Hemorrhagic Diathesis Due to PTC (Plasma Thromboplastin Component) Deficiency.* (20142)

JESSICA H. LEWIS AND JOHN H. FERGUSON.
From the Department of Physiology, University of N. Carolina, Chapel Hill.

Aggeler, White, Glendening, Page, Leake, and Bates(1) described for the first time a congenital hemorrhagic disease resembling hemophilia which they have called PTC (plasma thromboplastin component) deficiency. This disease is similar to hemophilia in exhibiting a prolonged coagulation time and abnormal prothrombin consumption. Prothrombin, proaccelerin (AcG or labile factor), proconvertin (pro-SPCA, factor 7, co-thromboplastin of Mann), fibrinogen and platelets are described as normal. The clotting defect differs from hemophilia in that it can be corrected by the addition of hemophilic as well as normal plasma and by the plasma factor, PTC. At the time the above-cited paper was published, we were studying a patient with a circulating anticoagulant. This patient (R.J.) resembled some of the described cases of hemophilia with circulating anticoagulants (2,3), in that he apparently suffered from a congenital hemorrhagic disease and had developed the anticoagulant subsequent to transfusion. Studies on this patient, reported here, were made at a time when the anticoagulant had almost completely disappeared and show that his basic disease was PTC deficiency and not hemophilia. The characteristics of the anticoagulant will be described in a separate communication(4).

In the last few months, we have had the opportunity to study 4 additional patients with prolonged coagulation times. Two of these, E.W. and Q.G., proved to have PTC deficiency and the others, W.B. and T.G., to have true hemophilia. These findings suggest that PTC deficiency may be a fairly common hemorrhagic disorder, previously unrecognized or confused with hemophilia.

Methods. All blood samples were carefully collected by venipuncture into silicone syringes and transferred to glass or silicone containers as desired. *Platelet "thromboplastin"* was measured by preparing washed (8x) platelet suspensions from normal and patients' citrated plasmas and observing their ability to restore the coagulation of normal platelet poor plasma (centrifuged at 20,000 *g* for 90 minutes). *Coagulation time* was measured by the Lee-White method(5) at 37°C. *Prothrombin consumption* was performed by a modification of the method of Quick(6). Blood samples were oxalated (one-tenth volume of 0.1 M sodium oxalate) after standing at 37°C for one hour after clotting. One-tenth ml of serum obtained by centrifugation was added to 0.1 ml of $BaSO_4$ plasma plus 0.1 ml thromboplastin (soluplastin, Schieffelin and Co., courtesy Dr. E. W. Blanchard). The clotting time was determined after addition of 0.1 ml 0.04 M calcium chloride. *Prothrombin concentration* was determined by the one-stage method of Quick(7), employing soluplastin, human brain thromboplastin or acetone dried dog brain obtained from a dog pretreated with dicumarol until only traces of prothrombin could be detected in his blood. The dog was anesthetized, exsanguinated and the brain was thoroughly perfused *in situ* before removal. *Labile factor* was assayed by mixing equal quantities of normal or patients' $BaSO_4$ plasmas with a standard aged plasma and comparing the reduction in "prothrombin time." *Co-thromboplastin* was assayed by the method of Mann(8). *Two-stage prothrombin* concentration tests were performed by the method of Ware and Seegers(9) modified to supply AcG. *Fibrinogen* was clotted by thrombin and determined gravimetrically. *Anticoagulant* titer was measured by a method to be described in detail(4). *Plasma antithrombin* was determined by adding 0.1 ml of thrombin (Parke, Davis and Co., courtesy Dr. E. A. Sharp) of various concentrations (10 u, 5 u, 2.5 u, 1.25 u/ml), prepared in silicone tubes to prevent rapid de-

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TABLE I. Historical Data on 5 Patients.

	PTC deficiency			Hemophilia	
	E.W.	R.J.	Q.G.	W.B.	T.G.
Age	4	16	40	20	40
Sex	♂	♂	♂	♂	♂
Race	C	C	W	W	W
Age at onset of symptoms	1½	3	4	Infancy	6
Symptoms:					
Epistaxis	+	0	0	+	+
Hematoma	+	+	+	+	+
Hemarthroses	+	+	0	+	+
Hematuria	+	0	+	+	+
Relative severity of bleeding tendency	4+	4+	+	4+	2+
Previous response to blood or plasma	+	+	+	+	+
Family history	+	Neg	+	+	+
Transmission	♀		♀*	♀*	♀
Sex	♂		♂	♂	♂

* or affected ♂.

