APPLICATION OF CONTINUOUS FLOW ELECTROPHORESIS TO THE STUDY OF THE BLOOD COAGULATION PROTEINS. II. THE HEMOPHILIAS (A AND B) *

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(Submitted for publication May 28, 1959; accepted June 19, 1959)

A previous communication (1) concerned the application of continuous flow electrophoresis to the fractionation of normal blood preparations and the study of the coagulation proteins in these fractions. It was considered of some interest to apply the same techniques to pathological plasmas obtained from patients suffering from congenital deficiencies of AHF (antihemophilic factor A) and PTC (plasma thromboplastin component, Christmas factor, antihemophilic factor B). In addition, the isolation of an anti-AHF from the plasma of a hemophiliac with an acquired inhibitor is described.

MATERIALS AND METHODS

The methods have been described previously (1, 2). Plasmas were obtained from six patients, five of whom had suffered from severe and one (C.F.) from moderate hemorrhagic symptoms throughout their lives. Table I shows coagulation studies carried out on these patients on or near the date on which the blood for electrophoresis was obtained. Four patients suffered from AHF deficiency and two from PTC deficiency. All plasmas for electrophoresis were citrated except that of Patient R.R. This patient showed an acquired anti-AHF which we wished to isolate by electrophoresis. Thus, his plasma was prepared essentially free of other coagulation factors by employing sodium oxalate as anticoagulant, adsorbing with barium sulfate and heating to 56° C. for 10 minutes. This patient (R.R.) was interesting in that the appearance of anti-AHF could be shown to relate directly to transfusion of normal AHF. On his first three studies (age two and one-half) in January to June, 1956, no anti-AHF was demonstrable. In August, 1956, he received approximately 1,500 ml. of plasma over a five day period, and study three weeks later revealed anti-AHF titer of 1:64. During the next four months this gradually fell to zero and remained so for 17 months during which time he did not receive any blood or plasma. He was then transfused with 500 ml. fresh whole blood and tests six days later showed an anti-AHF titer of 1:64. It was at this time that blood for electrophoresis was obtained.

RESULTS

Hemophilia A. Figure 1 illustrates the results in one experiment (Patient T.M.) Paper strip electrophoretic patterns run on samples from each tube showed γ-globulin present in tubes 5 through 14, β in 15 through 17, α_2 in 18 and 19, α_1 in 21 and 22, α_1 plus albumin in 23 and albumin in 24 through 27. AHF activity was absent from the whole plasma and all fractions. Each fraction and the mother plasma were incubated with normal AHF and the residual AHF titer measured. No loss of activity was observed; thus no anti-AHF was present. The other coagulation proteins appeared normal in their distribution and concentration. Profibrinolysin and "glass factor" were in the γ area; heparin cofactor in the β area; prothrombin, proconvertin, PTC, antifibrinolysin and antithrombin in the α_1 to α_2 areas and proaccelerin in the albumin area. Quantities of substrate for measuring Hageman factor and PTA (plasma thromboplastin antecedent) were available for only a few tubes. These activities appeared primarily in the γ area.

The two other experiments on hemophilic plasma were very similar. The study on Patient B.T. showed no AHF or anti-AHF in the plasma or any fraction. The study on Patient C.F., a rather mild bleeder, showed some AHF activity (about 1.5 per cent of normal) in the whole plasma and in one fraction in the α_1 area.

Hemophilia A with anti-AHF. Anti-AHF activity (titer 1:2 to 1:8) was found in four tube fractions. The four tubes (10 through 13) were in the γ-globulin area and separate paper strip electrophoresis also showed these fractions to contain γ-globulin.

* This investigation was supported in part by a research grant (H-2254) from the Division of Research Grants of the National Institutes of Health, United States Public Health Service, Bethesda, Md.

TABLE I

Blood coagulation studies

Test*	Patient					
	B. T.	C. F.	T. M.	R. R.	J. P.	G. C.
Coag. time—glass (*min.*)	96	18	48	93	27	120
silicone (*min.*)	<300	90	<300	<240	<300	<240
Serum prot. time (*sec.*)	11.4	16.8	11.4	11.2	14	14.2
Prothrombin consumption (%)	0	17	0	0	10	14
Thromboplastin generation (%):	0	5	0	0	0	0
with normal $BaSO_4$ plasma	95	100	90	†	0	0
with normal serum	0	5	0	0	100	100
AHF assay (%)	0	1.5	0	0	60	100
PTC assay (%)	100	100	100	70	0	0
Anti-AHF	0	0	0	1:64		
Anti-PTC					0	0

* Serum prot. designates serum prothrombin; AHF, antihemophilic factor A; PTC, plasma thromboplastin component.

† Normal $BaSO_4$ plasma restored thromboplastin generation unless the patient's serum and the normal plasma were allowed to preincubate, in which case marked inhibition of the normal plasma was observed.

Hemophilia B. Figure 2 illustrates the results found in one experiment (Patient C.G.); the other was similar. Paper strip electrophoresis carried out on each tube showed Tubes 5 through 13 to contain γ-globulin; 14 through 16, β-globulin; 19 through 20, α_2-globulin; 21, α_1-globulin; 22 through 24, α_1 plus albumin and 25 through 29, albumin. PTC activity was absent from all fractions. No anti-PTC could be demonstrated in the whole plasma or the fractions. Profibrinolysin, glass factor, Hageman factor and fibrinogen were found primarily in the γ area; antithrombin, heparin cofactor, antifibrinolysin, proconvertin, prothrombin and AHF in the α_2 to α_1 areas and proaccelerin in the albumin areas. PTA activity was tested in three tubes and appeared in the γ area.

