THE MEASUREMENT OF PROTHROMBIN IN PLASMA
A CASE OF PROTHROMBIN DEFICIENCY

BY

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Quick's (1935) one-stage prothrombin test is invaluable as a routine method, and it has been carried out on the plasma of patients with many types of clotting defect. When the one-stage clotting time is lengthened the patient is often said to have "hypoprothrombinaemia." This is misleading, because the test cannot be relied upon to measure prothrombin. Patients with a marked reduction in prothrombin may show little change of clotting time by Quick's method, and patients with no significant reduction in prothrombin may have a grossly lengthened one-stage clotting time. Quick's test is influenced by accelerators of blood coagulation, by the concentration of fibrinogen, and by the presence of inhibitors, such as heparin, in the blood. At present the one-stage test cannot be used as a measure of any specific substance.

The important accelerators which influence the one-stage test are factors V (Owren, 1947) .and VII (Koller, Loeliger, and Duckert, 1951). Factor V is a protein in the globulin fraction of plasma which is not adsorbed by inorganic precipitates such as $Ca_3(PO_4)_2$, $BaSO_4$, and $Al(OH)_3$. This substance has been given various names, accelerator globulin (Ware and Seegers, 1948), prothrombin accelerator (Fantl and Nance, 1948), and the labile factor (Quick, 1943). Factor V may be deficient from birth or childhood (Owren, 1947; Frank, Bilhan, and Ekren, 1950; Brink and Kingsley, 1952), or may become deficient as a result of acute infection (Koller, Gasser, Krüsi, and de Muralt, 1950), or liver disease (Owren, 1949). Factor VII (Koller *et al.*, 1951) is a protein in the globulin fraction of serum which is adsorbed by $BaSO_4$ and $Al(OH)_3$. The activity of factor VII may be identical with the phenomena attributed to prothrombin conversion factor (Owen and Bollman, 1948), serum prothrombin conversion accelerator (de Vries, Alexander, and Goldstein 1949), proconvertin and convertin (Owren, 1951a, 1951b), and co-thromboplastin (Mann and Hurn, 1951). The term factor VII is preferred because Koller *et al.* made the most illuminating

study of its properties. Factor VII is important because the plasma of patients under treatment with any of the dicoumarin group of drugs lacks this factor.

A rough, quantitative distinction between the effects of factors V, VII, and prothrombin can be achieved by the use of plasma adsorbed with $Al(OH)_3$, which contains factor V but not prothrombin or factor VII, and normal serum, which contains little factor V and prothrombin but much factor VII. The one-stage clotting time of a plasma deficient in factor V will be shortened by the addition of $Al(OH)_3$-treated plasma. The one-stage clotting time of a plasma deficient in factor VII will be shortened by the addition of serum but not by the addition of $Al(OH)_3$-treated plasma. The one-stage clotting time of a plasma deficient in prothrombin will not be shortened by the addition of $Al(OH)_3$-treated plasma or serum.

Although a number of patients with so-called idiopathic hypoprothrombinaemia have been described, in none can the defect be attributed with certainty to a lack of prothrombin alone. In five instances the defect was probably a reduction of factor V (Owren, 1947; Frank *et al.*, 1950; de Vries, Matoth, and Shamir, 1951; Stohlman, Harrington, and Moloney, 1951; Brink and Kingsley, 1952). In four instances factor VII may have been deficient (Giordano, 1943; Crockett, Shotton, Craddock, and Leavell, 1949; Landwehr, Lang, and Alexander, 1950; Alexander, Goldstein, Landwehr, and Cook, 1951). In nine instances the defect was not clearly defined (Rhoads and Fitz-Hugh, 1941; Plum, 1943; Murphy and Clark, 1944; de Marval and Bomchil, 1944; Hauser, 1945; Quick, 1947; Hagen and Watson, 1948; Covey, Cohen, and Papps, 1950; Ferguson, 1950).

The patient to be described had uncomplicated prothrombin deficiency which is, apparently, a very rare condition. The plasma of this patient was useful for the study of coagulation factors. From the two-stage method carried out on the patient's plasma a method for the measurement of

prothrombin was devised. This method will be described in this paper. The patient's plasma was also useful for the study of plasma thromboplastin, and this work is described elsewhere (Biggs, 1952 ; Biggs, Douglas, and Macfarlane, 1953).

