begin (to cite a specific observation) in 10 minutes but require 2 hours more before enough fibrin is formed for a solid clot. The tube with the glass rod should not be used for determining the final coagulation time.

A NEW THEORY OF BLOOD COAGULATION AND ITS BEARING ON THE COAGULATION TIME

As the result of recent studies, evidence has been obtained to show that platelets do not furnish thromboplastin, but in their disintegration liberate an agent, probably an enzyme, that activates thromboplastin which occurs in the plasma as a precursor and for which the term, thromboplastinogen, has been proposed. It is probably identical with the antihemophilic globulin of Minot and Taylor and the prothrombokinin of Lenggenhager. According to this new concept, the first step in coagulation can be expressed as follows:

\[
(1) \text{Thromboplastinogen} \overset{\text{platelet enzyme}}{\longrightarrow} \text{thromboplastin}
\]

The activated thromboplastin reacts immediately:

\[
(2) \text{Thromboplastin} + \text{prothrombin} + \text{calcium} = \text{thrombin}
\]

\[
(3) \text{Fibrinogen} \overset{\text{thrombin}}{\longrightarrow} \text{fibrin}
\]

The first and third equations are enzymatic, whereas the second is stoichiometric. Thus, even a small number of platelets are sufficient to activate enough thromboplastin to furnish a quantity of thrombin that will coagulate blood within the normal period of time. Such a quantity of thrombin may, however, be entirely inadequate, as will be discussed later, to meet the hemostatic requirements. To understand the significance of the coagulation time, it should be remembered that normal human blood could clot in 12 seconds if it had an optimum amount of thromboplastin and that furthermore, the curve correlating the coagulation time and the concentration of thromboplastin is a hyperbola with both asymptotes zero. This explains why the shortening of a coagulation time from 1 hour to 5 minutes can be brought about by an extremely small quantity of thromboplastin. The amount of thrombin formed depends not on the coagulation time but on the quantity of prothrombin, thromboplastin, and calcium in the plasma and this can be looked upon as the key to a better understanding of several important hemorrhagic diseases which will be considered in this presentation.

The coagulation time may be prolonged in four well known diseases or conditions: hemophilia, hypoprothrombinemia, afibrinogenemia and heparinemia. It is possible that a delayed coagulation may occur in other conditions, but these have not been studied sufficiently to permit critical analysis. Hypercoagulability remains a vague and as yet meaningless term.
THE COAGULATION TIME IN HEMORRHAGIC DISEASES

Hemophilia. With the exception of complete incoagulability of the blood as encountered in afibrinogenemia, the most prolonged coagulation times are observed in hemophilia. A coagulation time of one hour is not unusual, but a time of two hours or more is rather infrequent, provided the test is done carefully and at 37° C. It has been found in recent studies that the coagulation time of a hemophilic may be surprisingly constant for a relatively long period of time. Thus, the coagulation time of one subject has remained about 55 minutes with few exceptions during the past 18 months. Although it has been brought to normal several times with plasma transfusion it has always promptly returned to this rather fixed value. The same constancy has also been found in other hemophilics, but in no instance has the period been long enough to be significant.

To understand the coagulation time in hemophilia, it is necessary to understand the basic defect in this disease. In a recent study it has been found that hemophilic blood is almost completely devoid of thromboplastinogen; and even after all the fibrinogen has coagulated, no demonstrable consumption of prothrombin has occurred. All the coagulation is due therefore to a minute quantity of thrombin which is formed and which, because it is an enzyme, can convert all of the fibrinogen to fibrin in a relatively short time. The minuteness of the quantity of thromboplastin which can bring about a normal coagulation time is clearly demonstrated by the following experiment:

A stock extract of thromboplastin prepared by mixing 0.2 gm. of dehydrated rabbit brain in 5 cc. saline, was diluted 1 to 1000. On adding 0.1 cc. of this diluted thromboplastin to 1 cc. of a hemophilic blood which had a coagulation time of 2 hours and 15 minutes, the time was reduced to 5 minutes. This 0.1 cc. of thromboplastin contained only 2.5 gammas of solid material, of which a large fraction was inert. Obviously, the amount of thrombin formed must have been extremely small, yet it coagulated the blood in 5 minutes. The conversion of prothrombin, however, was so small that it could not be demonstrated.

