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CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XXXIII. THE COAGULATION DEFECT IN HEMOPHILIA: THE EFFECT *IN VITRO* AND *IN VIVO* ON THE COAGULATION TIME IN HEMOPHILIA OF A PROTHROMBIN AND FIBRINOGEN-FREE NORMAL PLASMA AND ITS DERIVED PROTEIN FRACTIONS [123]

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Research Article

CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XXXIII. THE COAGULATION DEFECT IN HEMOPHILIA: THE EFFECT *IN VITRO* AND *IN VIVO* ON THE COAGULATION TIME IN HEMOPHILIA OF A PROTHROMBIN AND FIBRINOGEN-FREE NORMAL PLASMA AND ITS DERIVED PROTEIN FRACTIONS [1, 2, 3]

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Patek and Taylor (1) in 1937 demonstrated that the activity of normal plasma in shortening the coagulation time of hemophilic blood could be found almost quantitatively in a derived globulin fraction. In 1939, Lozner, Kark and Taylor (2) showed that Berkefelded normal plasma free from fibrinogen and prothrombin had hemophilic clot-promoting power similar to that of untreated normal plasma. In unpublished investigations (3), they found that a globulin fraction derived from this fibrinogen and prothrombin-free plasma also had antihemophilic activity. Pavlovsky and Simonetti (4) in 1944, using the technique of Patek and Taylor, demonstrated that globulin substance prepared from the plasma of a patient with congenital fibrinogenopenia had normal antihemophilic activity.

Investigation in this laboratory of plasma protein fractions prepared by the Harvard Physical Chemistry Department showed marked *in vitro* (5) antihemophilic activity to be present in Fractions I and II + III. Fraction I studied *in vivo* (6) likewise showed marked power to shorten the coagulation time of hemophilic patient's blood.

This investigation was undertaken to study the antihemophilic activity of the various plasma protein fractions prepared by the Harvard Physical Chemistry Department from plasma free of fibrinogen and prothrombin.

METHODS

Fifteen hundred ml. of plasma obtained from normal human blood within 4 hours after venesection was heated to 56° C. for 2½ minutes. The precipitate was removed by centrifugation, and the supernatant plasma tested for fibrinogen and prothrombin. Prothrombin time was determined by a modification (7) of the Quick technique (8) using a human brain thromboplastin. Fibrinogen was measured by clot formation after addition of 0.1 ml. thrombin [4] to 0.1 ml. plasma.

The heated plasma was rapidly frozen and preserved at − 20° to − 40° C. until fractionation. The plasma protein fractions were dissolved in sufficient 0.85 per cent sodium chloride solution to make a 2 per cent solution by weight, and the pH adjusted at 7 to 7.4 before use. Samples of the original plasma and heated plasma were kept at − 20°, and tested for antihemophilic activity at the same time as the plasma protein fractions. The plasmas and plasma protein fractions were diluted by 0.85 per cent sodium chloride solution to make serial dilutions of 1/5, 1/50, 1/500, 1/5,000 and 1/50,000.

Antihemophilic activity was measured by 2 methods. In the first method, the time of coagulation was measured after 0.1 ml. of 0.25 per cent calcium chloride solution was added to a mixture containing 0.1 ml. of hemophilic plasma, 0.7 ml. of 0.85 per cent sodium chloride solution, and 0.1 ml. of the various dilutions of the 2 per cent solutions of the protein fractions. In the second method, whole blood was used instead of the diluted plasma. A modification of the Lee and White coagulation method (9) was employed, in which the coagulation of the hemophilic whole blood was measured after the addition of 2 ml. of the blood to 0.1 ml. of the various dilutions of the plasma protein fractions. All coagulation reactions were carried out at 37.5° C. in a constant temperature water bath.

[1] This is paper No. 45 in the "Studies of Plasma Proteins" of the Harvard Medical School, on products developed by the Department of Physical Chemistry, from blood collected by the American Red Cross.

[2] The expenses of this investigation were defrayed in part by gift from the Smith, Kline and French Laboratories, Philadelphia, and in part by a grant "In recognition of Dr. Francis W. Peabody's services to the Foundation" by the Ella Sachs Plotz Foundation.

