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Inherited haemorrhagic disease with abnormal prothrombin consumption

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SUMMARY. The propositus is a 4-year-old boy who presented with a history of excessive bleeding after surgical procedures as well as haematomas and epistaxis. The defect in haemostasis consisted in an anomaly of the prothrombin consumption tests as the only abnormality while all the other conventional coagulation and fibrinolysis tests as well as platelet function tests were normal. The father of the propositus had no previous history of excessive bleeding but was found to have an abnormal prothrombin consumption index. The reaction to prothrombin conversion, normal at onset, slowed down to less than normal and did not reach completion until 24 h. The *in vivo* studies suggest that the effect does not act on the interaction between platelet phospholipid and plasma. The factor II dosage and the electrophoretic mobility of prothrombin of the plasma were normal; nevertheless when studying the purified prothrombin by means of crossed immunoelectrofocusing there appeared an anomaly of pI. This result suggests the possible existence of an abnormal prothrombin molecule responsible for a slow prothrombin conversion.

Robinson *et al* (1967) studied a family presenting with a haemorrhagic disorder not described until then which proved normal in all coagulation screening tests except the prothrombin consumption test. The defect was thought to be due to an increase of factor Xa inactivation.

Later, Parry *et al* (1980) studied 10 individuals from three families suffering a defect similar but not identical to that described by Robinson *et al* (1967). From results obtained in laboratory studies and therapeutic assays it was suggested that the anomaly acted at the level of the interaction between platelet phospholipid and plasma, possibly due to a defect of γ-carboxy-glutamic residues of one of the vitamin K dependent clotting factors, although no functional alteration of these factors could be shown.

In this study we present the case of a patient with a genetically determined haemorrhagic disease characterized by normal results in all coagulation screening tests and platelet reactions, except in the prothrombin consumption test.

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PATIENT

The patient studied is a 4-year-old male who, from earliest infancy, presented with a history of frequent spontaneous haematomas after minimum trauma and sporadic epistaxis. Throughout his life the patient had presented with three episodes of profuse haemorrhage lasting several days following minor lesions. At age 4, after a tonsillectomy, he presented with a severe haemorrhage of a week's duration requiring an emergency tracheotomy and administration of 5 units of erythrocyte concentrate. At this time he was transferred to our centre for study.

The patient's father had no history of haemorrhagic tendency but blood studies confirmed the same anomalies *in vitro* with a prothrombin consumption index of 74%. The mother had no haemorrhagic history nor laboratory alterations. The patient's only brother had died at age 8 from a cerebral haemorrhage following a slight trauma.

METHODS

Conventional coagulation tests were measured using standard techniques (Biggs, 1976). Reptilase time was tested by the method of Straub & Funk (1971); quantitative assays of factors II, V, VII, VIII, IX, X, XI and XII (Hardisty & Ingram, 1965); factor XIII as described by Bohn & Haupt (1968); clot retraction according to the Hardisty & Ingram method (1965) platelet adhesiveness by the Salzman method (1963); platelet aggregation by the Born method (1962); platelet factor 3 availability as described by Hardisty & Hutton (1966); antithrombin III by the method of Abilgaard *et al* (1977) and the antifactor Xa activity by the method of Ødegard *et al* (1976); euglobulin lysis time by the von Kaulla & Schultz method (1958); fibrinogen degradation products as described by Merskey *et al* (1969); α_2-antiplasmin by the Teger-Nilsson *et al* method (1977); Von Willebrand factor by the method of Levy-Toledano & Bellanger (1975). Factor VIII related antigen and fibrinogen was determined by the method of Laurell (1966) and the electrophoretic mobility of both by Clarke & Freeman technique (1968); fibrin monomer aggregation by the Gruendlinger & Bouvier method (1964).

The prothrombin consumption index (PCI) was measured by the method of Parry *et al* (1980). Factor II was determined by the two-stage Biggs & Douglas method (1953), and using Taipan viper venom as thromboplastin according to Denson *et al* (1971), *Notechis scutatus scutatus* venom according to Jobin & Esnouf (1966) and *Echis carinatus* venom as described by Girolami *et al* (1975). Quantitative immunologic factor II assay was carried out according to the Laurell method (1966). Blood thromboplastin decay rate and factor Xa inactivation were measured by the method described by Parry *et al* (1980).

