Clotting factor V activity in plasma cryoproteins¹

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SYNOPSIS Plasma cryoprecipitates containing considerable quantities of clotting factor V were observed in five patients. The unusual factor V activity was not associated with significant amounts of cryofibrinogen. Clinically, the symptoms were not distinguishable from those of other cryopathies. The abnormality did not lead to a deficiency of factor V in the circulating blood nor to a haemorrhagic diathesis.

Serum proteins precipitating in the cold are called cryoglobulins. When a cold precipitate is formed in the plasma and not in the serum of a patient, the precipitate is usually clottable or exhibits other properties common to fibrinogen. This material has been called cryofibrinogen by Korst and Kratochvil (1955). Since then numerous publications concerning cryofibrinogenaemia have appeared (Smith and von Korff, 1957; Campbell, Hammack, and Frommeyer, 1959; Kalbfleisch, 1960; Kessel, 1960; Jager, 1962; McKee, Kalbfleisch, and Bird, 1963). It is tempting to speculate that in conditions predisposing to the development of cryoproteins, clotting factors other than fibrinogen might precipitate in the cold. The present report concerns five of 14 patients with plasma cryoproteinaemia in whom coagulation studies revealed moderate or considerable factor V activity in the plasma cryoprecipitate.

MATERIAL AND METHODS

For the preparation of oxalated and heparinized plasma, 9 parts of blood were collected into 1 part of 0.1M sodium oxalate and 100 mg % heparin respectively in siliconized glass tubes. Serum was obtained from blood collected in unsiliconized glass tubes. Immediately after venepuncture the samples were placed in a water bath at 37°C for three hr and centrifuged for 5 min at 3,300 rpm at room temperature. The supernatant plasma and serum were divided in two parts and stored at 4°C for 24 to 72 hours. At the end of this period, one tube was again incubated at 37°C to redissolve the precipitate. The cryoprecipitate of the other tube was separated by

centrifugation at 4°C, washed three times with cold 0.9% saline, dissolved in veronal-acetate buffer (Michaelis, pH 7.4) at 37°C and diluted to the original plasma volume. The cryoprotein solution was centrifuged for 15 min at 3,300 rpm in order to remove any undissolved protein and corpuscular elements. To avoid inactivation of the heat-labile clotting factors and loss of solubility, the cryoprecipitate was not chilled and warmed again for further purification.

Coagulation studies were carried out with fresh and rewarmed oxalated plasma ('plasma with cryoprotein'), with oxalated plasma after separation of the cryoprecipitate ('plasma without cryoprotein'), and with the cryoprotein solutions ('separated cryoprotein'). Heparinized samples were oxalated and treated with Polybrene before coagulation analysis.

Values for routine laboratory assays of blood coagulation factors (Duckert, 1958) are given in units (0.1 ml human oxalated plasma containing 100 units of each clotting factor). Thrombin-clottable fibrinogen was estimated according to the method of Clauss (1957). Immunoelectrophoresis was performed according to Scheidegger (1955), using home-made rabbit antihuman-fibrin antiserum and horse antihuman-globulin antiserum (from Institut Pasteur, Paris).

Heparin (Liquemin) from Hoffmann-La Roche, Basel, Switzerland, and Polybrene from Abbott Laboratories, were used.

RESULTS

In Table I the clinical data for the five patients with plasma cryoprecipitate are summarized. Table II gives the results of the coagulation studies.

The presence of factor V activity in the cryoprecipitate is not due to coprecipitation with other cryoproteins because the precipitate of the two cases with the highest cryofactor V activity (cases 1 and 2) contained the smallest amount of contaminating plasma proteins determined immunoelectrophoretically. Moreover cryofibrinogenaemia was minimal in the five cases presented.

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TABLE I
CLINICAL DATA

Case No.	Name	Sex	Age (yr)	Diagnosis	Clinical Symptoms Referring to Cryopathy	Cryoproteins		
						Oxalated Plasma	Heparinized Plasma	Serum
1	C.B.	♀	22	Rheumatic fever Pheochromocytoma	Acrocyanosis after exposure to cold; erythema nodosum-like efflorescence; transient cyanotic spots on the skin without necrosis	+	+	0
2	C.G.	♂	30	Rheumatic pericarditis	0	++	++	0
3	S.G.	♀	45	Pulmonary tuberculosis, necrotizing angitis	Cyanotic spots on the legs with subsequent ulceration; necrosis of several toes	+	+	0
4	F.T.	♂	64	Renal carcinoma without metastasis Multiple haemangioma of the spleen and intestines (necropsy)	Episodes of haemorrhage associated with increased fibrinolytic activity in the plasma	+	+	0
5	V.E.	♂	20	Periarthritis nodosa	Disturbed arterial circulation of the legs; coronary heart disease; thrombophlebitis	+	++	0

