Plasma Thromboplastin Component (PTC) Deficiency: A New Disease Resembling Hemophilia.* (19488)

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In current concepts of blood coagulation mechanism(1), thromboplastin is said to be liberated from fixed tissue sources through injury, or from blood itself by interaction of a platelet factor and a single inactive plasma factor(2,3). The coagulation defect in hemophilia is thought to be due to absence of the latter plasma factor. We obtained evidence from the study of the coagulation defect of a patient, who had previously been thought to suffer from hemophilia, of the presence of a second plasma factor which appears to be as important biologically as the anti-hemophilic factor for the production of thromboplastin in the blood.

The patient, a 16-year-old Caucasian male with no siblings, living or dead, had a negative family history of hemorrhagic diatheses. Clinical and laboratory findings in infancy suggested a diagnosis of thrombocytopenic purpura, and splenectomy was performed at the age of 2 years. Since then numerous tests of the platelet count and function, bleeding time and clot retraction and of the plasma fibrinogen and prothrombin and serum calcium concentrations have been normal, and venous blood coagulation times have been markedly prolonged. It is not known whether venous blood coagulation time was prolonged prior to splenectomy. The patient has been hospitalized over 100 times for major hemorrhagic episodes, including bleeding from lacerations and hemorrhage into subcutaneous tissues, muscles, joints, gastrointestinal tract, pharynx, and retroperitoneal region. In all instances prolonged coagulation time was markedly shortened and the hemorrhage rapidly controlled by the transfusion of whole blood, fresh or frozen plasma, or freshly lyophilized plasma. The first suspicion that the patient did not have true hemophilia arose when the coagulation time was not significantly shortened by potent preparations of anti-hemophilic globulin (Cohn's fraction I). The prolonged coagulation time is accompanied by a markedly defective prothrombin utilization(4,5), which can be corrected by addition of small quantities of normal plasma *in vivo* or *in vitro*. Further studies have shown that the patient's blood contains normal amounts of all previously described coagulation factors(1), and that abnormal amounts of circulating anticoagulants are not present(6). These findings indicate that the coagulation defect in this patient is due to the absence from his plasma of a previously undescribed coagulation factor. The fact that the defect can be completely corrected with accelerator-free tissue thromboplastin eliminates the possibility of a deficiency in prothrombin or prothrombin conversion accelerators and indicates that the missing factor is concerned with the production of thromboplastin. Accordingly, we have named it the *plasma thromboplastin component* (PTC).

Studies conducted in our laboratory show that PTC can be adsorbed by $BaSO_4$ from oxalated plasma, as are prothrombin(7) and serum prothrombin conversion accelerator (spca)(7,8). It can also be adsorbed by all of previously described prothrombin adsorbants(2) and by Seitz filtration(9). It can be concentrated relatively free of all previously described coagulation factors as follows: Carefully drawn blood is oxalated and centrifuged to remove platelets, using Conley's

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TABLE I. Effect of Various Blood Fractions on Patient's Coagulation Time and Prothrombin Utilization.

Coagulation time,* min	Fraction added (.1 cc added to 2 cc patient's blood)	Coagulation time of mixture, min	Residual serum prothrombin,† %
	Effective fractions		
60	Normal plasma	12	21
120	Supernatant from 20000 r.p.m. plasma (17)	12	25
64	Hemophilic plasma	10	12
60	Berkefeld filtered normal plasma	18	32
60	Hypoprothrombinemic plasma from dicumarol-treated patient	22	40
35	Plasma heated at 56°C 5 min	20	30
36	Normal serum (thrombin free)	11	15
36	Normal serum heated at 56°C 11 min	15	30
60	Euglobulin (9)	5	35
60	.12 mg accelerator-free tissue thromboplastin‡	2	20
	Non-effective fractions		
70	$BaSO_4$-treated normal plasma	70	80
70	Seitz-filtered plasma	70	85
70	Patient's plasma	70	87
70	Citrate eluate from patient's plasma	70	100
60	.04% to .4% AHG (Cohn's fraction I)	60	90

* Two cc venous blood in 12 × 75 mm glass test tubes at 37°C.
† Modified Ware and Seegers (11). Serum citrated 1 hr after above coagulation time started. (Patient's plasma prothrombin is 100%.)
‡ Supplied by Chilcott (CH-61).