Two-dimensional crossed immunoelectrophoresis of plasma and serum was performed (Clarke & Freeman, 1968) using rabbit anti-human prothrombin antiserum obtained from Behringwerke, A.G. The prothrombin of plasma was purified by affinity chromatography according to the following method: 3 ml of rabbit anti-human prothrombin antiserum were coupled with 16 g of CNBr-activated Sepharose 4B and this mixture was packed in a column of $0{\cdot}9 \times 14$ cm; 3 ml of plasma were eluted from the column using as eluent buffer phosphate

0·1 M, 0·15 M ClNa, pH 7·4; once the first peak of elution was discarded, the prothrombin fixed to the antiserum of the column was eluted with glycine 0·1 M, pH 2·5, therein appearing a second peak of elution which was immediately adjusted to pH 7·8. Crossed immunoelectrofocusing (Soderholm *et al*, 1977) was carried out with the prothrombin purified in plasma using Pharmalyte pH interval 3·5–10 and rabbit anti-human prothrombin antiserum.

RESULTS

All tests shown in Table I gave normal results. Factor VIIIR: Ag, factor VIIIR:WF and electrophoretic mobility of factor VIII, fibrinogen assays, thrombin time, reptilase time, aggregation of fibrin monomers in plasma and electrophoretic mobility of the patient's fibrinogen were normal.

Prothrombin assays and prothrombin consumption tests

Prothrombin assays using various methods were performed on patient's plasma and serum samples separated 1 and 48 h, at 37°C, after clot formation. Normal plasma and serum

Table I. Routine coagulation study

	Patient	Normal range
Bleeding time (min)	3	2–8
Whole blood clotting time (min)	7	4–12
Platelet count ($\times 10^9$/l)	300 000	150 000–400 000
Recalcification time (s)	122	118
Prothrombin time (s)	12	12
Activated partial thromboplastin time (s)	41	40
Factor V (%)	120	60–150
Factor VII (%)	105	60–150
Factor VIII (%)	100	60–150
Factor IX (%)	85	60–150
Factor X (%)	95	60–150
Factor XI (%)	100	60–150
Factor XII (%)	95	60–150
Factor XIII (%)	80	60–150
Clot retraction	Normal	
Platelet adhesiveness (%)	75	40–80
Platelet aggregation (ADP, adrenalin, collagen and ristocetin)	Normal	
PF3a (%)	90	>30
Antifactor Xa activity (%)	105	60–150
Antithrobin III (%)	100	60–150
Euglobulin lysis time (min)	270	>180
Fibrinogen degradation products (μg/ml)	5	<10
α_2-Antiplasmin (%)	140	60–150

samples were similarly tested. The results are shown in Table II. It can be seen that the prothrombin level in the plasma of the patient was normal. Nevertheless, serum separated at 1 h still contained large quantities of prothrombin, while prothrombin consumption was total at 48 h.

The prothrombin consumption index (PCI) was measured on five separate occasions and was intensely abnormal each time with values varying between 65% and 90%. Normal values of PCI were always below 20%. Prothrombin consumption tests (PCT) were made on platelet rich plasma (PRP) and the results are shown in Table III where it can be observed that the patient's PCT was abnormal. It was also possible to demonstrate an inhibiting effect when a fifth part of the patients's PRP was added to the normal PRP before performing the PCT. Similar results were obtained when PRP was substituted for platelet poor plasma (PPP) to which Bell and Alton platelet substitute was added at a dilution of 1/500.

Table II. Prothrombin assays

Method	Plasma		Serum separate at 1 h		Serum separate at 48 h	
	Patient	Normal	Patient	Normal	Patient	Normal
Classical one-stage method	100	100	28	0	0	0
Two-stage method	90	100	32	0	0	0
Taipan viper venom method	105	100	23	0	0	0
N. scutatus scutatus method	110	100	30	0	0	0
E. carinatus method	85	100	27	0	0	0
Immunologic method	120	100	—	—	—	—

Table III. Prothrombin consumption tests on platelet rich plasma

		10 min incubation	20 min incubation
Control PRP	Serum (s)	42	123
	Plasma (s)	14	14
Patient's PRP	Serum (s)	11	16
	Plasma (s)	13	13
1/5 Patient's PRP + 4/5 Control PRP	Serum (s)	21	41
	Plasma (s)	14	14

Thromboplastin decay rate and factor Xa inactivation

Thromboplastin decay rate was measured on three different occasions and results obtained in the patient's blood were always normal as compared to those of the control.

Factor Xa inactivation was studied on two occasions but no differences were found in the results obtained from the patient's plasma with respect to the control plasma.

Immunological studies

Two-dimensional crossed immunoelectrophoresis demonstrated no prothrombin abnormality in the patient's plasma (Fig 1). The same study in normal serum at 10 min and at 1, 6, 18 and 24 h after clot formation (Fig 1) showed that in normal serum two peaks appear at 10 min, one with faster mobility and the other with slower mobility than the prothrombin plasma corresponding to two prothrombin derivative products. These two products persist up to 24 h after coagulation is produced. However, in the patient's serum, besides the two peaks corresponding to the two prothrombin derivative products, there appeared a third peak of intermediate mobility, identical to that of the prothrombin plasma which is present up to the time of the 18 h sample and disappears 24 h after coagulation is produced. These results suggest that prothrombin conversion is initiated in a normal manner in the patient but is

Fig 1. Two-dimensional crossed immunoelectrophoresis of patient's serum (P) and normal serum (N) against antiprothrombin serum. Samples obtained at 0, 10, 60 min and 6, 18, 24 h after clotting.

produced at a much slower rate and is not completed until 24 h after coagulation is begun, while normally this completion occurs at 10 min.

