Technical Papers

Purification of Plasma Thromboplastin Factor B (Plasma Thromboplastin Component) and Its Identification as a Beta$_2$ Globulin

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A previously unknown blood-clotting factor necessary for the production of thromboplastin in the plasma was discovered by Aggeler *et al.* (*1*, *2*). In contrast to the antihemophilic factor, which serves a similar function, this factor is present in serum as well as in plasma, is stable on storage at refrigerator temperature, is completely adsorbed by barium sulfate, is present in the 45 to 50 percent saturated ammonium sulfate fraction of plasma rather than the 0 to 33 percent fraction, and is not present to any significant degree in fraction I of Cohn. This factor was named plasma thromboplastin component (PTC). Its characteristics have been confirmed by Biggs *et al.* (*3*), who called it Christmas factor, and by Soulier and Larrieu (*4*), who called it antihemophilic factor B.

Numerous reports of patients deficient in the new factor have now been published, and it is estimated that the disease may account for 10 to 20 percent of all patients thought to suffer from hemophilia. The disease has been found to be indistinguishable from hemophilia except by special laboratory procedures. Aggeler, White, and Spaet (*5*) have reviewed the relevant literature and have proposed a uniform nomenclature whereby this factor would be called plasma thromboplastin factor B (PTF-B) and the antihemophilic factor would be called plasma thromboplastin factor A (PTF-A).

Biggs *et al.* (*3*) found that PTF-B was contained in a crude ether fraction precipitated from plasma and consisting of alpha and beta globulins. White, Aggeler, and Emery (*6*) found it concentrated in fractions III and IV-1 of Cohn. Small quantities were found in fractions I and IV-4. None was found in fractions II or V.

White, Aggeler, and Glendening (*2*) prepared a potent PTF-B extract by adsorbing acidified serum with $BaSO_4$ and eluting the $BaSO_4$ with sodium citrate. In the present investigation, a modification of this process was used to obtain a purified extract from fraction IV. Here 100 mg of $BaSO_4$ per milliliter of 10 percent solution of the fraction was used for adsorption, and all elutions were accomplished into volumes of solutions equal to the original volume of the fraction. All adsorptions and elutions were continued for 30 min at 37°C. The $BaSO_4$ was separated from the supernatants by centrifugation at 2000 rev/min for 10 min. After the fraction IV was adsorbed, the supernatant was discarded. The $BaSO_4$ was eluted first with 0.1*M* sodium oxalate and then with 0.006*M* sodium citrate. These eluates were pooled (extract B). The $BaSO_4$ was then eluted with 0.34*M* sodium citrate (extract C) (*7*). These eluates were dialyzed against distilled water and then desiccated in a lyophilizer.

In the first preparation, made from 192 ml of fraction IV, the yield of solids was 500 mg in extract B and 78 mg in extract C. In the second preparation, made from 400 ml of fraction IV, extract B was discarded, and the yield of solids in extract C was 70 mg. The coagulating potency of these extracts was compared with that of normal plasma by testing their ability to shorten the coagulation time and to decrease the residual serum prothrombin (*8*) in patients suffering from PTF-B deficiency (Table 1). Extract B was inert, whereas both a 0.02 percent solution of extract C #1 and a 0.01 percent solution of extract C #2 were equal in potency to undiluted plasma.

The amount of extract C available was too small for accurate chemical analysis; however, an approximation of its protein content was made by eluting the bromphenol blue from an entire paper electrophoretic

Table 1. Comparative effects on clotting time and residual serum prothrombin of the addition of normal plasma and of extracts of plasma fraction IV in the PTF-B deficient patient.

Fraction added*	Concentration of solids in fractions added (g/100 ml)	Clotting time (min)		Residual serum prothrombin in percentage of patient's plasma volume†	
0.85% NaCl		23–33		72–78	
Normal plasma					
100%		8		10	
40%		9		20	
20%		9		31	
Fraction IV Extract B	4.0	32		83	
	0.8	28		67	
	0.2	26		77	
Fraction IV Extract C		#1	#2	#1	#2
	0.4	7	8	4	4
	0.08	8	8	4	3
	0.04	11	10	6	7
	0.02	12	9	11	7
	0.01	13	12	20	11
	0.005	15		47	
	0.0025	17		67	

* 0.1 ml of fraction added to 2.0 ml blood.
† Ware-Stragnell modification of Owren's method (*8*); blood incubated for 1 hr at 37°C after clotting.

