

## Mild PTC (Plasma Thromboplastin Component) Deficiency Occurring in Two Brothers

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**A** PREVIOUSLY UNRECOGNIZED FACTOR, essential for the formation of blood thromboplastin and normally present in serum as well as plasma, was described recently.<sup>1, 2</sup> Aggeler, et al.<sup>1</sup> were the first to record findings in a patient lacking this factor, which they named plasma thromboplastin component (PTC). Later in the same year, Biggs, et al.<sup>2</sup> reported seven cases of a hemophilia-like disease. These patients were not deficient in the antihemophilic globulin (AHG) but lacked another factor also essential for thromboplastin

TABLE 1.—*Properties of Plasma Thromboplastin Component (PTC), Christmas Factor and the Antihemophilic Globulin (AHG)*

	PTC <sup>1</sup>	Christmas factor <sup>2</sup>	Antihemophilic globulin
Inheritance.....	Sex-linked recessive <sup>1,2</sup>	Sex-linked recessive <sup>2</sup>	Sex-linked recessive
Clinical manifestations of deficiency...	Like hemophilia	Like hemophilia	Hemophilia
Activity in plasma...	Present	Present	Present
Activity in serum...	Present	Present	Absent
Effect of prothrombin adsorbants.....	Readily adsorbed by Seitz filter, tricalcium phosphate, Al(OH) <sub>3</sub> and BaSO <sub>4</sub>	Readily adsorbed by Seitz filters and Al(OH) <sub>3</sub>	Not adsorbed
Effect of ammonium sulphate fractionation.....	Present in the 40-50% saturated fraction	Present in the 33-50% fraction	Precipitated by 25% saturation

formation. The English workers named this Christmas Factor (CF) after their first patient. From a comparison of the pattern of inheritance, the clinical manifestations and the similarities in the reported properties of PTC and CF (table 1), it now appears that these two factors are identical.

Hemophilia is now recognized<sup>4, 5</sup> as occurring in varying degrees of severity, from severe cases in which the blood clots only after many hours to a mild bleeding disorder with normal whole blood coagulation time and normal prothrombin consumption. Similar variations in the severity of PTC deficiency

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TABLE 2.—Reported Cases of PTC Deficiency and Christmas Disease

Author	Case	Age	Sex	Coag. time (min.)	Prothrombin consumption	Remarks
White, Aggeler, and Glendening <sup>3</sup> .....	1	16	M	30-135	Impaired	Coagulation defect corrected by normal serum, hemophiliac serum, or a partially purified preparation of PTC from serum
Biggs et al. <sup>2</sup> .....	1	5	M	10-15	Impaired	Serum deficiency demonstrated by the thromboplastin generation test. (Christmas disease)
Biggs et al.....	2	7	M	39-72	Impaired	Same as above
Biggs et al.....	3	14	M	14-16	Impaired	Same as above (sex linked recessive)
Biggs et al.....	4	6	M	13-16	Impaired	Same as above (sex linked recessive)
Biggs et al.....	5	28	M	28-45	Impaired	Same as above
Biggs et al.....	6	6	M	9	Impaired	Same as above
Biggs et al.....	7	21	M	7-10	Normal	Same as above
Rosenthal, Dreskin and Rosenthal <sup>11</sup> .....	D. R.	4	M	35-47	Impaired	Coagulation defect was not corrected by plasma from White, Aggeler, and Glendening's patient
Lewis and Ferguson <sup>12</sup> .....	E. W.	4	M	2-8 hrs.	Impaired	Coagulation defect corrected by PTC factor
Lewis and Ferguson.....	R. J.	16	M	>8 hrs.	Impaired	Coagulation defect corrected by PTC factor (sex linked recessive)
Lewis and Ferguson.....	Q. G.	40	M	24	Impaired	Coagulation defect corrected by PTC factor (sex linked recessive)
Van Creveld <sup>13</sup> .....	1	10	M	Several hrs.	Impaired	
Present.....	1	6½	M	14-20	Normal	Serum deficiency demonstrated by the thromboplastin generation test. Deficiency corrected by PTC
Present.....	2	13	M	14-21	Normal	Same as above

exist. In the thirteen reported cases in the literature (table 2), the severity of the bleeding disorder varied from that in a patient in whom the whole blood coagulation time exceeded eight hours to a mild case reported by Biggs, et al.<sup>2</sup> with normal whole blood coagulation time and normal prothrombin consumption.