TABLE II

COAGULATION STUDIES

Case No.	Oxalated Plasma with Cryoprotein	Oxalated Plasma without Cryoprotein	Separated Cryoprecipitate ¹ from Oxalated Plasma	Separated Cryoprecipitate from Heparinized Plasma
Fibrinogen (mg%)				
1	500	520	0	Trace
2	1,300	1,400	20	100
3	650	700	10	0
4	450	450	Trace	20
5	720	570	Trace	—
Factor V (units/ml)				
1	100	100	14	45
2	95	100	10	16
3	56	58	10	2.2
4	100	85	5	1.5
5	68	61	6.4	—
Factor II (units/ml)				
1	100	100	0.8	0
2	78	100	0.7	0
3	86	90	1.8	0.6
4	92	100	0.9	2.0
5	89	76	0.6	—
Factor VII Complex (units/ml)				
1	100	100	0.8	0
2	100	100	0.8	0
3	70	41	0	0
4	100	100	0.8	0
5	92	76	0.7	—
Factor X (units/ml)				
1	100	100	0.6	0
2	72	77	0.4	0
3	80	73	1.0	0.1
4	95	88	0.4	1.0
5	70	80	0.8	—

¹The washed cryoprecipitate was redissolved in a quantity of oxalated buffer corresponding to the original plasma volume. Clotting factor determinations were done on plasma or cryoprotein solutions diluted 1/10 with buffer. Values below 5 u/ml were ascertained in the undiluted cryoprecipitate solution.

It is also clear that the factor V activity of cryoprecipitate was not due to absorption to platelets because one would have expected a similar activity in control or cryofibrinogenemic plasmas and because the 'separated cryoprotein' solution was virtually free of platelets.

It is extremely unlikely that the factor V activity of the cryoprecipitate was artefactual because the activity was only found in the factor V assay. In the presence of contaminating thrombin or thromboplastin the test systems for factors II, VII-complex, and X would have

given high false activity and this was not the case.

By analogy with the experience with cryofibrinogenæmia (McKee *et al*, 1963; Kalbfleisch, 1960) there was no correlation between factor V activity in fresh plasma, in the precipitate, and in the supernatant. The instability of factor V and some alteration during washing of the precipitate might explain some of the discrepancy. Relatively great fluctuations were encountered when determinations of cryofactor V were repeated in the course of weeks in individual patients. Similar results have been reported in cryofibrinogenæmia (Jager, 1962) and may be due to the spontaneous course of the disease and to therapy.

DISCUSSION

To our knowledge, this is the first report of pathological human plasma cryoprecipitates containing considerable amounts of clotting factor V. Clinically, the symptoms were not distinguishable from those of other cryopathies. The abnormality did not lead to a deficiency of factor V in the circulating blood nor to a haemorrhagic diathesis.

The present five cases certainly do not belong to the group of cryofibrinogenæmias of Kalbfleisch and Bird (1959) and Campbell *et al* (1959) or to that of the cryoglobulinaemia of Klesper (1960), in which a complex precipitation of various clotting factors together with other cryoproteins is postulated. The observation that recalcification and prothrombin time determinations can be done on plasma cryoprecipitate solutions (Kalbfleisch, 1960) is explained by the presence of contaminant trace amounts of all

clotting factors in cryoprecipitates. We confirmed this observation in seven cases of cryofibrinogenæmia with coprecipitation of traces of all clotting factors but without sizeable amounts of cryofactor V.

Since only coagulation activities can be determined, it is obvious that nothing can be said on the nature of the alteration of the factor V molecule which leads to abnormal cryoprecipitability. Moreover we cannot exclude the possibility that other pathological proteins present in the cryoprecipitate simulate factor V activity. By analogy with fibrinogen, factor V is consumed during coagulation. The cryoprecipitability might be due to alterations similar to those of fibrinogen, where transition products of the fibrinogen-fibrin reaction are known to be precipitated in the cold (Shainoff and Page, 1960).

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