PROTHROMBIN CONSUMPTION, SERUM PROTHROMBIC ACTIVITY AND PROTHROMBIN CONVERSION ACCELERATOR IN HEMOPHILIA AND THROMBOCYTOPENIA [1, 2]

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INTRODUCTION

Attention has recently been focused on the consumption of prothrombin during blood coagulation. Studies of the velocity of prothrombin conversion to thrombin have yielded important information concerning the clotting defect in various hemorrhagic disorders. Thrombin evolution is retarded in the coagulation of hemophilic blood (1–5), thrombocytopenic blood (3, 4, 6, 7) and blood exposed to siliconized surfaces (8). This is reflected in the high residual prothrombic activity of the serum. Precisely how much prothrombin is consumed per unit of time is, however, not clear.

Obviously, prothrombin consumption can be computed from the difference between the prothrombin concentration of serum and that of its parent plasma. The reliability of this computation rests upon the specificity of methods for measuring the concentration of prothrombin in both plasma and serum. The one-stage procedure measures the velocity of thrombin formation in the presence of optimal thromboplastin and calcium. In applying it to serum one must exclude the possibility that in the transition from plasma to serum substances arise which affect the velocity of prothrombin conversion. Convincing evidence exists, however, that during coagulation prothrombin conversion accelerators evolve which are demonstrable in serum (9, 10).

Observations presented in this paper show that such an accelerator can be obtained from hemophilic, thrombocytopenic and silicone [3] sera and that it contributes toward their high one-stage prothrombic activity. Accordingly, the validity of computing prothrombin consumption from one-stage prothrombin determinations is questioned. The two-stage method provides more reliable data since the serum accelerator is inert in the two-stage system which measures the total yield of thrombin rather than the velocity of its evolution.

METHOD

Venous blood was drawn from normal, hemophilic and thrombocytopenic subjects with a chemically clean syringe rinsed with physiological saline. Care was taken to exclude tissue juice. In some experiments syringe, needle and test tubes were coated with silicone according to the technique of Jacques *et al.* (11). Clotting times were measured by a modification of the Lee and White method (12).

Plasma prothrombic activity was determined by the one-stage procedure as modified by Rosenfield and Tuft (13) in which fresh prothrombin-free ($BaSO_4$ adsorbed) normal plasma is used as diluent. In all instances plasma was oxalated (1 volume of 0.1 M sodium oxalate to 9 volumes of blood).

The activity of serum or serum fractions was similarly determined; the proportion of serum or serum fraction to $BaSO_4$ plasma was 1 : 3, 1 : 10 or 1 : 20 depending upon the activity. Serum was obtained one hour after blood was allowed to clot spontaneously at room temperature. The clot was rimmed and centrifuged; the supernatant serum was separated, oxalated (1 oxalate to 4 serum), and incubated for 30 minutes at 37° C to inactivate thrombin.

In some experiments prothrombin was simultaneously measured by the two-stage modified technique of Ware and Seegers (14),[4] which measured the total yield of thrombin elaborated instead of the velocity of its evolution.

The serum prothrombin conversion accelerator (spca) was determined by the accelerating effect of serum or fractions thereof on the prothrombin time of a mixture of normal plasma and prothrombin-free ($BaSO_4$) normal plasma (10).

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[2] An abstract of this work was presented before the American Physiological Society at the Meetings of the Federation of American Societies for Experimental Biology in Detroit, April, 1949.

[3] The term "silicone serum" refers to serum obtained from blood handled in siliconized apparatus.

[4] We are grateful to Dr. Walter H. Seegers of Wayne University Medical School for a sample of bovine serum Ac-globulin used in the modified two-stage procedure.

TABLE I

One-stage prothrombic activity of hemophilic plasma and serum

Subject	Cl. T.	Prothrombic activity (%)*	
		Plasma	Serum
	minutes		
J. G.	170	64	90
J. G.	100	82	110
J. G.	126	—	155
R. R.	110	48	75
R. R.	32	78	84
R. R.	120+	48	68
R. R.	—	87	107
R. R.	—	46	75
R. R.	120+	65	118
I. G.	90	96	118
I. G.	53	98	108
I. G.	32	90	93
I. G.	32	90	116
I. G.	—	95	78
I. G.	120+	95	108
Average		77	100

* On the basis of normal plasma containing 100% prothrombic activity.