It is the purpose of this paper to describe two additional cases of mild PTC deficiency, observed in brothers, in whom the whole blood coagulation time was

only slightly prolonged and prothrombin consumption was normal. In these patients, the coagulation defect was detected and studied by the thromboplastin generation test.<sup>7</sup>

### CLINICAL MANIFESTATIONS

#### Case 1

This boy, age 6½ years, was examined because of profuse bleeding following dental extractions. He had always bruised easily, although cuts stopped bleeding promptly. Following the extraction of sixteen teeth for severe caries, clots formed promptly in the tooth sockets. On the following day, however, oozing began under the original clots and bleeding became profuse on the second day after the tooth extractions. When he was admitted to the Pediatric Service, fifty hours after the dental extractions, his hemoglobin had decreased from 13 to 7.0 Gm. per cent. Following transfusion of 500 ml. whole blood, bleeding gradually ceased.

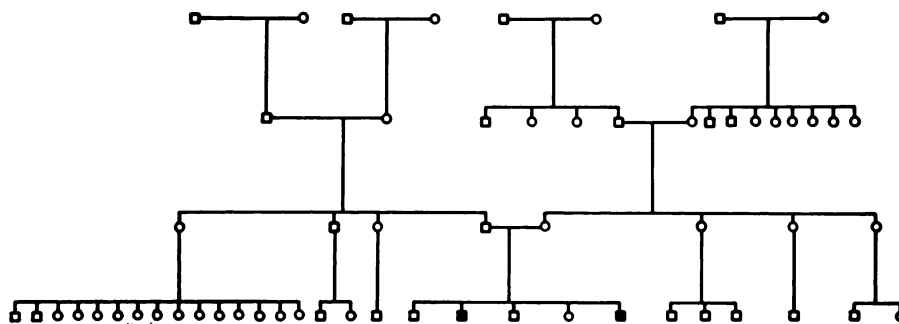


FIG. 1.—Family pedigree. □ Normal male. ○ Normal female. ■ Male bleeder.

#### Case 2

The brother of our first patient is 11 years of age. His bleeding disorder has been somewhat more severe than that of his younger brother, and on one occasion he developed a swollen, tender joint following trauma to the right knee. Although blood was not aspirated from the joint, this was assumed to be a hemarthrosis. He bruises easily and bleeds excessively following tooth extractions.

The three other siblings in this family, aged 8, 10, and 13 years, give no history of abnormal bleeding. All laboratory studies, including the thromboplastin generation test, have been normal in them as well as in the mother, father, and maternal grandmother. As determined by history there have been no other known bleeders in four generations of this family (fig. 1).

### METHODS

*Bleeding time* was measured by the method of Ivy,<sup>8</sup> using a stylet guarded at 2 to 3 mm. Tests of capillary fragility were performed by the application of a blood pressure cuff on the arm at a pressure midway between systolic and diastolic for 5 minutes.

The *coagulation time* of whole blood was determined by a modification of the Lee-White method in which three 11 × 75 mm. tubes are used. Two ml. of blood obtained by clean venepuncture are placed in each tube, and these are deposited in a 37 C. water bath. The first tube is partially inverted every 30 seconds until complete inversion is possible. The procedure is next repeated with the second tube and then the third. The recorded coagulation time refers to the time from venepuncture until the third tube can be completely inverted.

