The Thromboplastinogen*) activity time (TAT) Test

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The basic principles of the classical theory of blood coagulation which were formulated in 1904 by Morawitz and by Fuld and Spiro have been the guide to much of the modern research in this field. This theory, however, was not fully appreciated until the one- and two-stage prothrombin methods were developed in 1935 and 1936. The nature of the second factor in this theory, namely thrombokinase or thromboplastin, remained poorly understood until the prothrombin consumption time was described in 1947 (2). In that procedure the prothrombin (measured by the one-stage prothrombin time) remaining in the serum after the blood is clotted under standard conditions is taken as a quantitative measure of the thromboplastin activity of the blood. In the development of the test, it was established that the thromboplastin activity of the blood does not come from preformed thromboplastin in the platelets but that these cells supply a factor which reacts with a plasma constituent to form thromboplastin. Subsequent studies by various investigators showed that actually several plasma factors play a role in the generation of thromboplastin.

The prothrombin consumption test is obviously not a specific quantitative measure of any one particular clotting factor. In the initial study of 1947, it was shown that a poor prothrombin consumption occurs both in thrombocytopenia and in hemophilia. The test could be made more specific for hemophilia by repeating the determination after adding a known adequate quantity of normal platelets to the blood before it is clotted.

The task of preparing platelets for a routine test is too laborious and time-consuming to be practical. It was fortunate and important that a simple means was discovered for preparing a satisfactory platelet substitute (5). It was observed that when an extract of rabbit brain (the reagent employed in the one-stage prothrombin time) is heated to 60° C, it loses its holothromboplastin activity but retains properties that closely resemble those of a platelet extract. By holothromboplastin activity is meant the ability of an agent to bring about consumption of prothrombin when added to plasma from a severe hemophiliac,

*) Thromboplastinogen = Factor VIII; as Factor IX was not yet discovered when the term thromboplastinogen was proposed in 1947, the latter applies to Factor IX as well.

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while platelet activity is the ability to cause consumption of prothrombin in normal platelet-poor plasma.

The platelet-like activity of heated rabbit brain extract makes this reagent valuable in the differentiation of hemophilia from platelet deficiencies such as thrombocytopenia and thrombasthenia. In the latter conditions, the addition of heated rabbit brain to the blood before it is clotted will correct the defective consumption of prothrombin. Failure to correct indicates a defect in the plasma. Since at the time that the basic prothrombin consumption test was developed, only one plasma factor was known, namely thromboplastinogen — the agent lacking in hemophilia — the modified test with added heated rabbit brain was named the „thromboplastinogen activity time". Although it is now established that other plasma factors in addition to thromboplastinogen are necessary for the generation of thromboplastin, it seems advisable to retain the name of the test with the understanding that it measures several components of the thromboplastinogen complex, rather than any specific agent. For convenience, heated rabbit brain extract is designated as TAT reagent.

In this paper the usefulness of the test in differentiating bleeding conditions due to platelet defects from hemophilia and hemophilia-like conditions will be shown, and the employment of the test for differentiating hemophilia from other conditions caused by lack of plasma thromboplastin factors, particularly PTC deficiency (or Christmas disease), will be outlined.

Experimental

Prothrombin Consumption Time (Basic). Blood is obtained by venipunctures with a silicone-coated syringe and needle and transferred to test tubes similarly coated which are kept in an ice bath. The blood in one tube is allowed to sediment until sufficient plasma has separated to permit the removal of 1 ml. This platelet-rich plasma is transferred by means of a silicone-coated pipette to a glass test tube. In a second test tube, 1 ml of blood is placed. Both tubes are put in a water bath at 37° C and the time required for a solid clot to form is recorded. 15 minutes after clotting, the tubes are centrifuged for about 2 minutes at 2500 rpm to separate the serum from the coagulum. The tubes are again placed in the water bath for 45 minutes. The prothrombin of the serum is determined as follows: 0.1 ml of the serum is blown from a pipette into the following mixture:

0.1 ml — thromboplastin reagent (rabbit brain extract)

0.1 ml — fresh deprothrombinized rabbit plasma

0.1 ml — 0.02 M $CaCl_2$ solution.

The preparation of these reagents has been described in detail (1). The time of clotting is determined with a stopwatch.

Preparation of TAT Reagent. Acetone-dehydrated rabbit brain extract prepared according to the directions of the writer is incubated at 60° C for 20 minutes. It is convenient to mix 200 mg of the dehydrated rabbit brain powder with 5 ml of 0.85% sodium chloride solution. It is important to note that human brain cannot be substituted in this test. The reagent is stored at — 20° C.

Thromboplastinogen Activity Time (TAT) on Whole Blood. One ml of freshly-drawn blood is added to a small test tube containing 0.025 ml of TAT reagent. After thorough

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mixing, the tube is placed in a water bath at 37° C; at 15 minutes after clotting, it is centrifuged and again incubated for 45 minutes. The prothrombin of the serum is determined as in the prothrombin consumption test.

The technique for carrying out the test on native plasma is the same except that 0.05 ml of the TAT reagent is mixed with 1 ml of plasma.

