A Possible New Thromboplastin Deficiency Occurring in Five Siblings

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THE INCREASING USE of the thromboplastin generation test has revealed instances of faulty coagulation in which all other tests have failed to demonstrate any abnormality. The purpose of the present report is to describe a defect in coagulation which has not been reported previously and which was only demonstrated by means of this test. We have studied five siblings in a family, all of whom have had excessive bleeding following surgical procedures, and two of whom have developed bleeding of an alarming nature requiring hospitalization. In addition, a sixth sibling has a suggestive history of bleeding, but is not available for study. The parents have also been investigated and are normal. The determinations of the clotting times and the prothrombin consumption tests were normal in all of the patients on whom they were done and it was not until the thromboplastin generation test was performed that the nature of the defect was discovered. The factor which is deficient in this family is present in normal serum, it has been proven not to be PTC,[6, 7, 8] PTA,[9, 10] Hageman factor[11, 12] or Stuart factor[17, 18] by cross-correction studies and it does not have the properties of Duckert's factor X.[15] We cannot differentiate it from the factors described by Grieg et al.[16] since they do not give a detailed account of the properties of their factors.

Methods

Standard methods were used for the platelet counts, clot retractions and the venous coagulation times. Prothrombin was measured by the one-stage method of Quick.[2] Serum Prothrombin was measured one hour after coagulation by the method of Stefanini.[3] The thromboplastin generation test was performed by a previously described modification of the method of Biggs and MacFarlane.[4, 5]

Case Histories

The family consists of the parents and six children, of whom four are women and two men. The parents are both from Italy; they had the same last name and come from adjoining towns. However, they deny consanguinity. Neither parent has experienced any bleeding difficulty.

Case 1. P. P., a 34 year old white woman, had no bleeding difficulty until the age of 18 years, at which time she developed a severe hemorrhage eight days after a tonsillectomy. Bleeding was controlled without transfusion, and she remained asymptomatic for five years. At 23 years of age a severe post-partum hemorrhage occurred seven days after an uneventful full term delivery. The bleeding ceased following the insertion of a vaginal pack and a transfusion of 500 ml. of stored whole blood. Two subsequent deliveries were without difficulty.

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At the age of 31 years she developed menometrorrhagia for which dilatation and curettage and a cervical biopsy were performed. On the tenth postoperative day severe vaginal hemorrhage occurred and was controlled by a vaginal pack. Prothrombin time, bleeding time, venous clotting time and platelet counts were normal at that time. On the twentieth postoperative day she was admitted in shock due to vaginal hemorrhage. Despite numerous transfusions of whole blood, insertion of vaginal packs and ligation of both deep hypogastric arteries, the bleeding continued and necessitated a total hysterectomy. Examination of the uterus and cervix failed to show a local cause for the hemorrhage.

A laparotomy performed at 32 years of age was not associated with excessive hemorrhage; preceding and during the operation she received stored whole blood. She has not had any recent difficulty except that she has prolonged oozing from small lacerations.

She has three children—11, 8 and 3 years of age. The 8 year old boy had bleeding following a tooth extraction which required packing. None of the other children has evidenced any bleeding tendencies.

Case 2. M. D., a 42 year old white woman, was well until the age of 19 years when she bled excessively following the extraction of a tooth. At the age of 26 she bled profusely on the first and fourth day following a tonsillectomy. No further difficulty occurred until the age of 37 when she developed vaginal bleeding four days after a dilatation, curettage and cervical biopsy. Bleeding and clotting times were normal. She received 1500 ml. of whole blood and a vaginal pack was inserted, following which there was cessation of the bleeding. She has remained well since. The cervical biopsy revealed only chronic cervicitis.

The patient's daughters—age 17, 15 and 9 years—have had no bleeding difficulty.

Case 3. R. M., a 29 year old woman, had a tonsillectomy without hemorrhage at the age of 12 years. At 20 years of age she developed pulmonary tuberculosis for which a therapeutic pneumothorax was performed. This was followed in a few days by a hemothorax on the same side. She received one transfusion of whole blood following which there was no further bleeding.