The *coagulation time of recalcified plasma* is the coagulation time observed following the addition of 0.1 ml. of 0.025 M  $\text{CaCl}_2$  to 0.1 ml. citrated plasma (1 vol. 3.8 per cent citrate to 9 vol. blood) obtained by centrifugation at 1000 rpm for 5 minutes.

The *one-stage prothrombin time* was determined by adding to 0.1 ml. oxalated plasma (1 vol. 0.1 M potassium oxalate to 9 vol. blood), 0.1 ml. 0.025 M  $\text{CaCl}_2$  and 0.1 ml. thromboplastin extract.\*

Serum prothrombin activity was measured by the two-stage method of Herz, et al.,<sup>9</sup> using alcohol to block antithrombin activity. The original procedure was modified by the use of prothrombin-free beef plasma as a source of fibrinogen for the thrombin titration. Sufficient prothrombin-free beef plasma† for this study was prepared and lyophilized in one lot. A portion of the plasma was reconstituted with distilled water immediately prior to each

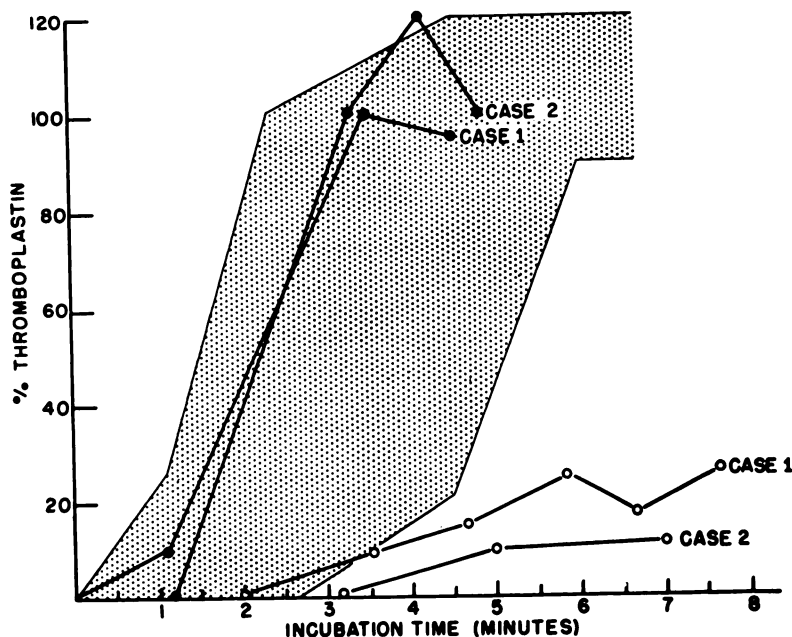


FIG. 2.—The curves represent the results of thromboplastin generation tests carried out with serum from cases 1 and 2 and normal  $\text{Al}(\text{OH})_3$ -treated plasma and platelets (○—○); and with  $\text{Al}(\text{OH})_3$ -treated plasma from cases 1 and 2 and normal serum and platelets (●—●). The shaded area represents the range observed in twenty-five normal tests.

determination. In twenty-six determinations on twenty normal subjects the prothrombin content of normal serum 1 hour after coagulation in glass tubes was found to range from immeasurable amounts to 12 per cent.

The *thromboplastin generation test*, as described by Biggs and Douglas,<sup>7</sup> was applied to serum and  $\text{Al}(\text{OH})_3$ -treated plasma from both cases. In our experience with this test, satisfactory curves can be obtained with platelet suspensions, substrate plasma and  $\text{Al}(\text{OH})_3$ -adsorbed plasma stored at  $-20^\circ\text{C}$ . for as long as ten days. In order to insure an adequate concentration of prothrombin, factor V and factor VII, for substrate plasma, great care was taken to use plasma with a one-stage prothrombin time of 15 sec. (control 13.0 to 15.5 sec.).

\* Bacto-thromboplastin, a rabbit brain thromboplastin prepared by Difco Laboratories.

† We are indebted to Dr. C. W. Christensen, Difco Laboratories, Detroit, Michigan for generously supplying this material.