[3] We are indebted to Professor Edwin J. Cohn and Dr. John T. Edsall for furnishing the material on which these observations were made.

[4] Lederle clotting globulin.

RESULTS

Table I shows the prothrombin and fibrinogen content of the plasma before and after heating to 56° C. for 2½ minutes. After heating, there is no clottable fibrinogen, and only a trace of prothrombin in the plasma. Table II shows the distribution of protein in the fractions of normal and heat defibrinogenated plasma. Part of the albumin, as well as the fibrinogen and prothrombin, had been coagulated by the heat processing. No fibrinogen or thrombin was found in any of these plasma protein fractions, and in Fractions I, II + III and IV only traces of prothrombin were detected.

Tables III and IV show the hemophilic clot promoting activity of the original plasma, heated plasma and plasma protein fractions as measured by hemophilic plasma recalcification, and whole blood coagulation times. These data indicate that some of the antihemophilic activity of whole plasma is lost by the heating to 56° C. for 2½ minutes, although the prothrombin and fibrinogen free plasma thus obtained still has marked hemophilic clot promoting powers. The fractions derived from this material show marked activity in Fractions I and II + III, and are very similar to fractions obtained from normal plasma (5).

Sterile preparations of normal Fraction I were heated unopened to 56° C. for 4 minutes, and the supernatant fluid collected sterily and injected intravenously into a hemophilic patient. Figure 1 shows a comparison of the response of one hemophilic patient to intravenous injection of 0.8 gram of Fraction I and the supernatant obtained after heating the same amount of the same Fraction I. The protein contents of these solutions vary markedly, the total protein of the former being 450 mgm. injected, and of the latter 190 mgm. injected; but the antihemophilic activities are very similar, demonstrating that the fibrinogen-free Fraction I has not lost much activity in the heating process.

TABLE I

The effect of 56° C for 2½ minutes on the prothrombin and fibrinogen of normal citrated plasma

	"Prothrombin time"*	"Fibrinogen time"†
Normal plasma	13 seconds	4 seconds
Heated plasma	‡ 10 minutes	no clot in 24 hrs.

* Human brain thromboplastin.
† Lederle clotting globulin used as thrombin.
‡ 0.1 ml. standard fibrinogen solution added.

TABLE II

Distribution of protein among fractions of normal and heat defibrinogenated plasma

Fraction	Normal protein	Heat defibrinogenated protein
	grams per 1000 ml.	*grams per 1000 ml.*
I	4.3	2
II+III	16.3	14
IV-1	9.7	3
IV-4		3
V (albumin)	29.6	15
Total	59.9	37.0

TABLE III

Recalcification time (in minutes) after addition of various dilutions of whole plasma, plasma freed from fibrinogen and prothrombin, and derived plasma protein fractions, to diluted hemophilic plasma

Dilution	0	1/5	1/50	1/500	1/5000
Saline (control)	26				
Whole plasma	2	2½	4½	8	14
Heated plasma	3½	5	9	14	21
Fraction I	4	5	6	9½	15½
Fraction II+III	4	6	10	16	23
Fraction IV-1	17	20	25	27	27
Fraction IV-4	21	21½	29	26	26
Fraction V	27	26	26	26	26

TABLE IV

Coagulation time (in minutes) of hemophilic whole blood after addition of various dilutions of whole plasma, plasma freed from fibrinogen and prothrombin and its derived plasma protein fractions

Dilution	0	1/5	1/50	1/500	1/5000	1/50,000
Saline (control)	123					
Whole plasma	9	9½	19	19	21	56
Heated plasma	12	12½	19	48	102	97
Fraction I	10	16½	65	74	105	112
Fraction II+III	9	17	51	102	112	112
Fraction IV-1	74	102	112	112	128	123
Fraction IV-4	84	102	123	123	123	123
Fraction V	92	93	112	117	123	117