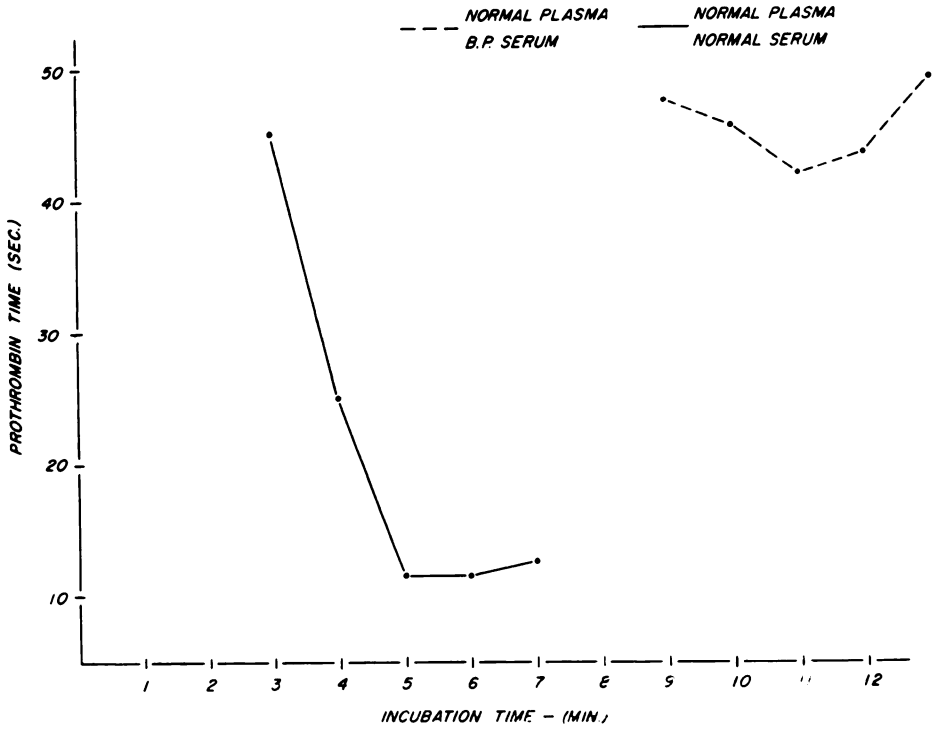

FIG. 1.—The effect of substituting serum from B.P. for normal serum in the Thromboplastin generation test.

About four years later, at the age of 25, following the excision of a nodule in the breast, she had excessive loss of blood which necessitated the administration of 1000 ml. of whole blood. Her menstrual periods have been normal, and she has had no further difficulty.

Case 4. B. P., a 36 year old woman, had no difficulty from bleeding until approximately four years ago when she developed severe epistaxis. This has become increasingly frequent, and two to four such episodes occur weekly. Occasionally these are severe enough to require nasal packs. She has been examined repeatedly during and between attacks without finding a local site of hemorrhage. Her blood pressure is normal. She has noted a tendency to bleed excessively from minor lacerations during this same period of time. Recently her menses have become abnormally long with excessive flow. Bleeding time, clotting time, and prothrombin times obtained during periods of active nasal bleeding have been normal.

She has two sons, 11 and 8 years of age. The older child has shown petechiae around the neck when he vomits. The younger son bled following simple extraction of a tooth and required closure of the wound by sutures to effect hemostasis. Both children have normal prothrombin consumption and thromboplastin generation tests.

Case 5. J. C., a 40 year old man, bled at the age of 21 on the second day following tonsillectomy. This was controlled by local measures. At the age of 35 he had an episode of hemoptysis. Bronchoscopic examination and roentgen films of the chest obtained then and during the following six months failed to reveal the cause of this hemoptysis. No further episodes of hemoptysis have occurred, and there have never been other manifestations of a hemorrhagic tendency.

Case 6. C. C., a 30 year old white man, has had one episode of hemorrhage from a tooth socket at 19 years of age. This stopped after the gum was sutured. No further hemorrhagic episodes have occurred. He has not been available for study.