Methods

Collection of Plasma.—Venous blood is collected and mixed with 3.8% sodium citrate in the proportion of 1 part of citrate to 9 parts of blood. The plasma is separated after centrifuging for five minutes at 2,000 r.p.m.

The one-stage method is carried out essentially as described by Quick (1942). A full account of the method, including the preparation of brain emulsion and M-40 calcium chloride, is given by Biggs (1951).

Two-stage Method.—Fibrinogen is prepared by the phosphate-buffer method of Jaques (1943) described by Biggs (1951).

Test.—For the test 0.4 ml. of undiluted citrated plasma is mixed with 0.4 ml. of brain emulsion warmed to 37° C. in a water-bath and 0.4 ml. of M-40 CaCl₂ added. As the CaCl₂ is added a stop-watch is started, and at 15, 30, and 45 seconds, 1 minute, and thereafter at minute intervals 0.1-ml. samples are removed from this incubation mixture which is forming thrombin and added to 0.4-ml. amounts of fibrinogen warmed to 37° C. in small tubes. The clotting times of the fibrinogen samples are recorded and the test continued until these exceed three minutes. When the two-stage method is carried out on undiluted plasma thrombin formation and its neutralization by antithrombin are both very rapid. Usually in normal plasma no significant amount of thrombin can be detected after four minutes' incubation. A technical difficulty results from clotting in the incubation mixture. When this occurs the clot must be removed. It has been found that the removal of the clot can be achieved quite easily by winding it on to a wooden swab stick, the end of which has been split and the halves slightly separated. Inexperienced workers may find that this test is best carried out by two operators. One worker removes the 0.1-ml. samples from the incubation mixture and the other records the clotting times of the fibrinogen samples.

Thrombin-Fibrinogen Dilution Curve.—The preparation of this curve is described by Biggs (1951). The clotting times are read from the thrombin-fibrinogen curve in terms of thrombin units. A curve of thrombin generation and disappearance can then be drawn and the area enclosed by this curve is computed either with a planimeter or by the more laborious method of counting the squares on the graph paper. The procedure is carried out on normal and abnormal plasma samples and the area obtained from the abnormal is expressed as a percentage of the normal. As an example the figures obtained as an average of 15 normal plasma samples are given in Table I. These can be converted to

TABLE I
RESULTS OF A TWO-STAGE PROTHROMBIN TEST ON NORMAL PLASMA

	Incubation Time in Minutes						
	¼	½	1	2	3	4	5
Clotting time (seconds) ..	20	17	18	31	77	170	180÷
Thrombin units	6·2	7	6·8	3·5	1·2	0·2	0

thrombin units from the thrombin-fibrinogen dilution curve (Table I). From these figures the curve of thrombin generation and its disappearance can then be drawn (Fig. 1).
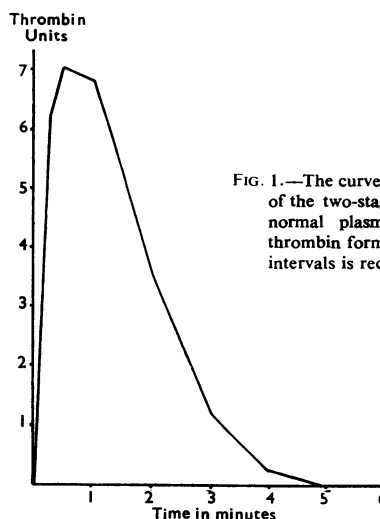

FIG. 1.—The curve represents the results of the two-stage test carried out on normal plasma. The amount of thrombin formed after various time intervals is recorded.

Antithrombin.—It is an essential prerequisite of this test that the antithrombin content of the normal and test plasmas should be similar. The antithrombin content of plasma can be tested by the method of Astrup and Darling (1942). To ensure that the antithrombin is sufficiently normal for the two-stage test a simpler technique may be employed. Sufficient thrombin to clot 1 ml. of plasma in three to four seconds is added to 1 ml. of plasma. At half-minute intervals thereafter 0.1 ml. of the thrombin-plasma mixture is added to 0.4 ml. of fibrinogen and the clotting times are recorded. The lengthening of the clotting time with the passage of time should be similar in the normal and patient's plasma. " Thrombin topical " prepared by Roche may be used for this test.