From the results observed in hemophilia and in hypoprothrombinemia it seems definite that hemostasis is not dependent on the clotting time but on the quantity of thrombin supplied during the clotting process. In hemophilia little thrombin is formed since the plasma lacks the thromboplastin precursor. Even if the plasma contains enough thromboplastinogen to cause a normal coagulation time, it may not be sufficient to supply enough thrombin for the hemostatic needs. This explains why a normal coagulation time may be found in known hemophiliacs suffering from repeated hemorrhages. In a limited number of such patients, the senior author could demonstrate no consumption of prothrombin after coagulation had been completed. Such patients are a problem to the surgeons since the normal coagulation may create a false sense of security. Furthermore, not every measure which reduces the coagulation time of a hemophilic is necessarily effective in controlling hemorrhage. Just as the effectiveness of vitamin K cannot be established

* A new procedure named the prothrombin consumption test has been developed. It consists in determining (by the senior author's method) the prothrombin remaining in the serum 1, 3, and 14 hours after the blood has clotted.
by the coagulation time but only by the decrease in the prothrombin time, so the assay of any antihemophilic agent cannot be made with an absolute degree of certainty by the coagulation time, but will probably require the measurement of the prothrombin consumption.

The coagulation time is obviously of limited value in hemophilia either in the diagnosis or in the treatment. A prolonged value is suggestive of hemophilia provided other causes are ruled out. A normal coagulation time does not exclude a diagnosis of hemophilia. A history of bleeding and a markedly poor consumption of prothrombin during coagulation appears to be much more reliable evidence on which to base a diagnosis.

The coagulation time, is, however, of some practical and theoretical value. A hemophilic with a coagulation time that is nearly normal usually has mild attacks of bleeding and only encounters serious trouble when relatively large vessels are damaged. The severity of the bleeding tendency appears to be relatively independent of the coagulation time when the value of the latter exceeds 15 to 20 minutes. In three hemophils having average coagulation times of 25, 55 and 120 minutes respectively, the frequency and severity of the bleeding episodes during a period of observation of 6 months, was roughly the same. Theoretically, the coagulation time is of value since it offers the only means to grade the severity of the hemophilic defect. Thus the difference in availability of thromboplastin between the three hemophils mentioned is so small that no other test, including the prothrombin consumption, can detect the difference.

The coagulation time has, it should be mentioned, served not only in establishing the presence in plasma of an antihemophilic agent, but has enabled Minot, Taylor and their associates to concentrate it. They wisely depended not so much on a transient lowering of the coagulation time but on a sustained normal value.

Hypoprothrombinemia. Prior to the advent of vitamin K, it was very puzzling to the surgeon why the jaundiced patient bled postoperatively in spite of a normal coagulation time. The senior author, on the basis of his early studies on vitamin K, concluded that the hemorrhagic danger level was indicated by a prothrombin time of about 25 seconds, which corresponds in man to a prothrombin activity of 20 per cent of normal. At this level the coagulation time is so little increased that unless the test is done with great care it escapes detection, since it is still well within the normal range. In fact, it has been found that in dogs an increase of the prothrombin time from the normal of 6 seconds to 60 seconds is accompanied by a change of the coagulation time only from 3½ to 3½ minutes; i.e., an average increase of only 2 minutes. Even with extremely low concentrations of prothrombin, the coagulation time is rarely as prolonged as in moderately severe hemophilia. At very low levels, the prothrombin time and the coagulation time tend to become identical. Thus, on reducing the prothrombin in a dog with dicumarol until the prothrombin time was 20 minutes, a coagulation time of 19 minutes and a clotting time for recalcified plasma of 30 minutes was obtained. The likely reason for such a result is that the limiting factor is prothrombin and that under such circumstances the thromboplastin of the plasma is adequate, and therefore additional amounts of the latter have no further effect.
Early in the work on toxic sweet clover poisoning, one of us\textsuperscript{17} discovered that a heart puncture in a rabbit with a reduced prothrombin caused fatal hematopericardium. This serves, therefore, as a useful means to study hemostatic effectiveness, and in Table 2 a correlation is made between the prothrombin time, coagulation time, and the hemostatic breakdown. A study of these results clearly shows that when the prothrombin time is less than 19 seconds the animals' blood could prevent cardiac bleeding. With a prothrombin time of 24 seconds or more, hematopericardium

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* Control: the other five rabbits were given 2 mg. per kg. of body weight of dicumarol daily by stomach tube.
† Blood was obtained from the median artery of the ear with a silicone coated syringe, which probably accounts for the long normal coagulation time.
‡ The heart punctures were made with a No. 21 needle and always approximately in the same position.
invariably occurred. While there was a slight increase in coagulation time, no clear cut relation between it and fatal hemorrhage could be found. Interestingly, one of us has observed a series of cases with congenital hypoprothrombinemia and has found that those with a prothrombin time of 16 seconds showed no hemorrhagic tendency, two cases with a prothrombin time of 19 seconds showed a distinct bleeding tendency, and one case with a value of 30 seconds showed a very severe hemorrhagic condition.