The study of plasma prothrombin purifed through crossed immunoisoelectrofocusing showed that while in normal prothrombin a single peak corresponding to a pI 4·7 appeared, the patient's prothrombin presented three separate peaks corresponding to pI 4·2, 4·7 and 5·0 respectively (Fig 2).

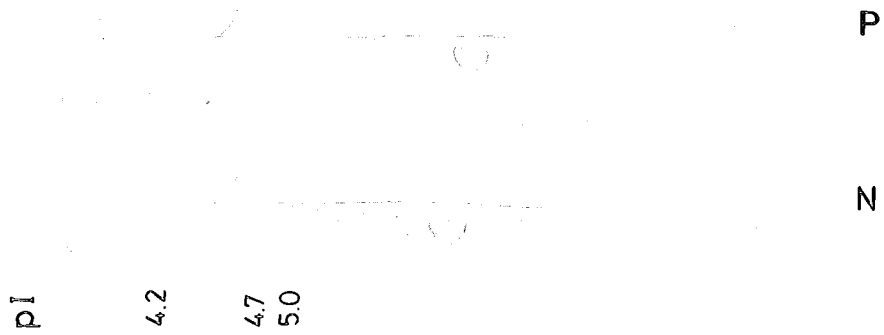

Fig 2. Crossed immunoelectrofocusing of patient's (P) and normal (N) prothrombin purified of plasma.

Therapeutic studies

Replacement therapy was performed during the haemorrhagic episode by infusion of 500 ml of fresh frozen plasma (FFP) which was effective in staffing the haemorrhage and the PCI decreased from 78% before infusion to 32% 2 h after its initiation, maintaining similar values 24 h following FFP administration.

DISCUSSION

In this study we present two patients, members of the same family, with a haemostasis alteration consisting in an anomaly of the prothrombin consumption index as the only defect while all other conventional tests of coagulation and fibrinolysis as well as platelet function tests were normal. Studies were carried out for the purpose of eliminating dysfibrinogenaemia and von Willebrand's disease were likewise normal. One of the patients presented with a history of serious haemorrhage caused by lesions of slight importance and a minor surgical procedure.

We have found only two publications in the literature concerned with an anomaly similar to that described in this study. The first involves a family described by Robinson *et al* (1967) in which abnormal PCI is related to an increase of factor Xa inactivation as a consequence of a natural inhibitor which would impede the complete conversion of prothrombin to thrombin. In our case the existence of an inhibiting effect of the patient's plasma was able to be demonstrated by adding a fifth of the volume of the patient's PRP to normal PRP before

performing the PCT. However, the normality of antithrombin III levels, of antifactor X activity in plasma and of the factor Xa inactivation test and thromboplastin decay rate discount the increase of a natural inhibitor of factor Xa as the mechanism responsible for the alteration.

The second publication is concerned with three non-related families presenting with a similar defect which was described by Parry *et al* (1980). Our case coincides in many aspects, such as the persistence of residual prothrombin in serum, measured by different methods, obtained 1 h after clotting had occurred. Likewise similar to Parry's case is the study of the two-dimensional crossed immunoelectrophoresis in serum obtained on various occasions during the first 24 h after clotting had occurred which showed that the reaction to prothrombin conversion, although normal at onset, slowed down to less than normal and did not reach completion until 24 h. However, *in vivo* studies on our patient show that FFP infusion corrects the haemorrhagic disorder and the haemostatic defect without need for additional platelets as was so in the case described by Parry. This would suggest that in our patient's case the defect does not act on the interaction between platelet phospholipid and plasma as Parry *et al* suggest.

A possible explanation for our case might be the existence of an abnormal prothrombin molecule responsible for a slow prothrombin conversion. Factor II dosage by various methods in the patient's plasma as well as the study of the electrophoretic mobility of prothrombin of the plasma were normal; both facts would suggest the non-existence of an abnormal prothrombin molecule. Nevertheless, when studying the purified prothrombin by means of crossed immunoelectrofocusing there appeared an obvious anomaly of pI with respect to the normal prothrombin, which indicates the existence of a heterogeneity of the prothrombin molecule and might be related to the abnormal prothrombin molecule.

Data available at this time does not permit greater precision as to the exact nature of the alteration but it seems clear that there exists an anomaly in prothrombin conversion responsible for a clinically significant haemorrhagic disorder.

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