TAT Test-Modification 1. The procedure is the same as the basic test except that 0.1 ml of fresh normal deprothrombinized plasma is mixed with the TAT reagent and the native plasma. The deprothrombinized plasma is prepared by treating fresh oxalated plasma with $BaSO_4$ or $Ca_3(PO_4)_2$ (1).

TAT Test-Modification 2. The same as the above except that 0.1 ml of aged serum from normal blood is mixed with the TAT reagent and the native plasma. The aged serum is prepared by clotting normal blood at 37° C, centrifuging after 15 minutes and incubating the serum at 37° C for 1 hour, at 4° C for 24 hours and then storing it at — 20° C.

Results

In uncomplicated thrombocytopenic purpura, a close relation exists between the prothrombin consumption time and the platelet count. Since the prothrombin consumption is corrected by the TAT reagent, one may conclude that lack of platelets is directly responsible for the poor consumption of prothrombin. In table 1, results are presented which were obtained on a patient who has had thrombocytopenic purpura for about 30 years. The close relation of the platelet count and the consumption of prothrombin in strikingly illustrated.

Table 1: The Interrelation of the Prothrombin Consumption Time, TAT and Platelet Count in Thrombocytopenic Purpura

Patient G, age 50:

Date	Clotting time min	Prothrombin consumption time sec	TAT sec	Clot retraction	Tourniquet test	Platelet count
13 Jan. 1954	10½	10	29	—	+++	12 000
31 Jan. 1955	6½	20	21	+	+	126 000
4 Dec. 1955	9	11	29	+	+++	33 000
10 Jan. 1956	9	14	30	+	++	45 000
3 Mar. 1956	10	10	43	+	+++	33 000
6 Aug. 1956	10½	9	40	—	+++	3 000
5 Nov. 1956	7	16	47	+	+	117 000

Bleeding time has occasionally been moderately prolonged and the prothrombin time has been normal

In table 2 results of studies on three patients whose bleeding tendencies were found to be due to defective platelets are recorded. The defect in the first patient is congenital and was not corrected by splenectomy. His platelets are exceedingly minute. In the second patient, the defectiveness of the platelet is

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shown by the low prothrombin consumption time which is corrected by the TAT reagent. This patient has a prolonged bleeding time, a positive tourniquet test and presents a clinical picture resembling thrombocytopenic purpura. It is clear that the quality and not the quantity of her platelets is responsible for the purpuric state. The third patient presents the most striking example of thrombasthenia. In spite of a normal platelet count, his prothrombin consumption was very low. It was acquired following prostatectomy and became corrected spontaneously.

Table 2: The Prothrombin Consumption Time and TAT in Thrombasthenia

Patient	Age	Clotting time min	Prothrombin consumption time sec	TAT sec	Platelet count	Clinical findings
D. K.	4	5	11	39	120 000	Ecchymoses; epistaxis
R. L.	60	14	14	61	163 000	Epistaxis; menorrhagia
W. G.	70	6½	12	46	260 000	Bleeding from prostate bed

The value of the TAT test in differentiating true hemophilia from PTC deficiency or Christmas disease is shown in table 3. It will be observed that in hemophilia both the prothrombin consumption time and the TAT values are low and that the addition of deprothrombinized plasma from a normal subject effects a significant correction, whereas aged serum is completely ineffective. In marked contrast, the reverse results are obtained in PTC deficiency: no correction with deprothrombinized plasma and a good response with serum.

Comment

The basic prothrombin consumption test, like the one-stage prothrombin time, is important in establishing a coagulation defect but, like the latter test, it is not by itself specific for any one clotting factor. With simple modifications, however, the specificity can be greatly increased. By adding a small quantity of rabbit brain extract heated to 60° C before the blood or plasma is clotted, the test is made responsive to lack of thromboplastinogen, PTC and, perhaps, to other plasma factors. By this means, platelet dysfunction can be quantitatively determined. It is to be emphasized that the platelet count, even when accurately made (which unfortunately is not always done in the clinical laboratory) does not supply in all instances the essential information, namely: the true clotting activity of these cells. The term „thrombasthenia" has remained hypothetical because no simple test was available for its determination. By carrying out a prothrombin consumption time and a TAT test, the diagnosis of thrombasthenia can now readily be made.

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Table 3 : The Application of the TAT Test for the Differential Diagnosis of Hemophilia Vera and PTC Deficiency

	Hemophilia Vera			PTC Deficiency	
	Moderate	Mild	Severe	Mild	Severe
Clotting time	min 7½	27	60	12	59
Prothrombin consumption time (basic)	sec 14½	9	8	14	12
TAT	sec 10½	10½	8	12	10
TAT + plasma $Ca_3(PO_4)_2$*)	sec 24	20	25	13	12
TAT + aged serum	sec 10½	11	8	90	28

The differential diagnosis for distinguishing hemophilia vera and PTC deficiency can easily be made with the prothrombin consumption time and the TAT tests. If the prothrombin consumption is corrected by mixing the blood or plasma with a small amount of normal plasma treated with $Ca_3(PO_4)_2$ or $BaSO_4$, the diagnosis of hemophilia is fairly certain. These adsorbents remove PTC but not thromboplastinogen, therefore one may assume that if correction occurs with adsorbed plasma, the condition is hemophilia, since the lack of thromboplastinogen in this disease has been experimentally established (3). A correction of the prothrombin consumption time with aged serum strongly suggests that the disease is PTC deficiency, since that principle is present in the serum and is stable on storage.