Obviously the coagulation time is of little or no value in the study of hypoprothrombinemia. It cannot be used for controlling dicumarol therapy. Again basically the fact is brought out that hemostasis depends on the amount of thrombin formed, and when the prothrombin is reduced to about 20 per cent insufficient thrombin is furnished for stanching.

Afibrinogenemia. When total incoagulability of the blood is found, afibrinogenemia should be suspected and a qualitative test for fibrinogen made. Recently, Pinniger and Prunty demonstrated experimentally that the prothrombin time remained approximately normal in the blood of their patient until the fibrinogen fell below 50 mg. per 100 cc. of plasma, and that the Lee-White coagulation was 5 minutes when the fibrinogen concentration was as low as 30 mg. It is obvious that the coagulation time has little practical value in this hemorrhagic condition except in the initial detection of a coagulation defect.

Heparinemia. Animals, particularly dogs subjected to peptone or anaphylactic shock, respond by an outpouring of histamine and heparin into the blood, and by a marked thrombocytopenia. The resulting heparinemia may be so great that the blood is rendered incoagulable. In man, the appearance of heparin in the blood has not been unequivocally demonstrated although there is a good probability that it can occur. The increase of the coagulation time is not necessarily proportional to the concentration of heparin. The latter can be much more accurately determined by titration with progressive dilutions of a standard thrombin solution. The therapeutic use of heparin in the prophylaxis of thrombosis is successfully controlled by the coagulation time, but this is entirely on an empirical basis, since it has not been accurately determined how much heparin is needed for this purpose. It is probable that the effective action of heparin consists in neutralizing thrombin, and thus reduces the effective quantity of the latter.

From the foregoing discussion, it becomes clear that the coagulation time has limited value in the study of the known hemorrhagic diseases. It has, however, an important function in the possible discovery of new hemorrhagic diseases. On finding a prolonged coagulation, a concise diagnosis can be made only by specific tests such as the prothrombin time, and the prothrombin consumption test. A little over a decade or two ago, hemophilia was the waste basket for nearly all hemorrhagic diseases characterized by a coagulation defect. Since then hypoprothrombinemia, afibrinogenemia, and heparinemia have been recognized as separate entities. It is highly probable that other hemorrhagic conditions having a prolonged coagulation time exist but thus far have not been recognized and defined because of a lack of suitable methods of study.
COAGULATION TIME AND HEMORRHAGIC DISEASES

SUMMARY

The coagulation time is a measurement of the intrinsic power of the blood to convert fibrinogen to fibrin. It is an empirical test no matter how performed, and therefore in order to be reliable requires that the test be done on venous blood under strictly controlled conditions. A recommended procedure is outlined in detail.

The coagulation time is prolonged in hemophilia, hypoprothrombinemia, afibrinogenemia and heparinemia. In hemophilia, the coagulation time theoretically is a measure of the severity of the disease but practically is of limited value since the coagulation time may be within normal limits in some patients; the prothrombin consumed in the coagulation of hemophilic blood is therefore a better guide for diagnosis. The coagulation time in hypoprothrombinemia is relatively little prolonged until a drastic reduction occurs. The test is therefore of no value for establishing a hemorrhagic condition in hypoprothrombinemia. In afibrinogenemia the blood is incoagulable. A small amount of fibrinogen restores the coagulation time to normal.

The presence of heparin increases the coagulation time. The test is therefore useful in controlling the therapeutic action of this drug.

The senior author, in making a survey of the literature on hemorrhagic diseases in preparation of his monograph, was impressed by the significant and diverse contributions which Dr. George R. Minot made to this field of medicine. We feel honored to contribute this study to the collection of papers offered as a fitting tribute to Dr. Minot, who has so successfully and productively combined science and clinical medicine.

REFERENCES

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