technic (10). The "platelet-free" plasma is then heated to 56°C for 5 minutes and the coagulated fibrinogen is removed by centrifugation. The supernatant plasma contains most of the original PTC and anti-hemophilic globulin (AHG) (9) and only traces of prothrombin (11), spca (12,8), Factor V (13), and labile factor (12). The PTC fraction is separated from the AHG by adsorbing it on $BaSO_4$, from which it is eluted with 5.8% sodium citrate.‡ The citrate eluate is then dialyzed against 0.9% NaCl. The final product is free of thrombin. It can be lyophylized without loss of potency. Analysis of one of the products showed that the amount of protein obtained from 1 ml of plasma was equivalent to 8.1 μg of tyrosine (14). The *PTC content* of various plasma or serum fractions can be assayed against the patient's blood in a manner similar to that reported by Graham *et al.* (5) for AHG, using a "2-stage" prothrombin utilization test. The patient's PTC fraction when so assayed is inert, while that obtained from normal, hemophilic, platelet-deficient, or dicumarolized hypoprothrombinemic plasma is significantly active (Table I). PTC concentrate is now under study, using PTC-free "purified" coagulation factors in a system similar to that described by McClaughry and Seegers (15). PTC is associated with the plasma euglobulins obtained either by dilution and acidification of plasma (9) or by 50% saturation of plasma with $(NH_4)_2SO_4$ (16). Only traces remain in the other plasma fractions.§ It is most stable in the neutral ranges, being least stable at pH 11.0. Unlike AHG, PTC remains in the serum even after prothrombin, thrombin, and spca have been removed by heating at 56°C for 5 minutes.

PTC is not a platelet factor, AHG, or "complete" thromboplastin, such as that derived from fixed tissue sources, since it will not correct the defect (4) present in "platelet-free" normal plasma or in platelet-rich hemophilic plasma. It does not appear to be the plasma

‡ This method of adsorption and elution is similar to Alexander's method (7) for the preparation of prothrombin, except that we start with "platelet-free" heated plasma.

§ Subsequent work has revealed that PTC is precipitated by 50%, but not by 40%, $(NH_4)_2SO_4$ saturation. This further excludes the anti-hemophilic factor which is associated with the proteins precipitated between 0% and 33% $(NH_4)_2SO_4$ saturation (16).

thromboplastin reported by Chargaff and West (17), since the supernatant from plasma centrifuged at 20000 r.p.m. for 150 minutes completely corrects the patient's defect. PTC also is not to be confused with TPC(18), which is Shinowara's term for anti-hemophilic globulin(19). As suggested by Koller(20), the prothrombin conversion factor of Owen and Bollmann, the prothrombin accelerator of MacMillan, the co-thromboplastin of Mann and Hurn, factor VII of Koller, Owren's proconvertin, and spca are probably the same. Since these factors can be adsorbed by $BaSO_4$ and eluted with sodium citrate, it would also seem probable that they contain PTC. A study of this possibility is now in progress. The fact that preparations of PTC do not accelerate a one-stage prothrombin determination, as do the above factors, indicates that our preparation of PTC is free of these factors.

PTC deficiency resembles hemophilia both clinically and in the results of the usual laboratory investigations. It can be distinguished from hemophilia by the following features: 1) the coagulation defect of PTC deficiency cannot be corrected by anti-hemophilic globulin (Cohn's fraction I), Seitz-filtered plasma, or $BaSO_4$-adsorbed plasma; 2) it can be corrected by normal serum, as well as plasma, and by the citrate eluate of $BaSO_4$-adsorbed plasma or serum; 3) hemophilic and PTC-factor deficient bloods correct each other's coagulation defects.

It has been reported that blood from some cases of "hemophilia" is capable of correcting the coagulation defect in other cases of hemophilia *in vitro*(21). This has not been our experience, and it appears more likely to us that such results may be due to mixtures of blood from patients with PTC deficiency and true hemophilia. It is not known whether PTC deficiency is hereditary, and, if hereditary, whether it follows the genetic pattern of hemophilia.

Summary. 1. A severe hemorrhagic disease, characterized by a prolonged whole blood coagulation time due to the delayed formation of thrombin, has been described. The patient with this disease was found to have normal plasma concentrations of all the previously described coagulation factors. 2. The corrective factor in normal plasma or serum can be removed by barium sulfate adsorption. A method for the partial purification and concentration of the patient's missing factor has been outlined. The defect in the patient's blood may be corrected by the addition of plasma free of previously described thromboplastin components (*e.g.* platelet-free hemophilic plasma), as well as by small amounts of tissue thromboplastin. The name *plasma thromboplastin component* (PTC) has been assigned to this previously undescribed coagulation factor.

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