Test for Factor V Deficiency.—The preparation of Al(OH)₃α is by the method of Bertho and Grassmann (1938). Al(OH)₃-adsorbed plasma was prepared and described by Biggs (1951).

Test.—To 0.9 ml. of the plasma to be tested is added 0.1 ml. of Al(OH)₃-treated normal plasma. The mixture is tested by the one-stage prothrombin test. Al(OH)₃-treated plasma is rich in factor V but poor in prothrombin and factor VII. If the clotting

time of the mixture is appreciably shorter than that of the patient's plasma factor V is deficient.

Test for Factor VII Deficiency.—Normal serum is prepared by collecting 3 ml. of normal blood into a glass tube containing three glass beads. The tube is shaken during clotting to ensure that most of the prothrombin is converted to thrombin. After one hour or more at 37° C. the serum can be separated by centrifuging.

Test.—To 0.9 ml. of the plasma to be tested is added 0.1 ml. of serum. The mixture is tested by the one-stage method. Serum is rich in factor VII, but contains little factor V or prothrombin. A marked reduction in clotting time indicates factor VII deficiency.

Case Report

G. W. was a man aged 26.

The patient had been well until 15 months before investigation. He then developed haematuria, investigation of the renal tract revealing no cause. Nine months later he developed large spontaneous bruises on the right calf, on both arms, and in the neck. There was a history of epistaxis and bleeding from the gums, and also of transient pains in the knees. As a child the patient underwent tonsillectomy, and two years before the onset of his present illness a tooth was removed with no excessive bleeding. There was no history of abnormal bleeding in his relatives. At the time of onset of the bleeding the patient was working in a brewery. Soon afterwards he went to a rubber factory, where he was employed in cutting out from sheets of rubber.

The abnormality in this patient was acquired in adult life and could not easily be attributed to his occupation ; nor did he admit to taking any form of medicine.

Clinical examination was negative.

Laboratory Investigations. — Haemoglobin was 13.7 g.%, the white-cell count 5,100 per c.mm. The blood film appeared normal.

Plasma proteins were 6.6 g./100 ml. (albumin, 3.8 g. ; globulin, 2.4 g. ; fibrinogen, 360 mg.). The thymol turbidity test was negative, as also was the colloidal gold test. The sucrose tolerance curve was normal. The bilirubin level was 0.3 mg.%.

Fat excretion was normal, with an intake of fat of 280 g. per day and an excretion of fat of 7.2 g. (2.6%). The bleeding time (Ivy's method) was 5 minutes (normal 2½–7 minutes). A tourniquet test was negative. A platelet count gave 645,000–1,121,000/c.mm.

Investigation of Coagulation Defect.—The whole-blood clotting time, determined by the modified method of Lee and White (1913), was 13½–20 minutes (normal 5–10 minutes) ; the one-stage prothrombin time was 18–22 seconds (normal 14 seconds). The antithrombin measured by Astrup and Darling's (1942) method was 179 units (normal plasma 193 units). The reaction of plasma to thrombin was normal.

Test for Factor V.—The one-stage clotting time of the patient's plasma was not shortened by the addition of normal plasma treated with Al(OH)₃. Since the Al(OH)₃-treated plasma contains an excess of factor V the patient's plasma did not appear to be deficient in factor V.

Tests for Factor VII.—The one-stage clotting time of the patient's plasma was not shortened by the addition of normal serum, which contains an excess of factor VII. Additions of the patient's plasma shortened the clotting time of plasma from a patient under treatment with " tromexan " (which lacks factor VII) as well as did similar additions of normal plasma.

It appears that the patient's plasma lacked neither of the known accelerators of blood coagulation.

Two-stage Prothrombin Test.—The curves illustrating thrombin formation in normal and in the patient's plasma, using the two-stage method, are shown in Fig. 2. The interpretation of this test is considered in detail in a later part of this paper.
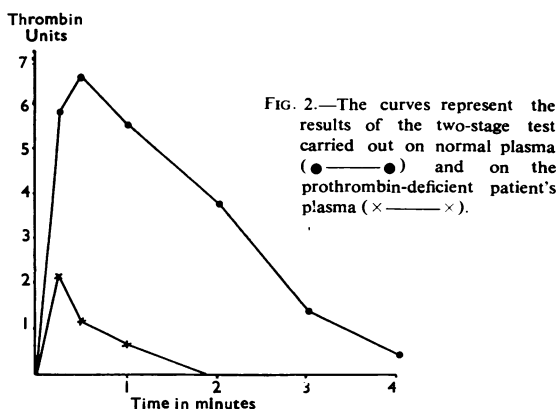

FIG. 2.—The curves represent the results of the two-stage test carried out on normal plasma (●———●) and on the prothrombin-deficient patient's plasma (×———×).