The range of normal observed in twenty-five tests is shown in figure 2. The coagulation time representing 100 per cent thromboplastin varied from 13 to 19 seconds and for this reason a thromboplastin dilution curve was prepared each time the test was performed.

PTC was adsorbed by barium sulfate from normal serum.<sup>3</sup> This factor was eluted from the barium sulphate with a quantity of 5 per cent sodium citrate equal to one-half the original volume of plasma. This eluate was then dialyzed for 72 hours at room temperature against 0.9 per cent saline and the resulting dialysate was used as the stock solution. When tested by Owren's methods,<sup>10</sup> no prothrombin or factor VII activity could be detected in this preparation.

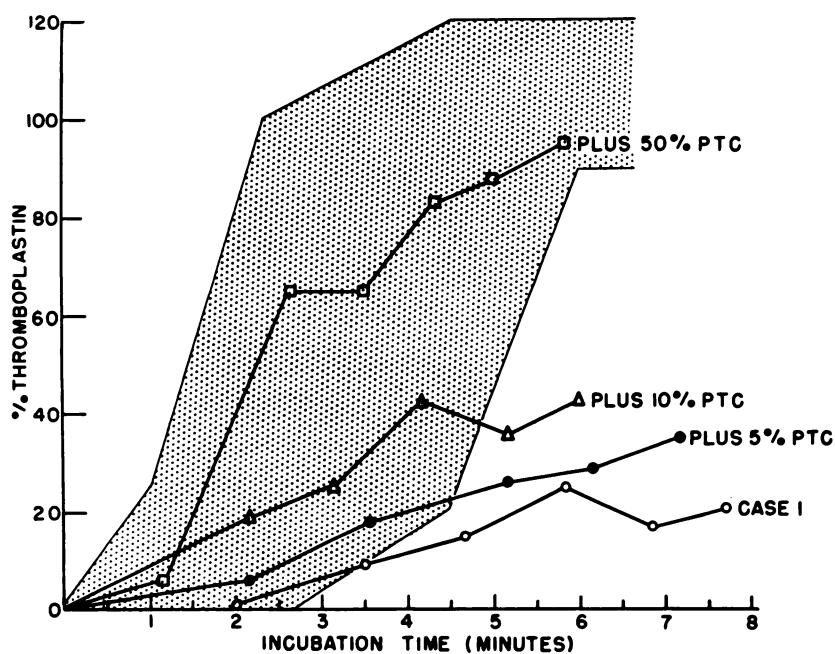


FIG. 3.—The curves represent the results of thromboplastin generation tests carried out with serum from case 1 and normal platelets and  $\text{Al}(\text{OH})_3$ -treated plasma (○-○); and mixtures of serum from case 1 with 5 per cent PTC (●-●), 10 per cent PTC (△-△) and 50 per cent PTC (□-□). The shaded area represents the range observed in twenty-five normal tests.

To determine whether or not the addition of PTC to serum from our patients would correct the serum deficiency, the following mixtures were made. The stock PTC solution was used undiluted and, in addition, was diluted 1:5 and 1:10 with saline. Of a 1:10 dilution of the patient's serum, 0.15 ml. was added to 0.15 ml. of the undiluted, 1:5, and 1:10 solutions of PTC to give final concentrations of 50 per cent, 10 per cent, and 5 per cent of the stock preparation of PTC, respectively. These mixtures were then tested by the thromboplastin generation test (fig. 3).

## RESULTS

The results of tests of the coagulation mechanism in our patients are shown in table 3. The platelets were normal in number and function. Morphologically they appeared normal on blood smears. Tests of capillary fragility, clot retraction,

tion, and bleeding time were also normal. The fibrinogen\* content was somewhat low. However, this did not appear to interfere with the coagulation mechanism since a firm, normally retractile clot was formed each time blood was drawn. The one-stage prothrombin time was normal in both cases and therefore the prothrombin, factor V, and factor VII content of the plasma may be assumed to have been within normal limits. In addition, prothrombin was converted to thrombin in a normal fashion during coagulation since, one hour after clotting, less than 1.25 per cent remained in the serum. Recalcified clotting times were within the limits of normal found by this method. The only suggestion of abnormality was in the coagulation time of whole blood which on several occasions was observed to be at the upper limits of normal or slightly prolonged.