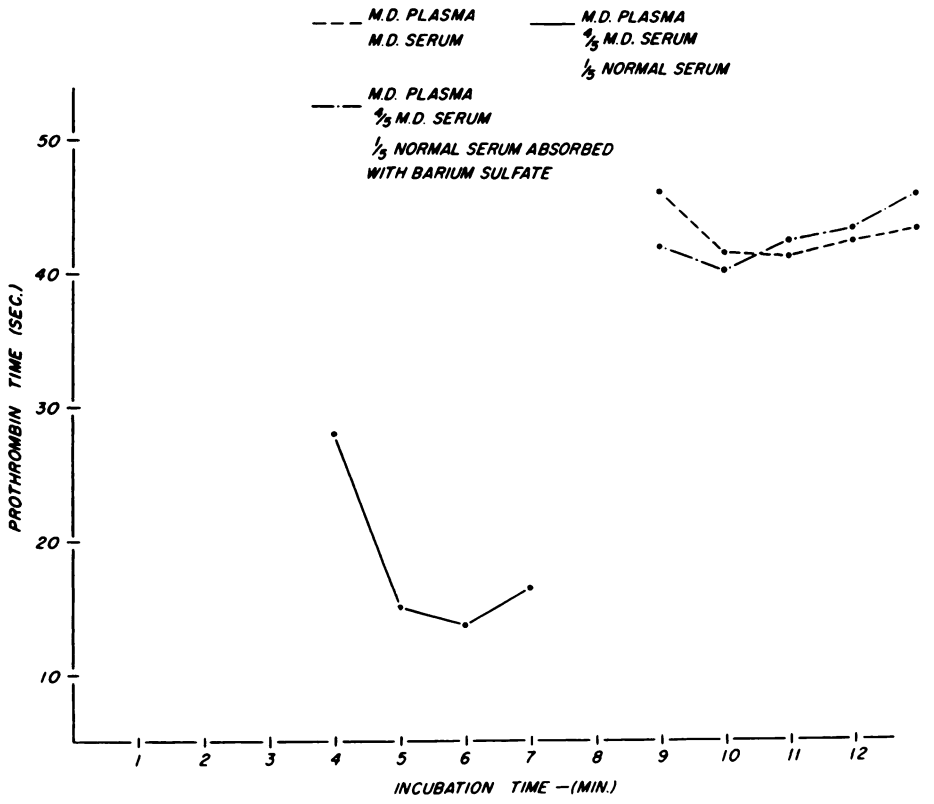

FIG. 2.—The effect of normal serum and of serum absorbed with barium sulfate upon the thromboplastin generation test of plasma and serum from M. D.

Results

Coagulation Studies

Normal values were obtained for the bleeding times, clotting times and prothrombin times of all patients. Patients P. P. (case 1) and M. D. (case 2) have had normal platelet counts. Serum prothrombin times were determined on patients P. P., M. D., and R. M. (cases 1, 2 and 3): less than 10 per cent of residual serum prothrombin was present in each instance.

The thromboplastin generation test was performed with fresh plasmas and serums which were drawn simultaneously from five members of the family (cases 1–5). These tests were uniformly abnormal and revealed poor generation in regard to both rate and total amount of thromboplastin formed. When the serums from patients 1 and 2 were substituted for normal serum, the generation of thromboplastin from normal plasma was similarly poor. This is shown in fig-

TABLE 1.—*Clotting Time of Normal Blood Diluted with Car. Serum and with Saline*

Ml. Normal Blood	Saline	Car. Serum	Clotting Time	Serum Prothrombin Time (Sec.)
1	0	0.1 ml.	3′ 30″	47
1	0.1 ml.	0	4′	49
0.5	0	0.5 ml.	6′ 30″	54
0.5	0.5 ml.	0	6′	57

TABLE 2.—*Results of the Thromboplastin Generation Test Using the Mixtures Described*

The results are expressed as coagulation time in seconds

Reaction Mixtures	Incubation Time min.						
	3	4	5	6	7	8	9
A. Normal plasma							
Car. (M. D.) serum	>50	>50	>50	>50	>50	>50	>50
B. Normal plasma							
⅘ vol. Car. serum plus:							
1. ⅕ vol. normal serum (average of 12)	>50	28	15.2	14.2	15.8		
2. ⅕ vol. 0–25% $(NH_4)_2SO_4$ precipitate from serum	>50	32	23	20.5	20.5	21	
3. ⅕ vol. 0–33% $(NH_4)_2SO_4$ precipitate from serum	>50	35	26	22	20	23	
4. ⅕ vol. 33–40% $(NH_4)_2SO_4$ precipitate from serum	>50	>50	46	26	26	28	
5. ⅕ vol. 40–50% $(NH_4)_2SO_4$ precipitate from serum	>50	>50	>50	>50	>50	40	42
6. ⅕ vol. serum deficient in PTA	>50	37	21	16.4	16.2	17.2	
7. ⅕ vol. normal serum heated to 60 C. for 3 min.	>50	>50	>50	>50	46	42	40
C. PTC plasma							
⅘ vol. serum deficient in PTC plus:							
1. ⅕ vol. normal serum	39.2	23	17.6	19	19.8		
2. ⅕ vol. Car. serum	37.6	25.2	19.4	21.6			