From these curves it was calculated that the patient's plasma contained about 11% of the normal amount of prothrombin.

The Effect of Vitamin K.—Vitamin K was given to the patient in three different ways with an interval of three days between each trial: 100 mg. of a water-soluble analogue, " synkavit," was given intravenously ; 1,000 mg. of vitamin K₁ was given orally ; and 500 mg. of vitamin K₁ was given intravenously by the method of Davidson and MacDonald (1943). In none of these trials did the vitamin K cause a rise in prothrombin tested by the two-stage method.

Effect of Intravenous Administration of Normal Plasma.—Fresh citrated plasma, 860 ml., was given to the patient. The plasma was given within two hours of its collection from three donors. On a second occasion 500 ml. of stored citrated plasma was given to the patient. On each occasion the rise in prothrombin as tested by the two-stage method was slight and the effect transient (Fig. 3). The ineffectiveness of transfusion suggests either that the normal rate of prothrombin utilization was very high or that it was unusually fast in this patient.
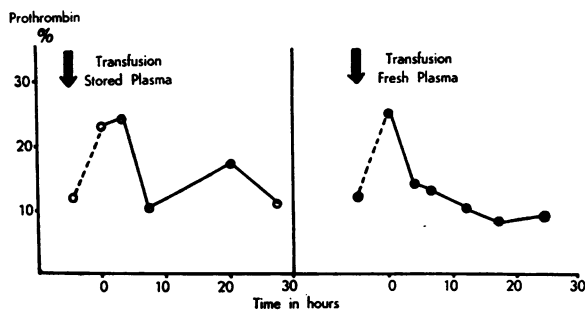
B

FIG. 3.—The curves show the level of prothrombin (measured by the two-stage test) in the patient's plasma before and after plasma transfusions.

This patient is apparently the first to be described in whom a coagulation defect can be attributed to an uncomplicated deficiency of prothrombin. The unusual features of the case are the relatively slight alteration in the one-stage " prothrombin " time and the lengthened whole-blood clotting time. The slight lengthening of the one-stage clotting time suggests that the one-stage test may be relatively more sensitive to changes in the accelerators of blood coagulation than it is to prothrombin. The lengthened whole-blood clotting time may be related to a very low level of thrombin generated by the plasma thromboplastin system when prothrombin is grossly deficient.

The Measurement of Prothrombin

The two-stage prothrombin test is usually considered to give the most reliable measure of prothrombin, but it is to be doubted whether this test, as usually interpreted, often gives a true measure of prothrombin in plasma. In experiments in which prothrombin is separated from antithrombin the test can be used to give a measure of prothrombin (Biggs, 1951 ; Biggs and Macfarlane, 1953). In these artificial conditions prothrombin is quantitatively converted to thrombin, and the amount of thrombin formed gives a measure of prothrombin. In plasma thrombin is neutralized by antithrombin as it is formed, and the amount of thrombin detected in a plasma mixture forming thrombin is not the total amount of thrombin formed, but the amount from which the amount neutralized has been substracted.

The two-stage test is therefore usually carried out on high dilutions of plasma samples to avoid the effects of antithrombin. But it can be shown (Biggs, 1951 ; Biggs and Macfarlane, 1953) that dilution does not remove the effects of antithrombin. Moreover, plasma samples in which prothrombin is reduced cannot be greatly diluted because the amount of thrombin to be detected is too low in these diluted samples. If the effects

of antithrombin cannot be eliminated by dilution, it is obviously necessary either to remove antithrombin by some method other than dilution or to devise a test in which the effect of antithrombin is allowed for.