The results of thromboplastin generation tests using the patients' serum and  $\text{Al(OH)}_3$ -treated plasma are shown in figure 2. The plasma of these two patients generated thromboplastin normally when incubated with normal platelets and serum, indicating that no deficiency of the antihemophilic globulin existed.

TABLE 3.—*Tests of Bleeding and Coagulation Function*

Test	Case 1	Case 2	Normal controls
Platelet count (per cu. mm.)	220,000	250,000	200,000-500,000
Bleeding time (Ivy) (min.)	3	2.5	1-5
Coagulation time (Lee-White, 3rd tube) (min.)	14-20	14-21	8-14
Recalcified clotting time (seconds)	128	110	70-120
Fibrinogen (mg. %)	130	156	180-300
One-stage prothrombin time (seconds)	15.5	15.3	15.0
Serum prothrombin 1 hr. after clotting (%) <sup>9</sup>	<1.25	<1.25	<1.25 to 12

Their serum, however, was deficient in a factor necessary for the rapid generation of a potent thromboplastin.

The serum deficiency observed in these two patients was modified considerably by the addition of various amounts of a partially purified preparation of PTC derived from normal serum.<sup>3</sup> In case 1, thromboplastin generation became normal with the addition of 50 per cent PTC (fig. 3). The serum deficiency could also be corrected by the addition of 50 per cent normal serum but not by  $\text{BaSO}_4$  adsorbed serum.

Plasma and serum from case 2 were sent to Dr. Paul Aggeler. The addition of 5 per cent of either plasma or serum from our patient to plasma from his patient with PTC deficiency<sup>1, 3</sup> failed to alter the residual serum prothrombin. On the other hand, normal plasma in a concentration of 5 per cent reduced the residual serum prothrombin from 53 to 4 per cent and, even in a concentration of 0.6 per cent, decreased the residual serum prothrombin to 32 per cent.

In summary, the routine tests of the coagulation mechanism were essentially normal in our two patients except for slight prolongation of the coagulation time of whole blood. Thromboplastin generation studies, on the other hand,

\* We are indebted to Dr. B. V. Jager of this Department, for the fibrinogen determinations. These were carried out by a modification of the method of Morrison (J. Am. Chem. Soc. 69: 2723, 1947).

revealed a deficiency of a serum factor. This serum deficiency could not be corrected by BaSO<sub>4</sub> adsorbed serum, but was significantly decreased by the addition of normal serum or a partially purified preparation of PTC.

#### DISCUSSION

In the first year following the original description of PTC deficiency,<sup>1</sup> thirteen cases have been described (table 2). In addition, we report here two cases. It appears very probable that a re-evaluation of some of the cases previously considered to be hemophilia and re-examination by means of the thromboplastin generation test of cases with a mild bleeding disorder in which no abnormality has been detected, will reveal a number of unrecognized cases of PTC deficiency.

Like hemophilia, PTC deficiency appears to be inherited through a sex-linked recessive gene.<sup>2, 12</sup> Cases hitherto described have all been in males. However, it is probable that the disease will be discovered in a homozygous female. The bleeding manifestations in this disease are identical with those of hemophilia. Excessive bleeding can be controlled by the transfusion of variable amounts of whole blood or plasma but not by administration of Cohn's fraction I.<sup>3</sup> On the basis of theoretic considerations, the administration of normal serum would appear to be good treatment.

