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THE ONE-STAGE PROTHROMBIN CONSUMPTION TEST

Clinical Value in the Identification of Thromboplastin-Deficiency Diseases

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THE FORMATION of a solid clot does not represent the end point of the intricate reactions which are preliminary to the coagulation of blood or plasma. After clotting has occurred, the various factors continue to interact, and slowly or rapidly dissipate themselves and one another. A study of these residual reactants in the serum is sometimes of great clinical value. Quick et al.[1-3] have demonstrated and emphasized the importance of the determination of *serum prothrombin activity* in the diagnosis of hemophilia, acquired hemophilia-like disease and severe thrombocytopenia. In all of these conditions there apparently is an impaired activation of thromboplastin (fig. 1). Without activation of thromboplastin, thrombin cannot be formed. With minimal activation of thromboplastin, enough thrombin may eventually form to clot fibrinogen, but a large residual of prothrombin remains in the serum. The *consumption of prothrombin* during such coagulation is slight.

Prothrombin activity of serum can be measured at various intervals after coagulation with both one-stage[3] and two-stage procedures.[4, 5] The one-stage technic (prothrombin consumption test of Quick) has the important advantage of being easily adaptable to clinical use. It is, however, a qualitative test and its limitations must be understood. Fundamentally, the test consists of adding thromboplastin, labile factor and fibrinogen to serum, and measuring the clotting time of the mixture. It has been assumed that the clotting time obtained with this procedure is a direct function of the prothrombin which has been left unconverted in the serum. As a matter of fact, at least three other factors may influence the prothrombin time of serum: (1) the thrombin formed during the process of coagulation and not yet neutralized by the natural antithrombin; (2) the "accelerator effect" of serum, due to one or more factors capable of accelerating the conversion of prothrombin to thrombin; a well defined accelerator is formed from the labile factor (plasma Ac globulin, factor V) through the action of thrombin (fig. 2) during coagulation[6, 7]; (3) the concentration of labile factor not utilized during coagulation.

An analysis of the individual importance of these different factors present in serum has been presented elsewhere.[9] It was shown that thrombin and labile factor can be controlled, thrombin by deactivation, labile factor by adding an excess. When the prothrombin consumption test is performed within two hours from the completion of clotting, the serum prothrombin and serum accelerator are the two determinants of the serum prothrombin time. Of these, the effect of prothrombin

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is by far the greater. The one-stage prothrombin consumption test does not eliminate the accelerator effect and, for this reason, the test has been criticized as non-

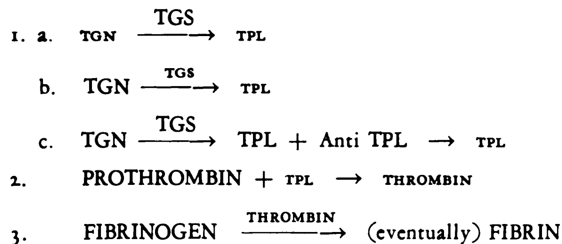

FIG. 1.—*Clotting reactions in thromboplastin-deficient conditions* (TGN: Thromboplastinogen, a plasma factor, precursor of thromboplastin. TGS: Thromboplastinogenase, a factor of enzymatic nature, the activator of thromboplastinogen. TPL: Thromboplastin. Small letters represent deficiency of the factor represented.)

Reaction 1a: *Hemophilia.* The plasma factor, TGN, is lacking. The platelet factor, TGS, is adequate. Result: TPL deficiency.

Reaction 1b: *Thrombocytopenic purpura.* Plasma factor, TGN, is adequate. The platelet factor, TGS, is lacking. Result: TPL deficiency.

Reaction 1c: *Acquired hemophilia-like disease* (antithromboplastinemia). Both plasma and platelet factors are adequate, but a thromboplastin inhibitor (antithromboplastin) is present. Result: TPL deficiency.

Reaction 2: With inadequate thromboplastin the consumption of prothrombin is slight and little thrombin results.

Reaction 3: With slow and inadequate evolution of thrombin, the clotting of fibrinogen is delayed, but it eventually occurs and goes to completion.