In Fig. 4 are shown the results of carrying out the two-stage test as described in this communication on an undiluted normal plasma sample, on the plasma of the patient with prothrombin deficiency, and on the plasma of a patient treated with " tromexan." In the prothrombin-deficient patient's plasma thrombin formation is complete in two minutes and the level of 2.2 units is maintained only for a few seconds, whereas in the " tromexan " plasma thrombin can be detected up to eight minutes, and a level of between 1 and
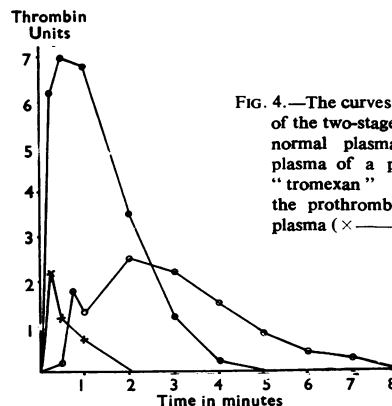

FIG. 4.—The curves represent the results of the two-stage test carried out on normal plasma (●———●), the plasma of a patient treated with " tromexan " (o———o), and the prothrombin-deficient patient's plasma (×———×).

2.5 units of thrombin is maintained for five minutes. The prothrombin-deficient patient's plasma has a rapid prothrombin conversion. In the " tromexan " plasma thrombin formation is delayed. These two curves suggest that much more thrombin is formed in the " tromexan "-treated patient's plasma than in that of the prothrombin-deficient patient. It would seem that, in assessing the amount of prothrombin present, the duration of thrombin formation must be as important as the level reached. The duration of thrombin formation and the level of thrombin attained can both be taken into account by computing the areas enclosed by the curves as a measure of prothrombin. When this is done the patient's plasma would be said to contain 11% of prothrombin and the " tromexan " plasma 68%. This assessment would appear to be more reasonable than the comparison of the levels of thrombin formed. Common sense suggests that this is a reasonable method of measuring prothrombin, but is it really more rational than the previous methods?

The use of the two-stage test on undiluted plasma involves an understanding of the effects of accelerating substances and antithrombin on the levels of thrombin detected when carrying out the test. To achieve this understanding it is convenient to consider a simplified theoretical model of the blood coagulation system. Suppose that a substance A is converted to another substance B by a first order reaction, and substance B is simultaneously converted to C by a first order reaction.

Then the progress of the reaction may be represented in Fig. 5a. The concentration of A falls as A is converted to B. The concentration of B rises until the conversion of B to C balances the conversion of A to B. Then when most of A is converted to B the concentration of B falls and that of C rises. In blood coagulation A would represent prothrombin, B thrombin, and C a neutralized thrombin-antithrombin association. In the two-stage method it is B which is measured. The speed of formation of B will depend on the presence of activating factors and the speed of disappearance of B on the concentration of neutralizing factors.

When the amount of A is varied, but the speeds of formation and neutralization of B are unchanged, the curves shown in Fig. 5b are obtained. In these curves the peak levels of B and the areas enclosed by the curves are both proportional to the amount of A initially present.

When the amount of A is constant and the speed of neutralization of B is unchanged, variation in the speed of formation of B gives curves such as those in Fig. 5c. The peak levels of thrombin reached are now no indication of the amount of A initially present, but the areas enclosed by the curves are proportional to the amount of A present at the beginning. From Fig. 5c it is clear that the constancy of the areas is due to the slower disappearance of B which leads to a crossing over of the curves during the decline of B. In the blood coagulation system this crossing over of the curves is often easier to detect than a change in the speed of appearance of thrombin.