terioration, to 0.1 ml oxalated plasma and determining the clotting time; at least one normal plasma was used as a control. *Antithromboplastin, immediate,* was determined by measuring the prothrombin time, employing dilutions of human brain thromboplastin and comparing these times to those of normal plasmas. *Antithromboplastin, progressive,* was determined by mixing silicone citrated plasma with an equal volume of a one to 20 dilution of human brain thromboplastin, incubating at 37°C and measuring the clotting time obtained when 0.2 ml of this mixture was added to 0.1 ml of 0.02 M $CaCl_2$, in silicone tubes, at intervals for 15 minutes. Simultaneous tests were performed on normal control plasmas. PTC was prepared as described by Aggeler *et al.*, and was concentrated by dissolving the dialyzed, lyophilized product in a volume equal to one-tenth of the original plasma volume. Tests showed that the PTC fraction contained no thrombin or SPCA(10). It did contain an insignificant trace of prothrombin. $BaSO_4$ plasma was prepared by mixing 100 mg of barium sulfate per ml of oxalated plasma for 10 minutes at room temperature and removing the barium sulfate by rapid centrifugation. This plasma would not clot on addition of thromboplastin and calcium.

Results. Pertinent data from the histories of the 5 patients studied are presented in Table I. All the patients were male and 2 of the patients with PTC deficiency were negroes. The type of hemorrhage, severity of disease, response to transfusion and familial transmission did not differentiate between the PTC and hemophilic groups. The incidence of hemorrhagic tendency in the families of 2 of the PTC deficient patients is shown in Fig. 1. R.J.'s mother died shortly after his birth and no adequate family history was obtained.

Routine hemostatic studies showed no detectable difference between the PTC deficient

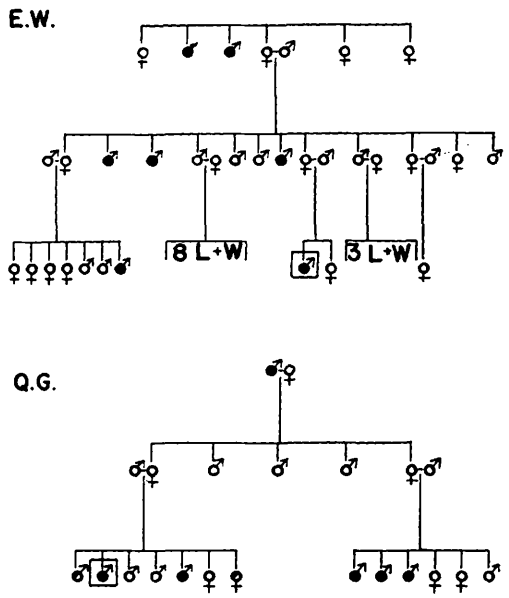

FIG. 1. Familial transmission in patients E.W. and Q.G. Open circle, no hemorrhagic history; half-closed circle, died in infancy; closed circle, hemorrhagic tendency; open square indicates patient studied.

TABLE II. Coagulation Studies.

Test	PTC deficient			Hemophiliac		Normal range
	E.W.	R.J.	Q.G.	W.B.	T.G.	
Clotting time:						
Glass	2–8 hr	>8 hr	24 min.	90 min.	24 min.	6–12 min.
Silicone	>8 hr	>8 hr	78 min.	>4 hr	4 hr	30–60 min.
Clot retraction	4+	3+	4+	3+	4+	4+
Clot lysis	0	0	0	0	0	0
Prothrombin consumption	10.6 sec.	13.8 sec.*	13.2 sec.	12 sec.	20.8 sec.	>50 sec.
Prothrombin time:	Sec.					
Soluplastin—Patient	12.4	14.4	15.2	15.0	17.5	
(Normal)	(15.0)	(15.6)	(17.9)	(15.0)	(15.0)	(15–18)
Human brain—Patient	17.4	17.3	21.2	22.3	15.3	
(Normal)	(17.1)	(17.3)	(17.5)	(19.3)	(17.4)	(17–20)
Dog brain—Patient	26.5	22.9	—	—	32.2	
(Normal)	(34.2)	(25.9)	—	—	(31.2)	(25–35)
2-stage prothrombin (u/ml)	300	276	280	—	280	260–300
Labile factor assay	100%	100%	—	—	—	100%
Co-Thromboplastin	100%	100%	—	—	—	100%
Fibrinogen (mg %)	230	440	320	380	360	250–500

* Due to the very long coagulation time, the usual method of measuring prothrombin consumption was unsatisfactory. Samples of normal and patient's blood were refrigerated 48 hr and residual prothrombin determined. Normal value was 26 sec.