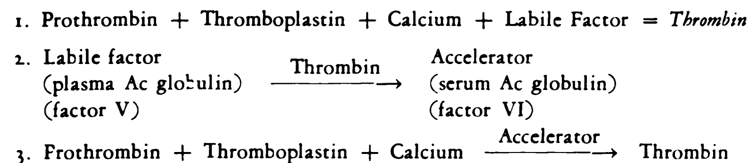

FIG. 2.—*St.p in the development of the accelerator effect of serum.*

The phenomenon of acceleration in coagulation has been studied in several laboratories. On the basis of changes in the clotting time of fibrinogen (accelerator effect) the presence of accelerator substances in serum has been deduced. From the conflicting evidence in the literature and from our experience, it appears that thrombin is first formed from the inter-reaction of prothrombin, thromboplastin, calcium and labile factor (plasma Ac globulin, factor V). Thrombin will then activate labile factor to an agent capable of accelerating the formation of thrombin from prothrombin in presence of thromboplastin and calcium. In this cyclical fashion, thrombin breeding accelerator and accelerator breeding thrombin, the reaction gains momentum.

This represents only one of the mechanisms of the "chain-reaction" which is known to take place during the coagulation of blood. Another is represented by the labilizing effect of thrombin on the platelets, which results in increased liberation of thromboplastinogenase and, therefore, in larger activation of thromboplastin.[1, 2]

specific.[10] The point is well taken, though of academic importance, as will be seen presently.

The serum accelerator effect does not mask defective prothrombin consumption.

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TABLE 1.—*The Relationship of the Prothrombin Activity of Plasma, Prothrombin Activity of Serum and Accelerator Effect of Serum in Healthy Subjects and Patients with Different Conditions*

	Prothrombin activity of plasma	Prothrombin activity of serum	Prothrombin activity of serum, corrected for accelerator effect	Accelerator effect of serum	Platelets	Hematocrit	Serum/plasma prothrombin activity ratio × 100
	%	%	%	%	cc. × 1,000		
1. Normal	100	4	2.3	75	450	43	2.3
2. "	100	2.5	1.3	98	372	41	1.3
3. "	95	1.2	0.5	120	385	46	0.52
4. "	100	16	8.2	95	503	39	8.2
5. "	100	2.1	1	107	475	41	1
6. "	90	3	1.5	98	398	44	1.6
7. "	100	1.2	0.6	102	480	46	0.6
8. "	90	1	0.5	89	524	38	0.55
9. "	100	6.3	3.6	73	286	43	3.6
10. "	100	12	6.4	87	358	42	6.4
11. "	100	15	9.9	48	616	40	9.9
12. Thrombocytopenic purpura: (a) before splenectomy	95	90	87	4	12	35	91.5
(b) after splenectomy (24 hrs.)	85	3	1.6	92	277	32	1.88
13. " " : (a) before splenectomy	100	70	63	12	57	36	63
(b) after splenectomy (24 hrs.)	90	1.6	0.8	96	407	34	0.88
14. Thrombocytopenia: acute lymphocytic leukemia	80	75	75	0	6	27	93.7
15. " : " " "	45	13	9.8	33	32	28	21.7
16. " : " " "	75	69	66	5	12	36	88
17. " : chronic myelogenous leukemia	100	87	62	41	64	35	62
18. " : " " "	95	22	15	50	69	38	15.7
19. " : " " "	100	53	37	42	68	41	37
20. " : " " "	95	46	31	50	52	34	32.6

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21. Aplastic anemia	35	35	34	2	4	26	97.1
22. Hemophilia: (a) before transfusion	95	79	68	16	512	41	71.6
(b) after transfusion (500 ml. of citrated plasma)	100	23	14	59	508	37	14
23. Hemophilia	100	95	91	4	432	36	91
24. Pseudohemophilia	100	9.2	4.5	102	198	37	4.5
25. "	87	5.2	3.1	69	256	40	3.5
26. Hypoprothrombinemia (dicumarol treatment)	62	13	7.3	79	367	43	11.4
27. " " "	18	3	2.4	26	465	45	13.3
28. " " "	9	0	0	16	346	42	0
29. " " "	36	10	6.8	43	478	42	19.1
30. " " "	26	1	0.7	45	383	39	2.7
31. Polycythemia vera	85	12	6.3	92	1,357	58	7.4
32. " "	100	8.5	4.5	87	2,476	57	4.5
33. " "	100	3.1	1.4	115	1,874	61	0.4

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It has been shown by differential examination for both factors that the accelerator and prothrombin activity of serum are present in an inverse proportion to one another.[7, 9] In *normal serum*, where prothrombin consumption has been good, the residual prothrombin activity is *low* and accelerator activity *high*. In *hemophilia* (and other thromboplastin-deficiency diseases) serum prothrombin remains *high*, but the accelerator is *low*. The purpose of the prothrombin consumption test is to identify a high residual prothrombin activity. In such a serum the amount of accelerator activity is negligible; it contributes very little to the one-stage serum prothrombin time. Normal serum, on the other hand, contains a high level of accelerator, but there is little prothrombin to act upon. Even 100 per cent acceleration of prothrombin is of no practical significance when the level of prothrombin activity is only 2 per cent.