When the amount of A is constant and the speed of formation of B is unchanged, but the speed of neutralization of B is varied, then the curves of
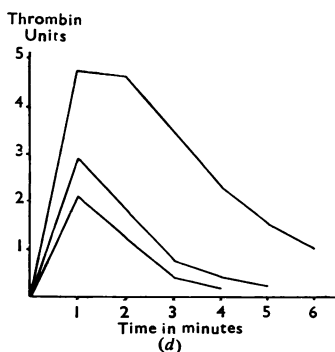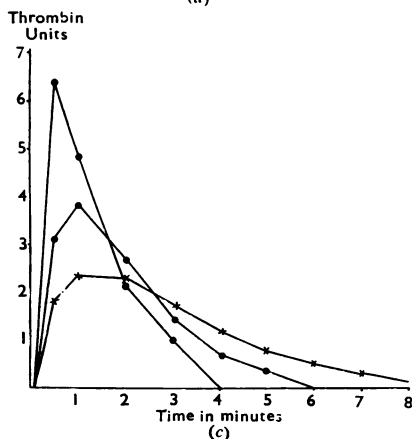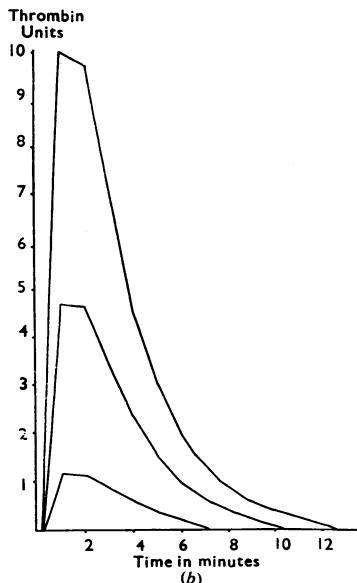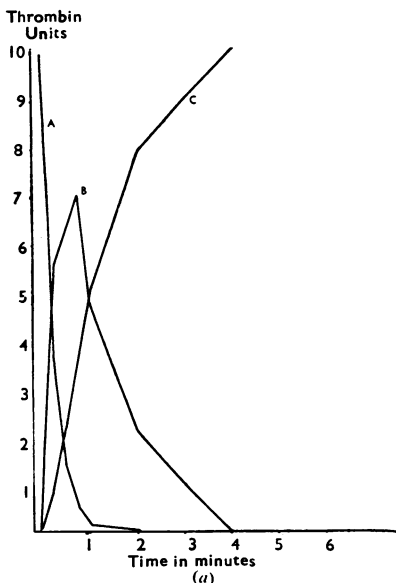

FIG. 5.—Theoretical curves to show the progress of two consecutive first order reactions: A———▶B———▶C. (a) These curves show the disappearance of A and the appearance of B and C in such a reaction. In the blood coagulation system A would represent prothrombin, B thrombin, and C neutralized thrombin. In the two-stage test B is measured. (b) These curves represent the progress of formation of B when three different initial concentrations of A are present. The speed of formation of B and its conversion to C are constant. (c) These curves represent the progress of formation of B when the initial amount of A is constant and the speed of neutralization of B is unchanged but the speed of formation of B is varied. (d) These curves represent the progress of formation of B when the initial amount of A and the speed of formation of B are constant but the speed of neutralization of B is varied.

Fig. 5d are obtained. In these conditions neither the peak levels of B nor the areas enclosed by the curves are proportional to the amount of A initially present.

If this theoretical model can be followed it is clear that the measurement of the area enclosed by the two-stage curve will give the most generally reliable proportional measure of prothrombin, but that prothrombin cannot be measured unless the level of antithrombin is normal.

It is obviously important to know how closely the blood coagulation reactions approach the theoretical model. When the average of 15 curves
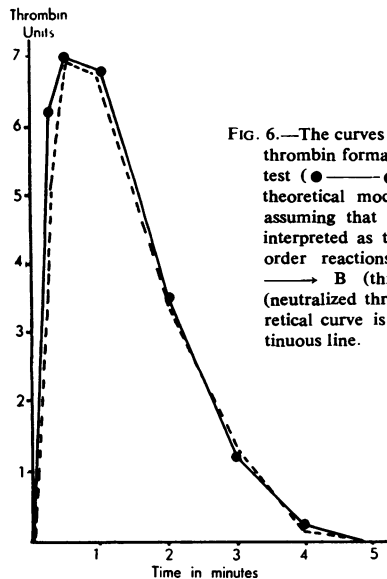
FIG. 6.—The curves represent progress of thrombin formation in the two-stage test (●———●) compared with a theoretical model of the reaction, assuming that the reaction can be interpreted as two consecutive first order reactions A (prothrombin) ———→ B (thrombin) ———→ C (neutralized thrombin). The theoretical curve is shown as a discontinuous line.

for normal plasma is compared with a theoretical model it is found that there is a surprisingly close agreement (Fig. 6).

The amount of prothrombin may be varied by adding prothrombin to the plasma of the prothrombin-deficient patient. When this was done it was found that the experimental curves agreed reasonably well with those of the theoretical model (Fig. 7).