and the hemophilic patients. Bleeding times, tourniquet tests and platelet counts did not differ significantly from the normal range with the exception of one prolonged bleeding time observed in patient Q.G. Plasma antithrombin and antithromboplastin titers were normal, and no anticoagulant could be detected with the exception of a trace in R.J., the patient who had previously shown a high titer. Additional clotting studies are shown in Table II. All patients showed normal clot retraction and their isolated platelets were able to restore the defect in platelet poor plasma. All patients showed prolonged coagulation times and abnormal prothrombin consumption. The one-stage prothrombin times were within the normal range even with the specially prepared dog brain thromboplastin. This finding suggested that prothrombin, proaccelerin, and proconvertin were normal in these patients. To confirm this we measured (a) the prothrombin by the 2-stage method, (b) the labile factor (proaccelerin, AcG) by observing the ability of normal and patients' prothrombin free plasmas to restore the "prothrombin time" of aged plasma, and (c) co-thromboplastin which is thought to be the same as proconvertin or SPCA precursor(11). All results were within the normal range. On one occasion attempts were made to assay SPCA in E.W. and R.J., but unfortunately the blood samples did not clot in 8 hours and tests made the following day showed only traces of SPCA activity. Normal serum incubated for a similar time at 37°C, also showed only traces of SPCA activity.

Many additional studies were done on these patients. Of particular interest are the plasma recalcification studies shown in Table III. Citrated plasma samples from all of the patients showed prolongations of their recalcification times (normal range = 2-2½ minutes). Clotting was markedly shortened in the plasmas of E.W., R.J., and Q.G. by addition of plasmas from W.B. and T.G., by normal plasma and by PTC factor. Clotting in patients W.B. and T.G. was shortened by addition of plasma from E.W., R.J., and Q.G., by normal plasma and by normal $BaSO_4$ plasma. These simple tests served to differentiate the 2 groups: the PTC deficient and the hemophilic. Normal plasma was able to restore the clotting defect in both groups. It was possible to fractionate this normal plasma into (a) the PTC fraction, which restored the defect in PTC deficiency, but did not shorten significantly the recalcification time of the hemophilic group and (b) $BaSO_4$ plasma,

TABLE III. Plasma Mixture Recalcification Times. Citrated plasmas, previously frozen in silicone, from the 5 patients were recalcified in glass, after mixture with one-fifth part of the listed reagents.

	0.2 ml citrated plasma											
	PTC deficient						Hemophiliac					
0.05 ml added reagent	E.W.		R.J.		Q.G.		W.B.		T.G.		Normal	
	Min.	Sec.	Min.	Sec.	Min.	Sec.	Min.	Sec.	Min.	Sec.	Min.	Sec.
Patient's own plasma	8	30	26	00	6	30	18	50	10	10	2	15
Saline	8	00	26	00	6	30	18	20	10	20	2	10
Plasma:												
E.W.		—	26	00	6	00	3	50	3	55	2	05
R.J.	17	30		—	7	30	4	00	4	00	2	05
Q.G.	6	30	20	30		—	3	50	3	55	2	05
W.B.	3	15	6	15	3	15		—	10	35	1	40
T.G.	2	50	7	00	3	20	13	30		—	1	55
Normal	2	55	7	30*	3	20	4	10	4	30		—
$BaSO_4$ plasma	8	10	26	00	6	30	4	40	4	25	2	15
PTC factor	1	40	3	20	1	55	12	30	7	10	1	50

* This figure suggests that R.J.'s plasma still contains a trace of anticoagulant.

which shortened the hemophilic clotting but not the PTC deficient.

A number of PTC fractions, free from SPCA and thrombin, were prepared both from plasma and from serum. Freshly prepared serum fractions were usually more active than plasma fractions, but after prolonged dialysis and drying, the 2 types of PTC showed equal activity.

Discussion. In considering the differential diagnosis of a hemorrhagic diathesis manifesting a prolongation of the whole blood coagulation time, the following possibilities must be considered: 1) hemophilia, 2) PTC deficiency, 3) qualitative or quantitative platelet deficiency (the Lee-White coagulation time is occasionally prolonged in severe deficiencies), 4) hypoprothrombinemia, 5) hypoproconvertinemia (deficiency of co-thromboplastin or SPCA precursor), 6) hypoproaccelerinemia (parahemophilia, deficiency of labile factor or plasma AcGlobulin), 7) hypo- or afibrinogenemia, and 8) circulating anticoagulant. The experimental data presented in Table II eliminate the last 6 possible diagnoses and suggest that the differential diagnosis lies between hemophilia and PTC deficiency. As Aggeler *et al.* have shown, this differentiation may be made by studying the ability of $BaSO_4$ normal plasma and the plasma fraction, PTC, to restore the coagulation defect. The plasma mixture recalcification test (Table III) proved a simple method for obtaining this differentiation.

The exact role of PTC in the coagulation mechanism has not been defined. Aggeler *et al.* chose the name PTC (plasma thromboplastin component) because tissue thromboplastin can substitute for or by-pass the role of PTC in clotting.

Summary. Five patients suffering from hemorrhagic diatheses manifesting prolongation of the whole blood coagulation time are presented. On the basis of the experimental evidence, 3 of these patients have been classified as PTC deficient and 2 as hemophilic.

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