ure 1. Interchanging serums and plasmas between different siblings failed to correct the defective thromboplastin generation. The addition of one-fifth volume of normal serum to serums and plasmas from cases 1 and 2 resulted in marked improvement in the generation of thromboplastin. However, the addition of normal plasma which had been absorbed with barium sulfate failed to correct the defective thromboplastin generation. The deficient factor, therefore, is present in serum but not in plasma which has been treated with barium sulfate. Similarly, absorption of normal serum for 30 minutes at 37 C. with barium sulfate removed the correction factor as evidenced by adding such serum to the serum of members of the family. This is shown in figure 2. Dilution of normal blood with serum from members of this family failed to prolong the coagulation time or change the prothrombin consumption of the normal blood as compared to the same normal blood similarly diluted with saline. These results are shown in table 1. The factor was present in all fractions precipitated from serum by ammonium sulfate, but was maximal in the 0–33 per cent saturated fraction. The factor has been found to be stable after storage at room temperature for 12 days and is not reduced by 11 days of Dicumarol therapy sufficient to produce a one-stage prothrombin of 17 per cent. It is destroyed by heating for three minutes at 60 C. These results are illustrated in table 2 and figure 3. These results differentiate this deficiency from those of antihemophiliac globulin, plasma thromboplastin antecedent and Hageman factor deficiency, since AHG is not present in serum

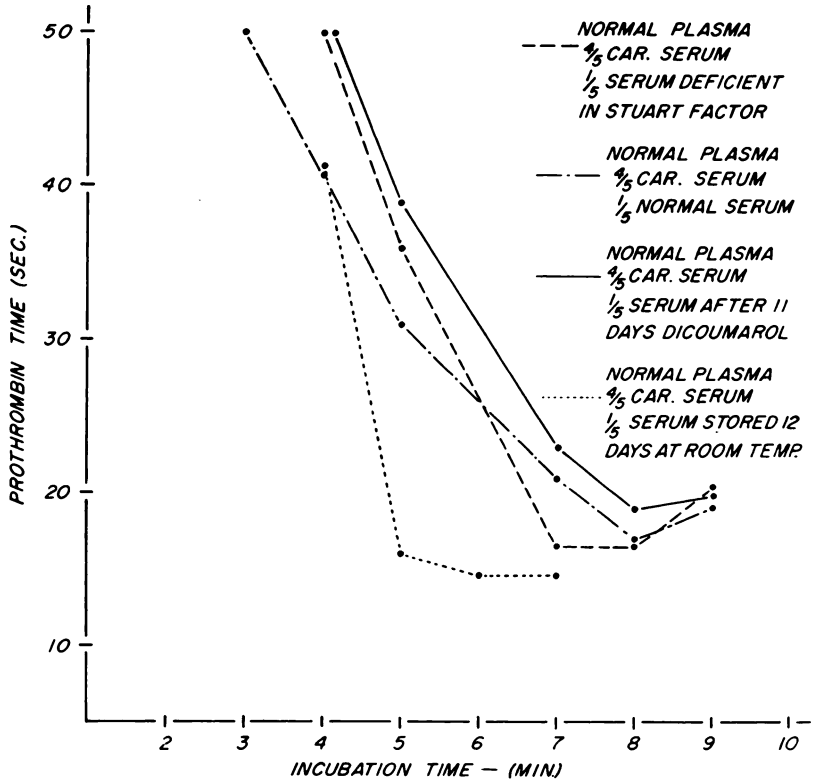

FIG. 3.—The effect of substituting various sera upon the thromboplastin generation of normal plasma and Car. serum.

TABLE 3.—*Recalcified Clotting Time of 0.1 ml. Hageman Plasma and 0.1 ml. Test Serum**

	Clotting Time (min.)
Normal serum, dil. 1:80	6,6
Car. serum, dil. 1:80	7,7
Buffer control	23,25

* Courtesy of Dr. Oscar Ratnoff.

and both PTA and Hageman factor are not absorbed by barium sulfate. They also demonstrate that the coagulation defect is not due to an anticoagulant in the blood of members of the family since addition of plasma or serum from members of the family did not retard the coagulation of normal blood as compared to blood diluted similarly with saline.

Studies of Known Coagulant Factors

1. Plasma thromboplastin component:[6, 7, 8] The prothrombin consumption and thromboplastin generation of blood from a patient* known to be deficient in PTC were corrected as well by serum from M. D. (case 2) as by similar amounts of normal serum. This is shown in table 2.

2. Plasma thromboplastin antecedent:[9, 10] The thromboplastin generation of blood from a patient* known to be deficient in PTA was corrected as well by serum from M. D. (case 2) as by normal serum in like amounts. This is shown in table 2.

3. Hageman factor:[11, 12, 13] Serum from patient M. D. (case 2) was sent to Dr. Oscar Ratnoff who very kindly tested it against that of a patient with deficiency of the Hageman factor. A normal amount of the Hageman factor was present as judged by the correction produced by serum from M. D. and by normal serum upon the recalcification time of plasma deficient in the Hageman factor. This observation is compatible with our finding of absorption of the factor by barium sulfate. These results are shown in table 3.