In the plasma of patients treated with " tromexan " thrombin formation is delayed, and the delay can be reduced by the addition of normal serum. The effect of accelerators on the two-stage test can therefore be studied by comparing thrombin formation in " tromexan " plasma with that in " tromexan " plasma to which serum has been added and with that in normal plasma. From Fig. 8 it is clear that the experimental curves are of the same general pattern as those of the theoretical model.
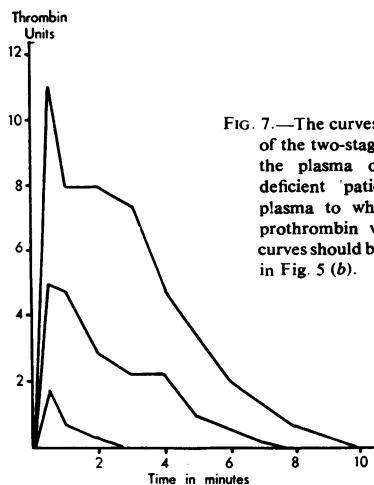
FIG. 7.—The curves represent the results of the two-stage test carried out on the plasma of the prothrombin-deficient patient and the same plasma to which two amounts of prothrombin were added. These curves should be compared with those in Fig. 5 (b).

The speed of neutralization of thrombin can be varied by adding various amounts of heparin to normal plasma. When the two-stage test is carried out on samples containing various amounts of heparin the curves resemble those of the theoretical model (Fig. 9).

The close agreement between the experimental results and the theoretical model does not imply that the reactions of blood coagulation can be explained in terms of this simple model. Other explanations may also be adequate. Slight alterations in the conditions of carrying out the tests may affect the results. From this close agreement all that can be deduced is that, provided the conditions for making the test are not altered, the area method of measuring prothrombin is likely to give more generally reliable results than other methods. The calculations for deriving the theoretical curves are described by Daniels (1948).
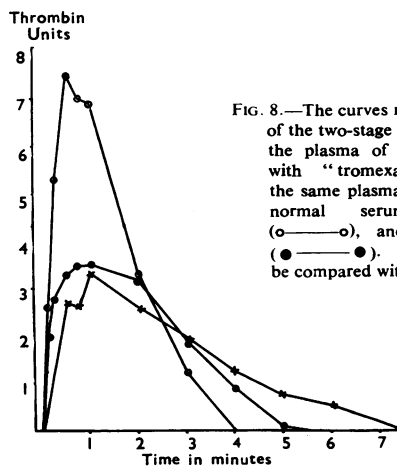
FIG. 8.—The curves represent the results of the two-stage test carried out on the plasma of a patient treated with " tromexan " (×———×), the same plasma to which 10% of normal serum was added (o———o), and normal plasma (●———●). These curves should be compared with those in Fig. 5 (c).
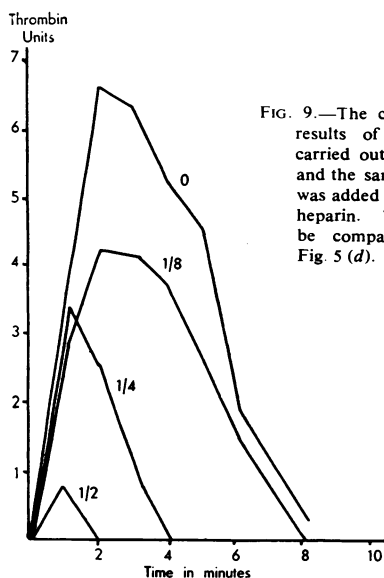
FIG. 9.—The curves represent the results of the two-stage test carried out on normal plasma and the same plasma to which was added ⅛, ¼, and ½ a unit of heparin. These curves should be compared with those in Fig. 5 (d).

There are two other methods by which the reliability of this method of measuring prothrombin can be assessed. The amount of prothrombin in a normal and prothrombin-deficient plasma sample can be measured. The two samples can then be mixed in known proportions and the amount of prothrombin in the mixtures can be measured and the measured results compared with those calculated from the original samples. When this is done there is a reasonable correlation between the observed and expected figures (Fig. 10).