This report presents a number of cases which involve a defect of the hemostatic mechanism. In each case, the serum prothrombin time was determined by the one-stage method and the result corrected for serum accelerator activity. It is shown that the results of the one-stage test arrange themselves in two groups, those with a normal prothrombin consumption and those which are definitely abnormal. The integrity of the two groups is not disturbed by correcting for accelerator activity. The diagnostic value of the prothrombin consumption test, therefore, is not compromised by the serum accelerator effect.

Materials and Methods

The blood of 11 healthy subjects and 22 patients was examined. The patients presented a variety of deficiencies of the hemostatic mechanism as shown in table 1. The tests which were performed are also indicated in table 1.

1. *Deprothrombinized plasma* was prepared from oxalated plasma by the method of Quick and Stefanini.[11] One liter of calcium chloride solution containing 66.6 Gm. of the anhydrous salt was added slowly with vigorous stirring to a solution of trisodium phosphate containing 158 Gm. of the salt in one liter of distilled water. The pH was adjusted to 7. The precipitate of $Ca_3(PO_4)_2$ was washed by decantation until all traces of sodium chloride were removed. The suspension was then made up to one liter and had therefore a concentration of 0.2 M. From this stock solution a preparation of 0.008 M was made by diluting 4 ml. with 96 ml. of distilled water. This suspension is stable at room temperature, but must be agitated vigorously before use. One ml. of the diluted suspension was then transferred to a small test tube and centrifuged to pack the gel. The water was poured off, the tube drained and 1 ml. of plasma to be deprothrombinized added. The tricalcium phosphate and the plasma were thoroughly mixed and allowed to stand at room temperature for ten minutes. The adsorbant was finally separated by centrifugation at 2,000 r.p.m. for twenty minutes and the supernatant deprothrombinized plasma transferred to another glass test tube.

2. *Thromboplastin* was prepared from human brain by the acetone-extraction method of Quick. The batch which was used throughout this work gave a prothrombin time of 12 seconds with normal fresh oxalated human plasma.

3. *Plasma prothrombin activity* was determined by the method of Quick. Normal plasma was serially diluted with deprothrombinized plasma and the clotting time of the various mixtures determined. Each mixture was taken to represent a percentage of normal prothrombin activity, depending upon the percentage of normal plasma. When the prothrombin time was correlated with prothrombin activity, an exponential curve resulted. This is the curve used in figure 3. By reference to this curve other prothrombin time values could be converted to percentages of normal prothrombin activity.

4. *Serum prothrombin time* was determined by the following method: A 20-gage needle on a glass syringe was neatly inserted into a vein and a few ml. of blood were drawn. The glass syringe was exchanged for a second one coated with Silicone (Dri-Film 9987, General Electric Company) and chilled prior to use.