RESULTS

The prothrombic activity (one-stage) of hemophilic serum obtained one hour after coagulation frequently exceeds that of its parent plasma (Table I). This is in striking contrast to the value found in normal serum (8).

It is noteworthy that due to the prolonged clotting time "coagulation" had proceeded in the hemophilic blood for approximately two or more hours before the addition of oxalate to the serum.

In some instances aliquots of the same oxalated serum were stored in the refrigerator (4° C) overnight. Their prothrombic activities were even greater than those determined one and one-half hours after coagulation.

By the two-stage technique no rise in prothrombic activity was demonstrable (Table II). The discrepancy between the results obtained by the two methods was even more striking when coagulation was markedly accelerated by the addition of purified spca [5] to the drawn blood. The two-stage serum prothrombin was lower than that of the parent plasma; by the one-stage method prothrombic activity still exceeded that of the plasma.

The high activity (one-stage) of hemophilic serum is due to the presence of both unconsumed prothrombin and some prothrombin conversion accelerator which evolves during coagulation. The accelerator can be adsorbed by $BaSO_4$ from which it can be eluted by solutions of sodium citrate (15). Hemophilic serum was subjected to this procedure as follows: 25 mgm. $BaSO_4$ (C.P.) was added to each ml. of oxalated serum, the mixture was shaken and incubated (37° C) for 15 minutes during which time it was frequently agitated. It was then centrifuged, the supernatant was separated, and the sediment washed twice by resuspension in 0.02 M acetate buffer (pH 5.2) totalling in volume that of the original serum. To the washed $BaSO_4$ was added the same volume of 5% sodium citrate in physiological saline. The mixture was shaken thoroughly for 15 minutes, centrifuged, and the solution separated (eluate).

Eluates thus obtained from the sera of two hemophiliacs had negligible prothrombic activity yet were capable of markedly accelerating prothrombin conversion when added to a mixture of normal plasma and prothrombin-free ($BaSO_4$) normal plasma (Table III).

Adsorbing these hemophilic sera with $BaSO_4$ also resulted in marked reduction of their prothrombic activities. The supernatant sera were essentially devoid of spca.

Similar observations were obtained on silicone

TABLE II

Comparative values of prothrombic activity obtained by one-stage and two-stage methods on hemophilic plasma and serum

Subject	Cl. T.	Prothrombic activity			
		One-stage (%)		Two-stage (*units*)	
		Plasma	Serum	Plasma	Serum
	minutes				
R. R.	—	46	75	138	160
R. R.	120+	65	118	138	123
	4*	65	75	138	66
I. G.	—	95	78	210	148
I. G.	120+	95	108	165	147
	4*	95	118	165	100

* Coagulation accelerated by addition to 2 ml. of hemophilic blood of 0.1 mgm. of purified serum prothrombin conversion accelerator in 0.1 ml. of saline.

[5] Spca was obtained by absorbing normal serum with $BaSO_4$ and subsequent elution with sodium citrate solution. The eluate was then fractionated with $(NH_4)_2SO_4$. Details of isolation and purification will be reported elsewhere.

TABLE III

Prothrombin conversion accelerator (spca) in hemophilic serum

Subject	Prothrombic activity* (%)			Mixture (parts)					Proth. time (*sec.*)
	Serum	Eluate	Supernat.	Norm. plasma	Sal.	Hemoph. serum el.	Hemoph. serum sup.	$BaSO_4$ Norm. plasma	
I. G.	125	6	40	1	1	—	—	18	44
				1	—	1	—	18	26
				—	—	3	—	7	87
R. R.	100	3	10	1	1	—	—	18	44
				1	—	1	—	18	28
				—	—	3	—	7	100

* One-stage.

serum (Table IV). Its prothrombic activity (one-stage) exceeded that of the parent plasma; by the two-stage method, however, it showed less prothrombin than the original plasma. $BaSO_4$ adsorption and elution with sodium citrate yielded two fractions approximately equal in prothrombic activity (Table V). The residual supernate had no demonstrable spca activity. The eluate, on the other hand, contained substantial amounts of spca.