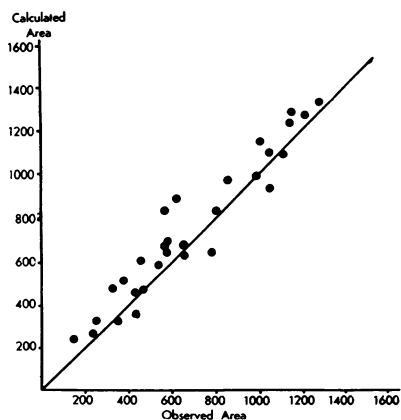The prothrombin content of a plasma sample can also be measured in the globulin fraction con-

FIG. 10.—This diagram represents the results of carrying out the two-stage test on normal and prothrombin-deficient samples and on mixtures of the two in known proportions. In the mixtures the calculated and observed values for the areas enclosed by the curves are compared.

taining prothrombin separated from antithrombin (Douglas, 1953). When this is done a constant level of thrombin is reached in the two-stage test. This constant level of thrombin is proportional to the amount of prothrombin present. The globulin fraction of 10 samples of plasma from patients treated with " tromexan " were prepared. The amounts of prothrombin found by two-stage tests on the original plasma samples agreed very well with the results of the two-stage tests on the globulin fractions.

From these results it appears that a simple two-stage procedure on unmodified plasma can be used to give a reasonably reliable measure of prothrombin.

Discussion

Very numerous methods for measuring prothrombin are now in use, but none of these can be relied upon to measure prothrombin in all plasma samples. Two-stage tests on diluted plasma to which various accelerator substances have been added (Ware and Seegers, 1949) are very complicated and depend on the assumption that an excess of activators is present. It is probable that in some plasma samples a very large excess of activators may be necessary and this large excess cannot be achieved easily.

One-stage tests in which a large excess of activators is added have also been devised (Owren and Aas, 1951 ; Koller *et al.,* 1951). These methods do not always give the same results as the two-stage test described in this communication (Douglas, 1953). The reason for the discrepancy is as yet unknown. The modified one-stage test is based on the assumption that its results must measure prothrombin if an excess of activators is present. If the excess of activator required is very large this assumption may not be justified. Moreover, the modified one-stage tests require reagents such as Seitz-filtered ox plasma and normal serum, which may vary in constituents from one sample to another, and the test is difficult to standardize.

The two-stage test described above requires no special reagents, and possible variations in the potency of brain thromboplastin do not affect the results. In addition there are methods of testing the validity of the two-stage test. The results of this test must at present be considered to give a more probable measure of prothrombin than modifications of the one-stage method. The only recognized limiting factor in the application of this test is variability in the antithrombin content of the control and test samples. For this reason the method cannot be used to compare the pro-

thrombin content of plasma and serum because plasma contains more antithrombin than serum.

The two-stage test is useful in distinguishing between the coagulation defects caused by reduction in factor V, factor VII, and prothrombin. The test is also useful for determining the extent of true prothrombin deficiency. It is perhaps too obvious to emphasize that the test will not be useful for measuring the extent of a coagulation defect that is not primarily one of prothrombin. In " tromexan " therapy, for example, the main abnormality is a reduction in factor VII which is reliably measured by the one-stage test. The prothrombin test would not be a good method for the control of anticoagulant therapy with dicoumarol or " tromexan." In this form of therapy the one-stage test should be used.

Finally, it must be emphasized that an isolated deficiency in prothrombin is an extremely rare condition. This fact may be difficult to appreciate because the term " hypoprothrombinaemia " has been used to describe many different coagulation defects and because the results of the one-stage test are so often erroneously reported in terms of " percentage of prothrombin." The one-stage test alone is a useful empirical test. When the coagulation defect has been defined in terms of factor V, factor VII, or prothrombin deficiency it may be possible to use the one-stage method as a quantitative measure of some specific substance. For example, in " tromexan " therapy the one-stage test probably gives a measure of factor VII deficiency. In the patient with 10% of prothrombin recorded in this communication the one-stage prothrombin time was little lengthened. In the plasma of other cases we have never found less than 25% of prothrombin by the two-stage test. Thus it is probable that the classical " prothrombin " test seldom gives a measure of prothrombin deficiency because prothrombin is not usually sufficiently reduced to influence the results of the test.

Summary

A case of prothrombin deficiency is described. In this patient prothrombin was reduced in amount, but factors V and VII were normal. It appears that isolated prothrombin deficiency is a very rare condition.

From a study of this patient a method for the measurement of prothrombin was devised. This test is simple, requires no special reagents, and, as far as can be determined, it gives a reliable measure of prothrombin.

This two-stage method is useful in distinguishing between deficiencies of prothrombin and

factors V and VII, reduction in any of which may lengthen the clotting time by the one-stage test.

The test is naturally not useful for following the course of " tromexan " or dicoumarol therapy because in this form of anticoagulant treatment the main abnormality is a reduction of factor VII and not of prothrombin.

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