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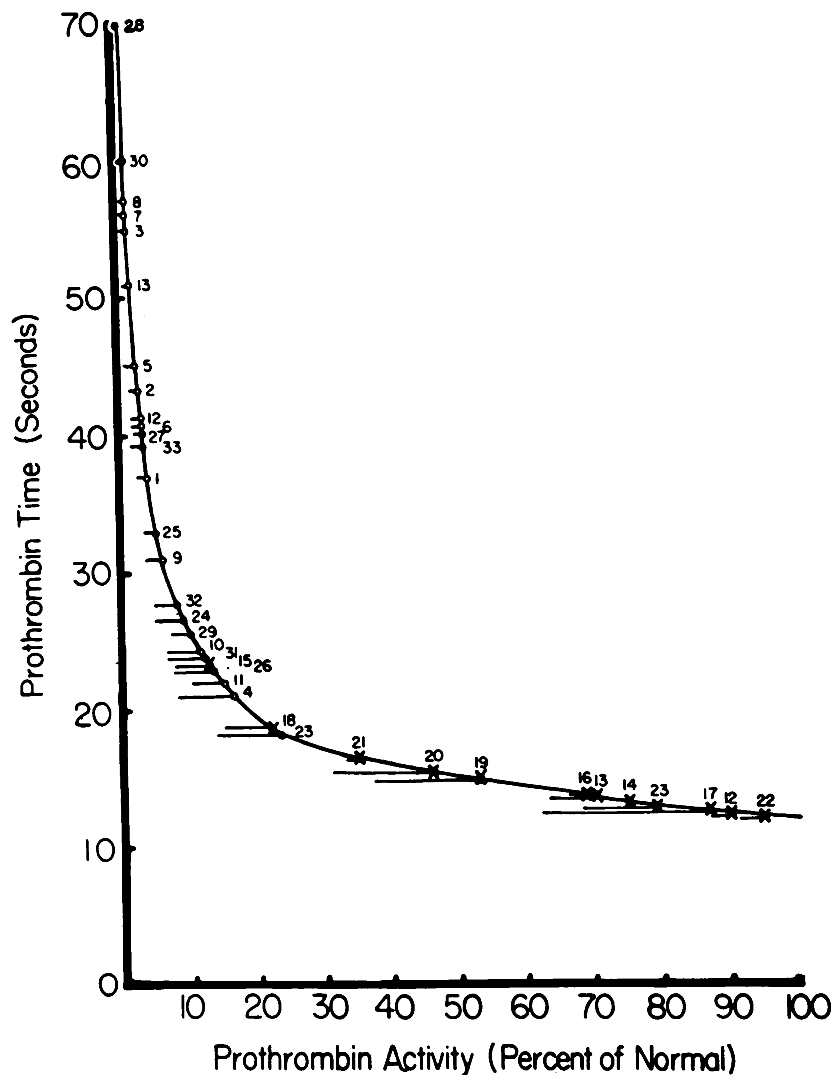

FIG. 3.—Calculation of the percentage of prothrombin activity from the prothrombin time and correction for the accelerator activity in serum. (Crossed circles are the patients with thromboplastin deficiency diseases. The number by each circle identifies it with the results presented in table 1.)

The curve was derived from prothrombin values of dilutions of normal oxalated plasma as described under Methods (paragraph 3). Serum prothrombin *time* was located on the curve and the uncorrected serum prothrombin activity was thereby obtained. This value was then corrected for accelerator activity as described under Methods (paragraph 4). The circles on the curve represent the uncorrected values for serum prothrombin activity. A line from each circle extends to the left to a point which represents the corrected serum prothrombin activity. Empty circles are the normal controls. Solid circles are the patients with various disorders of the hemostatic mechanism.

Two ml. of blood, apparently uncontaminated by tissue thromboplastin, were collected, transferred to a Pyrex tube and allowed to clot in a water bath at 37 C. One hour after the completion of clotting, the serum was separated and mixed with $\frac{1}{10}$ volume of sodium citrate and incubated for thirty minutes more

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to allow neutralization of thrombin. The prothrombin time of this serum was determined by the method of Quick et al.[3] In a test tube kept in a water bath at 37 C. the following were added in rapid succession: 0.1 ml. of deprothrombinized plasma; 0.1 ml. of 0.02 M $CaCl_2$; 0.1 ml. of Quick's thromboplastin; 0.1 ml. of the serum to be tested. The clotting time of the mixture was recorded.

Serum prothrombin activity was derived from serum prothrombin time by reference to the curve, figure 3. Correction for the serum accelerator effect was made with the following formula:

$$X = \frac{P}{(100 + A)} \times 100$$

where: X = corrected prothrombin activity in per cent; P = uncorrected prothrombin activity and A = accelerator activity.

5. *Serum accelerator activity* was derived by the one-stage method of de Vries et al.[12] The deprothrombinized plasma used in the reaction was prepared with $Ca_3(PO_4)_2$ gel instead of $BaSO_4$ powder. The results are expressed as per cent of acceleration. Thus when accelerator activity was able to reduce prothrombin time by half, acceleration was considered to be equal to 100 per cent.

6. Platelet counts were done by the indirect method of Dameshek,[13] which gives values of around 500,000 thrombocytes per cu.mm. in healthy subjects.