TABLE IV

Prothrombic activity of serum from blood clotted in silicone

	Prothrombic activity				
	Cl. T.	One-stage (%)		Two-stage (*units*)	
		Plasma	Serum	Plasma	Serum
	minutes				
Expt. 1	60	72	90	140	70
Expt. 2	57	57	82	225	150
Expt. 2a*	7	57	15	225	50

* Coagulation accelerated by addition of 0.1 mgm. purified spca to 2 ml. of blood.

Silicone surfaces delay coagulation presumably by retarding the evolution of thromboplastin. Removal of platelets similarly results in thromboplastin deficiency (16). It has already been shown that serum from thrombocytopenic blood is rich in prothrombic activity (one-stage) (4, 6, 7). The question arises whether here, also, the high activity is referable to the residual prothrombin plus some spca which evolves during coagulation or to unconsumed prothrombin alone.

The following experiment was performed: Normal blood, handled in siliconized apparatus, was centrifuged at 1,500 r.p.m. for 15 minutes to remove erythrocytes and leucocytes. The plasma, transferred with a siliconized pipette to another siliconized tube, was then centrifuged at 15,000 r.p.m. for 15 minutes at 5–6° C in an International Centrifuge with multispeed attachment. The supernatant plasma (essentially platelet-free) was carefully separated from the platelet pellet and transferred to an ordinary glass tube in which it clotted after 25 minutes at room temperature. After another hour the clot was rimmed and centrifuged. The supernatant serum was removed, oxalated and incubated for one-half hour. Its prothrombic activity was almost three-fold that of the parent plasma (Table V). As with hemophilic and silicone serum, fractionation yielded an eluate which had strong spca activity. The supernatant showed little prothrombin conversion accelerating effect.

Similar observations were obtained on a 55 year old male who showed the characteristic clinical manifestations of idiopathic thrombocytopenic

TABLE V

Prothrombic activity (one-stage) and spca in silicone serum and serum from deplatelelized blood

	Prothrombic activity (%)				spca (%)*	
	Plasma	Serum	Serum eluate	Serum supernate	Serum eluate	Serum supernate
Silicone	110	138	50	56	92	0
Deplate.	80	204	76	60	86	13

Silicone blood—Cl. T. 150 minutes, room temperature.
Deplate. plasma—Cl. T. (in glass) 25 minutes room temperature.

* Per cent enhancement of expected prothrombic activity of mixture containing normal plasma and serum fraction.

purpura (Table VI). The platelet count was 47,000–67,000 per cu. mm., the bleeding time (Duke) 20 +minutes and the clotting time normal. The high serum prothrombic activity, equalling or exceeding that of the parent plasma, confirmed the coagulation defect previously reported in this disease (6, 7). Spca was separated from the serum by adsorption with $BaSO_4$, and demonstrated in the eluate. Prothrombic activity was almost equally divided between the supernate and eluate.

TABLE VI

Serum prothrombic activity (one-stage) and spca in idiopathic thrombocytopenic purpura

Pat. A.D. (M4816)
Bl. T. 20+ minutes; Cl. T. 10 minutes
Plasma proth. activity—110%

Date	Blood platelets	Ser. proth. activity
	*thousands per cu. mm.**	(%)
3/10/49	49	140
3/11/49	64	90
3/22/49	50	150†
Splenectomy 3/22/49		
3/24/49	146	40
3/28/49	138	20
4/1/49	222	21
4/29/49	131	16

Spca of serum fractions 3/11/49

Mixture (parts)					Prothrombic activity (%)	
Norm. plasma	Sal.	Serum elu.	Serum sup.	$BaSO_4$ norm. plasma	Expected	Found
1	1	—	—	18	100	96
—	—	1	—	4	—	25
1	—	1	—	18	121	230
—	—	—	1	2	—	30
1	—	—	1	18	126	180

* Determined by the Rees-Ecker method.
† This value was obtained on serum from splenic vein blood obtained immediately before splenectomy. The prothrombic activity of serum simultaneously obtained from splenic artery blood was 142%.

Shortly after splenectomy, coincident with clinical improvement and return of the platelet count and bleeding time to normal, the clotting defect disappeared. It is of interest that the platelet count and serum prothrombic activity were the same in splenic vein blood as splenic artery blood obtained at the time of operation.