The limitations of using adsorbed plasma and aged serum in the prothrombin consumption time and TAT tests can readily be recognized. It is assumed that the first contains thromboplastinogen but no PTC, while the second contains PTC but no thromboplastinogen. While that assumption is correct, the possibility of other significant clotting factor being present in either plasma or serum cannot be ignored. Serum particularly is a complex mixture. It undoubtedly contains intermediary clotting factors; in fact, it is not entirely clear whether even PTC is a primary or a derived clotting agent. Perhaps, some agents, which in plasma are kept inactive by an inhibitor, are free in serum because the latter agent is destroyed or inactivated. Prothrombinogen (6) is an example and perhaps a similar type of precursor may be found for some of the factors in the thromboplastin complex.

For a more definitive diagnosis of hemophilia by the prothrombin consumption time test and its modifications, correction must be achieved by the addition of the specific clotting factor, thromboplastinogen, which is lacking in this disease

*) Plasma adsorbed with $Ca_3(PO_4)_2$

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(4). This has been accomplished since thromboplastinogen has been isolated in a relatively pure form and free from known clotting factors. Such a preparation corrects both the basic prothrombin consumption test and the TAT test. The present task is to isolate and purify the other clotting factors in the thromboplastin complex and to assay them on undiluted native plasma or whole blood to which no anticoagulants have been added.

The importance of using undiluted plasma or fresh whole blood as the testing material to evaluate hemostatic efficiency is gradually becoming recognized. The clinical success of the one-stage prothrombin time serves as an excellent example. Thus, a newborn infant with a normal prothrombin time (12 seconds) shows no bleeding tendency and can undergo surgery with safety; yet, the prothrombin as measured by the two-stage method may be only 25% of the adult level. The reason for the discrepancy is that the one-stage method measures active prothrombin which apparently is the fraction concerned directly in hemostasis, while the two-stage procedure measures total prothrombin — the sum of the free (or active) and prothrombinogen. Since the free prothrombin is the same in both the infant and the adult, their immediate hemastatic respone is the same even though prothrombinogen is absent in newborn blood. The two-stage method is theoretically correct as a measure of total prothrombin but in certain instances, as in the newborn infant, it may be misleading and erroneous as an evaluation of the prothrombin activity operative in hemostasis.

While the prothrombin consumption time and the TAT tests like all other clotting procedures are empirical, they, nevertheless, have the virtue of tending to maintain the physico-chemical balance of the blood. Under such conditions, the results are apt to reflect more specifically the hemostatic efficiency. The prothrombin consumption time and the various modifications outlined, therefore, have advantages over other procedures in which the plasma is diluted, the calcium removed, or chelated, and other drastic change introduced which alter the normal equilibrium and balance of the blood.

Summary

The thromboplastinogen activity time (TAT) test is described. The method is based on the finding that rabbit brain extract heated to 60° C loses its holothromboplastin activity but retains the properties of platelet extract and can, therefore, be used as a substitute for platelet extract in the prothrombin consumption test. The application of the test to differentiate hemophilia from thrombocytopenia and thrombasthenia is illustrated by case reports. The use of the test for the differential diagnosis of hemophilia and PTC deficiency is outlined and examples of its application given.

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Résumé

La technique du temps d'activation du thromboplastinogène (TAT) est décrite. Ce test est basé sur la découverte que les extraits de cerveaux de lapins, chauffés a 60° C. perdent leur activité thromboplastinique complète et peuvent être substitués aux extraits plaquettaires dans le test de la consommation de la prothrombine. Cette modification permet de différencier l'hémophilie de la thrombocytopénie et des troubles fonctionnels des plaquettes. L'emploi de cette méthode pour la distinction entre l'hémophilie et la déficience du PTC est démontré et illustré par quelques exemples.

Zusammenfassung

Beschreibung des Thromboplastinogen-Aktivitäts-Zeit-Tests (TAT). Die Methode beruht auf dem Befund, daß Kaninchenhirnextrakt, auf 60 Grad erhitzt, seine Aktivität als vollständige Thrombokinase verliert und nur die Eigenschaften eines Thrombozytenextraktes behält. Derartiger Kaninchenhirnextrakt kann daher beim Prothrombin-Verbrauchs-Test als Ersatz für den Thrombozytenextrakt verwendet werden. An Hand von Fällen wird die Anwendung des Tests zur Differenzierung der Hämophilie von einer Thrombopenie und Thrombastenie demonstriert, und schließlich die Verwendung des Tests zur Differentialdiagnose von Hämophilie und PTC-Mangel besprochen.

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