DISCUSSION

These investigations again emphasize that the substance present in normal blood which will shorten the coagulation time of hemophilic blood is independent of fibrinogen and prothrombin content of the blood, as well as the formed elements. This substance is asociated with the globulin fraction of the plasma proteins, and essentially with Fractions I and II + III as prepared by the Harvard Physical Chemistry Department. This

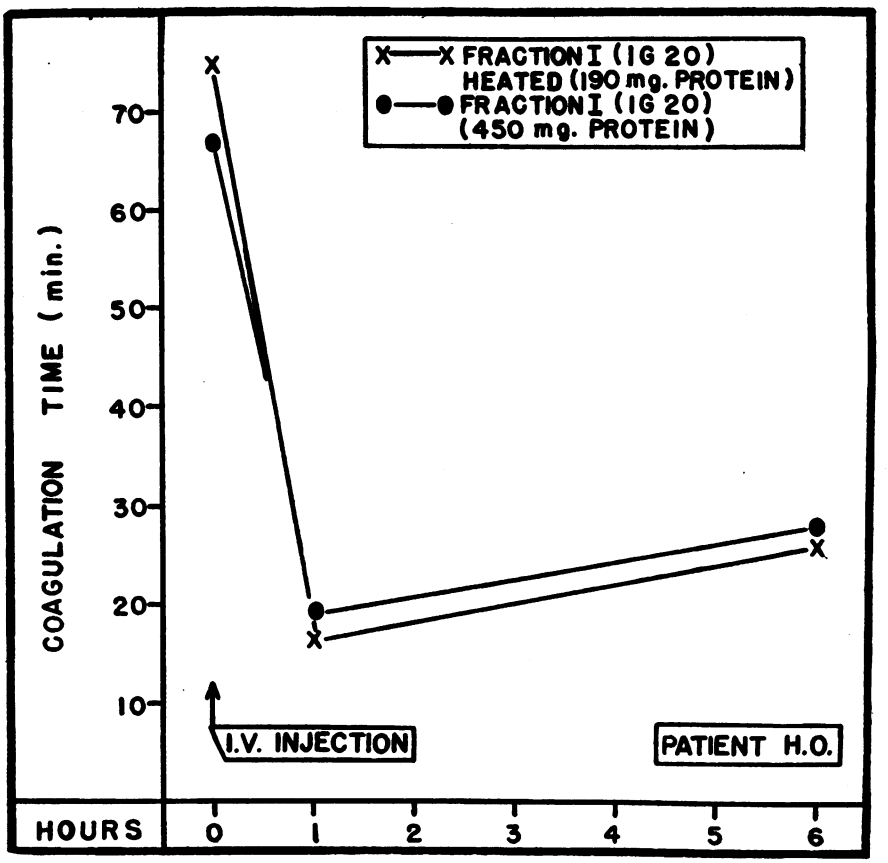

FIG. 1. A COMPARISON OF THE ANTIHEMOPHILIC ACTIVITY OF NORMAL FRACTION I AND HEAT DEFIBRINOGENATED FRACTION I IN THE SAME PATIENT

globulin substance is more thermostabile at 56° C. than the other known protein components of the blood coagulation reaction.

Fraction I prepared from this heated plasma contains no clottable fibrinogen, but is fairly insoluble in both water and various dilutions of saline which may be due to the presence of denatured fibrinogen. This material was not given to patients intravenously. Instead, Fraction I prepared from normal plasma and then heated to coagulate the fibrinogen was used. The supernatant contains no clottable fibrinogen and a low protein content, but is as active in reducing the coagulation time of a hemophilic patient as the same Fraction I before heating. This suggests that it may be possible to prepare an antihemophilic substance in a concentrated form.

CONCLUSIONS

1. Fifteen hundred ml. of normal plasma heated at 56° C. for 2½ minutes contained no clottable fibrinogen and only a trace of prothrombin.

2. This heated plasma and protein fractions prepared from it contain antihemophilic activity similar to normal plasma and plasma protein fractions.

3. A solution of Fraction I from which the fibrinogen has been removed by heat coagulation is as active as the original Fraction I in reducing the coagulation time of a hemophilic patient when injected intravenously.

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