4. Stuart factor:[17, 18] Lyophilized serum deficient in Stuart factor was obtained through the courtesy of Dr. John Graham. When $\frac{1}{5}$ volume of this serum was added to Car. serum in the thromboplastin generation test using normal plasma, the correction produced was similar to that produced by normal serum. This is illustrated in figure 3.

DISCUSSION

The family described here possesses a hemorrhagic tendency which is mild when compared to classic hemophilia, but which has been associated with bleeding following tonsillectomy in two patients and following extraction of teeth in two others. It has been associated with severe vaginal hemorrhage and abnormal menses in three of four affected women.

The affected members of the family who have been examined have normal

* We are indebted to Dr. Irving Waldow of Albert Einstein Hospital for allowing us to use these patients (patient with PTA also).

venous coagulation times, normal prothrombin times and normal prothrombin consumption tests. The defect is only demonstrable by means of the thromboplastin generation test. The factor responsible for this deficiency has been shown to be present in serum, to be absorbed by barium sulfate and to be labile on heating to 60 C. for three minutes. It is present in all fractions precipitated from serum by ammonium sulfate, but is present maximally in the 0–33 per cent fraction.

It is destroyed by heating to 60 C. for three minutes but is stable on storage at room temperature for 12 days. It is not reduced by 11 days of Dicumarol therapy. The defective coagulation has been shown not to be due to an anticoagulant. The presence of the factor in serum but not in barium sulfate–treated plasma or serum separates this factor from AHG, PTA, or the Hageman factor Moreover, serums from our patients have corrected the defect in the blood of patients known to be deficient in PTC, PTA, Stuart factor and the Hageman factor.

The fourth thromboplastin factor of Spaet[14] is not absorbed by barium sulfate and is thus different from the factor described here.

Duckert's factor X[15] is reduced by small amounts of Dicumarol and disappears from serum after storage at room temperature for ten days; whereas the factor described here is not influenced by Dicumarol and is stable for 12 days at room temperature.

Grieg et al.,[16] by cross-correction studies, have identified three serum factors. In one such factor the one-stage prothrombin time was also prolonged suggesting that they were dealing with the Stuart factor. The properties of their "factors" are not fully described as to storage, ammonium sulfate fractionation, etc., so that comparison is difficult. In addition, unlike the factor described herein, factor C is reduced by small amounts of Dicumarol. Until cross-correction studies can be performed or more information about their factors is available, we are not able to state whether or not we are dealing with one of the factors which they described.

The inheritance of this defect is not entirely clear. Since the parents bear the same last name and come from adjoining towns in Italy, it was believed initially that this was a recessive gene manifested in homozygous offspring of a consanguineous union. The proved occurrence of the defect in five of six children (and suggestively by history in the sixth) militates against this hypothesis. The problem was presented to Dr. Howard Levene of the Department of Mathematical Statistics at Columbia University who stated that the likelihood of this occurring in five of six siblings as a result of the homozygous inheritance of a recessive characteristic was .00098 and that this hypothesis could be rejected at the 0.1 per cent level. It is possible that the deficiency is inherited from one parent alone. Blood from both parents was examined and showed normal clotting times, normal prothrombin consumption and normal thromboplastin generation. Blood from two of the children of B. P. (case 4) has been studied and is normal.

The significance of the factor described in the mechanism of coagulation is undetermined. Owing to usual mildness of the symptoms, it is unlikely that it is as important as AHG or PTC. It would appear to be active as a thromboplastin

factor since the prothrombin has been repeatedly normal. Since it is possible that further studies may reveal other examples of this defect, lyophilized or frozen serum can be made available to those interested in testing it against other unusual deficiencies.

We have temporarily named this factor the Car. factor, an abbreviation of the family name of the persons in whom it has been found to be deficient.

Conclusions

1. A family with 6 siblings, four women and 2 men, is described in whom there is a mild coagulation defect characterized by the presence of abnormal thromboplastin generation.

2. Physical characteristics and cross-correction studies of this factor are described.

3. This defect appears to be unique and has not been described previously.

4. The defective factor has been temporarily named the Car. factor.

Summario in Interlingua

1. Es describite un familia con 6 fraternos—4 feminas e 2 homines—in qui il existe un leve defecto coagulatori characterisate per le presentia de un anormal generation de thromboplastina.

2. Es describite le characteristicas physic del factor in question, insimul con studios de correctibilitate reciproc.

3. Il pare tractar se de un defecto unic non previemente describite.

4. Le factor defective ha provisorimente essite designate como "factor Car". Isto es un abbreviation del nomine de familia del individuos in qui le defecto esseva studiate.

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