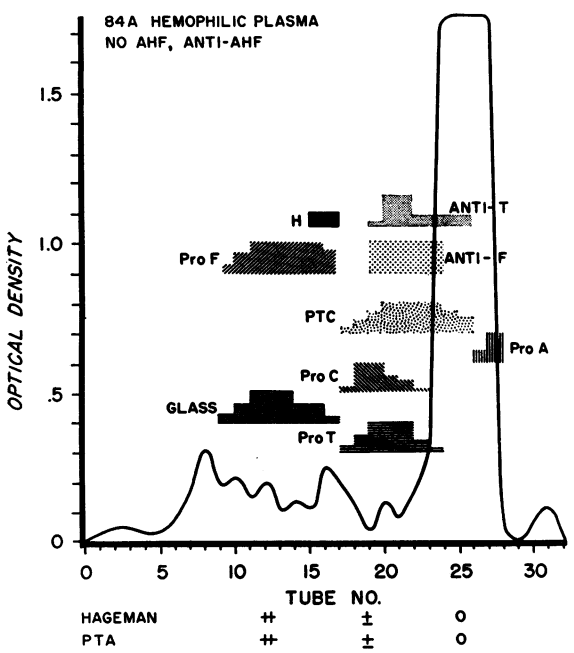

FIG. 1. CONTINUOUS FLOW ELECTROPHORESIS OF HEMOPHILIC PLASMA

The optical density curve indicates the relative protein concentration in each tube.

DISCUSSION

Most of the investigators in the field of blood coagulation feel that Hemophilia A and B are disorders due to genetically controlled deficiencies of two distinctly different plasma proteins. A divergent theory (3, 4) explains Hemophilia A as due to the binding of AHF by a circulating inhibitor. If such an AHF-anti-AHF complex exists in hemophilic blood, it is not dissociated by electrophoresis as neither AHF nor anti-AHF activity could be found in any of the fractions from the plasmas of the severe hemophiliacs. In the study on the mild hemophiliac a trace of AHF was found in the same location (α_1) in which it appears in normal plasma.

The inhibitor to AHF, demonstrated in the blood of Patient R.R., is probably not the same as that assumed to exist in the blood of all hemophilic patients by the exponents of the "inhibitor" theory of hemophilia. On two occasions it was possible to show a marked increase in the titer of this inhibitor after transfusions, followed by gradual diminution until no inhibitory effects on nor-

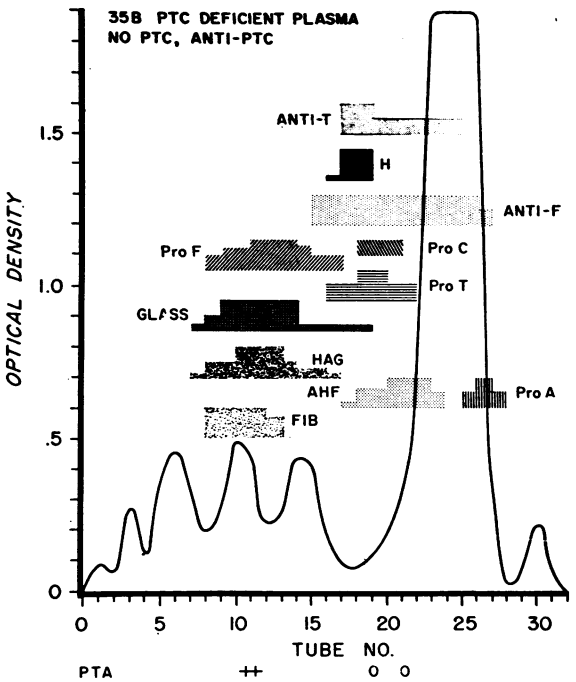

FIG. 2. CONTINUOUS FLOW ELECTROPHORESIS OF PLASMA THROMBOPLASTIN COMPONENT (PTC) DEFICIENT PLASMA

mal AHF could be demonstrated. In spite of these variations in inhibitor strength, AHF activity was never demonstrable. The inhibitory activity from the plasma of Patient R.R. was isolated in the γ-globulin fraction, confirming the previous findings of Craddock and Lawrence (5).

The abilities to correct the clotting "defects" in Hageman factor deficient plasma, normal silicone plasma ("glass factor") and PTA deficient plasma were present in the fractions from both Hemophilia A and B. The exact abnormality present in PTA plasma had not been completely defined. Rosenthal, Dreskin and Rosenthal (6, 7), who first described this disorder, felt that it was due to a deficiency of a unique clotting factor which they entitled PTA (plasma thromboplastin antecedent). On the other hand, Johnson, McClaughry and Seegers (8) described double deficiencies of platelet cofactors I and II (AHF and PTC) in PTA plasma. Our experiments suggest that both AHF and PTC deficient plasmas and certain of their fractions have the ability to correct the coagulation defect in PTA deficient plasma and, conversely, that plasma from a PTA deficient subject contains normal concentrations of AHF and PTC (9). The close similarity between the distribution of PTA, Hageman factor and "glass factor" in these and in normal plasma is being further explored.

SUMMARY

Continuous flow electrophoretic fractionation was applied to four AHF and two PTC deficient plasmas. One AHF deficient plasma showed the presence of anti-AHF. Fractions from the three ordinary hemophilic plasmas showed no anti-AHF and no AHF activity with the exception of one fraction from a mild hemophiliac which showed a trace of AHF activity. Fractions from the PTC deficient plasmas showed no PTC or anti-PTC activity. Other coagulation factors appeared normal in concentration and distribution. The anti-AHF activity of the plasma from the patient with an acquired inhibitor was concentrated in the γ-globulin area.

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