7. Hematocrit was measured in Wintrobe tubes after centrifuging at 1,500 rpm for sixty minutes.

Results and Discussion

The results are presented in table 1 and figure 3. Analysis of the data in figure 3 shows that the results of the prothrombin consumption test on all of the healthy controls lie in the left part of the curve. There is little prothrombin activity (a maximum of 15 per cent) even though the accelerator activity is quite high. Correcting for accelerator activity only slightly shifts the values further to the left. In patients with diseases characterized by thromboplastin deficiency[2] (hemophilia and severe thrombocytopenia) the values are well to the right of the curve. The prothrombin activity remains high even when corrected for the small degree of accelerator activity present. In thrombocytopenia, the prothrombin activity of serum is roughly proportional to the number of circulating platelets, being usually high when this is around or below a figure of 50,000 per cu. mm. At this level hemorrhagic manifestations are usually present.

More than the absolute value of serum prothrombin activity, however, the ratio, plasma/serum prothrombin activity, has diagnostic significance, because if the prothrombin activity of plasma is low the value of residual prothrombin in serum will also be low, independently from the efficiency of the mechanism of thromboplastin activation. Case 21 is a fair example of this situation. In this subject, suffering from aplastic anemia, the prothrombin activity of serum was equal to 35 per cent of normal, but the platelet count was practically nil. As, however, the plasma prothrombin level was also equal to 35 per cent of normal, the consumption of prothrombin during coagulation was practically nil, in agreement with the finding of a severe thrombocytopenia. An analogous situation, but due to a different mechanism, is found during dicumarol therapy, when both plasma and serum prothrombin activities are low, but the serum/plasma prothrombin activity ratio is essentially normal. A high value of this ratio has been so far, in our experience, related to a defective mechanism of thromboplastin activation.

A high serum prothrombin activity is of definite importance in both hemophilia

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and severe thrombocytopenia. In both conditions, the value of serum prothrombin activity is very close to that of the plasma and practically no accelerator can be demonstrated in the serum. A prompt and striking decrease in prothrombin activity of serum toward normal values follows transfusion of plasma in hemophiliacs, with a corresponding rise of accelerator activity in serum. This effect gradually fades out in three to four days. On the other side, a more pronounced and permanent normalization of the consumption of prothrombin during coagulation follows splenectomy in successfully operated cases of idiopathic thrombocytopenic purpura, and even immediately after ligature of the splenic pedicle, as reported by Quick et al.[3] It may be added here that in 8 cases of idiopathic thrombocytopenic purpura and 18 cases of symptomatic thrombocytopenia (leukemias, aplastic anemias) we have failed to demonstrate the presence of anticoagulants or inhibitors capable of influencing the determination of the prothrombin time of serum and plasma.

In the course of this work, we have confirmed the reported finding[7, 9] that values of prothrombin activity and accelerator activity of serum are indirectly proportional to each other. There is, however, an outstanding exception: During treatment with dicumarol, both these activities are decreased, but the ratio of prothrombin activity of serum to that of plasma is not greatly at variance with the one observed in healthy individuals, so that the limited amount of prothrombin available seems to be well utilized during the process of coagulation. The development of little accelerator effect with a normal consumption of prothrombin can be satisfactorily explained. As little prothrombin is available, a limited amount of thrombin will be formed. Since at least one of the serum accelerators derives from a plasmatic precursor through the action of thrombin,[6, 7] its production will be greatly decreased.

Table 1 and figure 3 also show the results obtained in 3 patients with polycythemia vera. These are representative of a group of 12, currently under our observation. It is well known that these patients bleed easily[14] and are subject to epistaxis, hemorrhage from peptic ulcer or following minor surgical procedures. In contrast, they are also inclined to vascular thrombosis. The hemorrhagic tendency has never been satisfactorily explained. It appears from our results that it is not due to abnormalities in the mechanism of activation of thromboplastin. The results of the prothrombin consumption test are within normal limits, but it should be noted that there is not the "superconsumption" of prothrombin which might be expected with extremely high platelet counts.

Summary

1. The results of the one-stage prothrombin consumption test, performed in a group of patients with various abnormalities of the hemostatic mechanism, are reported. It appears that the test accurately identifies those conditions such as hemophilia and thrombocytopenic purpura which involve a deficiency of available thromboplastin. Other conditions examined in which a hemorrhagic tendency may develop (hypoprothrombinemia, polycythemia vera, pseudohemophilia) showed a normal consumption of prothrombin. In the hypoprothrombinemia due

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to dicumarol therapy, the limited amount of available prothrombin appears to have been well utilized during the process of coagulation.

2. It appears that correction of the results obtained for accelerator activity of serum is hardly ever necessary, since this effect is not of such magnitude as to modify the results of the prothrombin consumption test to any extent. A formula for such correction is given.

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