Although PTC deficiency cannot be differentiated from hemophilia on the basis of the pattern of inheritance, the sex incidence, or the clinical manifestations, the laboratory establishment of the diagnosis is not difficult. In cases manifesting a prolonged whole blood and recalcified clotting time, the differentiation can be made by testing for the distinctive properties of PTC and AHG listed in table 1. The coagulation defect in PTC deficiency is corrected by normal or hemophilic plasma or serum but not by plasma or serum treated with various prothrombin adsorbants, such as barium sulphate or aluminum hydroxide, since these agents remove PTC. The coagulation defect in hemophilia, on the other hand, is corrected by normal or PTC deficient plasma or by plasma treated with prothrombin adsorbants. It is not corrected by serum.

The thromboplastin generation test<sup>7</sup> is very sensitive in detecting a deficiency of AHG or PTC. Hemophilia is due to the deficiency of a factor required in thromboplastin generation which is normally present in Al(OH)<sub>3</sub>-treated plasma, whereas in PTC deficiency a serum factor is lacking. Mild cases of hemophilia and PTC deficiency, in which the whole blood, recalcified clotting times and prothrombin consumption are normal, can be differentiated readily by the use of this test.

#### SUMMARY

1. Thirteen cases of plasma thromboplastin component (PTC) deficiency or Christmas disease are reviewed and summarized. Of these cases, only in one were the whole blood coagulation time and prothrombin consumption normal.

2. Two cases of a mild bleeding disorder, occurring in brothers with slightly prolonged whole blood coagulation times and normal prothrombin consumption are described.

3. In these two cases, the thromboplastin generation test revealed the deficiency of a serum factor essential for normal thromboplastin generation. A

mild deficiency of PTC was demonstrated by the correction of the serum deficiency by the addition to the patients' serum of a partially purified preparation of PTC.

4. The differentiation of PTC deficiency from hemophilia is discussed.

5. Mild bleeding disorders due to a moderate reduction of antihemophilic globulin (AHG) or PTC can be differentiated by the use of the thromboplastin generation test.

#### ADDENDUM

Since this paper was prepared three additional reports have appeared. Beaumont, et al.<sup>14</sup> studied a boy of 3 years with markedly prolonged coagulation time (35 to 210 minutes); Cramer, et al.<sup>15</sup> have reported two cases, a boy of 6 with coagulation time of 7 to 10½ minutes and another of 19 years with a coagulation time of 9 to 18 minutes. Soulier and Larrieu<sup>16</sup> described four cases, males, 12 to 20 years of age with markedly prolonged coagulation times. In all of the cases prothrombin consumption was impaired and other findings were consistent with those already discussed as characterizing PTC deficiency. Since this disorder cannot be distinguished from classical hemophilia on clinical grounds and since both diseases have the same hereditary pattern, Cramer, et al. and Soulier and Larrieu suggest that PTC deficiency be called hemophilia B to distinguish it from the classic disorder, hemophilia A. In this we agree.

#### SUMMARIO IN INTERLINGUA

(1) Es presentate un revista e summario de 13 casos del morbo Christmas, i.e., de deficientia del componente thromboplastinic del plasma. Le tempore de coagulation del sanguine integre e le consumption de prothrombina esseva normal in solo un de iste serie de casos.

(2) Es describe duo casos (occurrente in fratres) de un leve disordine sanguinante con leve prolongation del coagulation de sanguine integre e un consumption normal de prothrombina.

(3) In iste duo casos le essayo de generation de thromboplastina revelava un deficientia in un factor del sero le qual es essential pro le generation normal de thromboplastina. Un leve deficientia in le componente thromboplastinic del plasma esseva demonstrate per le facto que le deficientia in le sero del patientes poteva esser corrigite per le addition a iste sero de un partialmente purificate preparation del componente thromboplastinic del plasma.

(4) Es discute le differentiation inter hemophilia e deficientia del componente thromboplastinic del plasma.

(5) Leve disordines sanguinante, que es debite a un leve reduction de globulina antihemophilic o del componente thromboplastinic del plasma, pote esser differentiate ab hemophilia per le uso del essayo de generation de thromboplastina.

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