pattern with 0.1 percent NaOH (see next paragraph). The eluate was compared with a standard albumin solution, similarly eluted, in a Coleman junior spectrophotometer at a wavelength of 575 millimicrons (575 mμ). It appeared that only about 10 percent of the material in the C extracts was protein. Four percent solutions of these purified extracts contained no measurable quantities of thrombin, antihemophilic factor, labile or stable prothrombin conversion accelerators. Only a trace of prothrombin was found. Thrombin was tested by the addition of 0.1 ml of extract to 0.1 ml of normal oxalated plasma. Coagulation did not occur within 4 hr at 37°C. Labile prothrombin conversion accelerator was tested by the method of Quick (*9*); stable prothrombin conversion accelerator by the method of Owren and Aas (*10*); and antihemophilic factor by the method of Spaet *et al.* (*11*). In these tests, the addition of the extract gave results similar to the saline controls. Prothrombin was tested both by the method of Ware and Stragnell (*8*) and by the method of Owren and Aas (*10*). An amount less than 1 percent of that contained in normal plasma was found.

Paper electrophoresis was accomplished by a modification of the method described by Kunkel and Tiselius (*12*), in which the paper strips were suspended between glass plates coated with silicone. The system was supplied with Veronal buffer at a *p*H of 8.6 and an ionic strength of 0.05. Whatman #3 MM filter paper was used, and runs were of 4-hr duration at a potential difference of 500 v. Fraction IV and extract B contained all the electrophoretic components found in normal serum, although the relative proportions were altered. Extracts C #1 and C #2 were composed of alpha$_2$ and beta globulins and, perhaps, traces of gamma globulin.

In a separate experiment, 0.01-ml specimens of 40 percent extract C #2 were run in duplicate, together with a serum control. In this study, the filter paper strip was marked off in transverse divisions 1 cm apart, and the current was applied until the albumin of the prestained serum had traveled 12 cm from the origin. A portion of the paper containing one of the extract C #2 specimens was then cut from the rest of the strip and divided into segments corresponding to the transverse divisions. Each segment was eluted for 15 hr into 0.5 ml of isotonic saline; 0.1 ml of each eluate was then added to 2 ml of freshly drawn whole blood from a patient with untreated PTF-B deficiency, and the residual prothrombin in the serum was determined 4 hr after clotting. The remainder of the strip was stained with bromphenol blue in the usual way.

Figure 1 shows the results of these studies. The activity of the respective eluates is shown below that of the corresponding segment in graphic form. The results are expressed in terms of the percentage of increased prothrombin utilization in the patient's serum induced by each eluate. The PTF-B activity was found to be concentrated in the beta$_2$ globulins by this technique, and it can be assumed that the minor activity exhibited by the beta$_1$ and alpha$_2$ fractions was due to diffusion. The deeply staining material at the point of origin is congealed protein that failed to migrate. Less than half the protein present appears to be beta$_2$ globulin.

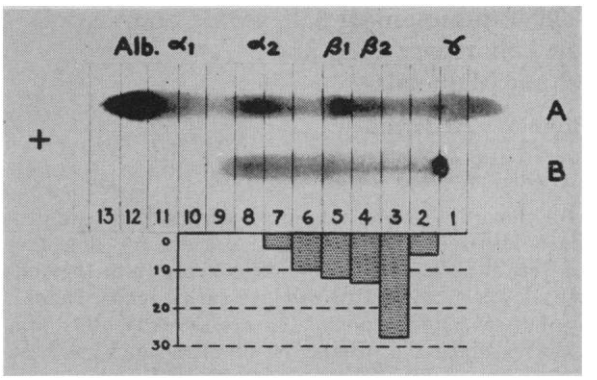

Fig. 1. Paper electrophoretic patterns of normal serum (A) and of extract C #2 (B). The PTF-B potency of the numbered segments contained in extract C #2 is shown in the lower graph. Potency is expressed in terms of the percentage of increased prothrombin utilization induced in the PTF-B deficient patient's serum by eluates from each segment of a parallel strip of extract C #2.

Since only about 5 percent of the total solids in the extract C preparations was beta$_2$ protein, and since the coagulating potency of extracts whose concentrations were 0.02 percent or less equaled the potency of normal undiluted plasma, the concentration of PTF-B in normal plasma is of the order of 1 mg or less per 100 ml. A favorable influence on blood clotting has been found for as long as 3 wk after transfusion of 500 ml of plasma in the PTF-B deficient patient weighing approximately 50 kg. This is in contrast to the disappearance of the AHF factor within a few days after a 500-ml plasma transfusion in a hemophilic patient of similar weight. The AHF concentration in normal plasma is unknown.

References and Notes

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