DISCUSSION

Hemophilic, thrombocytopenic and silicone sera, obtained one hour after coagulation, frequently show *more prothrombic activity* by the one-stage procedure than the parent plasmas. An agent (spca) can be separated from these sera which is capable of accelerating the conversion of prothrombin to thrombin in the one-stage method. Since it is highly unlikely that prothrombin is elaborated as a consequence of coagulation, it is concluded that the high prothrombic activity is due to the presence of this accelerator plus whatever prothrombin remains unconsumed, thus giving an apparently greater prothrombin "concentration" than actually obtains. This is further substantiated by the fact that values obtained simultaneously by the two-stage procedure are markedly lower. The specificity of the one-stage technique in measuring serum prothrombin concentration is, therefore, highly dubious since it reflects the activity not only of prothrombin but also of other moieties.

A reliable method for the determination of serum prothrombin would be of considerable practical value since it would permit accurate measurement of the effect of hemostatic agents on prothrombin consumption. Quick (2), using the one-stage method, reported that the clot-promoting effect of normal plasma on hemophilic blood could thus be estimated. Unfortunately, the lack of specificity of the one-stage technique invalidates the computation of prothrombin consumption from plasma and serum values obtained by this procedure.

The question arises whether the two-stage procedure is more specific in the determination of serum prothrombin. Earlier data on hemophilic serum (1) were obtained by the old two-stage method without supplements of serum Ac-globulin. It is now known that this clotting factor is necessary to assure most rapid prothrombin conversion and maximal yield of thrombin (17). Since nothing is known regarding the concentration of Ac-globulin in the plasma or serum of hemophilic or thrombocytopenic subjects, and in view of the remarkable lability of serum Ac-globulin in man, earlier observations may need reevaluation. A recent modification of the two-stage technique controls this possible variable by addition of purified bovine serum Ac-globulin or beef serum (14).

Furthermore, spca does not affect thrombin evolution in the two-stage system (18). Under such circumstances its presence in serum does not influence the results. Accordingly, we feel that the modified two-stage method is more reliable for the determination of serum prothrombin concentration, and provides a more valid basis for computing prothrombin consumption.

The prothrombic activity of serum (one-stage) is the resultant of unconsumed prothrombin and the amount of spca evolved during coagulation as well as other factors. We have previously reported on the small amount of this accelerator demonstrable in hemophilic, thrombocytopenic and silicone serum (5, 6, 8). This was based upon observations that the admixture of these sera with normal plasma (5, 6, 8), or with prothrombin-rich plasma fraction (18) failed to result in prothrombic activity much greater than could be accounted for by the sum of the activities of each component. In contrast, normal serum had a marked enhancing effect. The results in this paper show, nevertheless, that some spca was obtained from the abnormal sera. This suggests that the spca may be linked to the unconsumed prothrombin, and, accordingly, may be incapable of greatly affecting the velocity of thrombin evolution from *added* prothrombin.

Our observations indicate that in the clotting of hemophilic blood about 10–30% or even less of its prothrombin is consumed from the time it is drawn to one hour after coagulation. When coagulation of normal blood is retarded by silicone only 50% of the prothrombin is used whereas normal blood clotted in glass loses approximately 90%.

The clot-promoting effects of spca are of considerable interest. Not only is the coagulation of normal blood (8) accelerated but also that of hemophilic and silicone blood. The clotting time may be restored to normal without decreases in one-stage serum prothrombic activity. Indeed, in subject I. G. (Table II) added spca accelerated clotting while at the same time the one-stage serum prothrombic activity *increased*. The two-stage values, however, indicated substantial prothrombin consumption. These observations provide further evidence that the high serum prothrombic activity (one-stage) of hemophilic serum is due in part to spca.

The defect in the coagulation of hemophilic and silicone blood lies in the retarded evolution of thromboplastin. This results in slow prothrombin conversion. Clotting can be greatly accelerated by supplements of thromboplastin, or by accelerating in other ways the conversion of prothrombin to thrombin in the presence of limited amounts of thromboplastin. This appears to be the mechanism whereby spca promotes coagulation.

The question might be raised as to whether spca is thromboplastin. Against this concept is the fact that optimal amounts of thromboplastin are provided in both the one- and two-stage methods, and, furthermore, spca is highly active in the former and inert in the latter.

CONCLUSIONS

1. Studies are presented on the clotting defect in hemophilia and thrombocytopenia with reference to prothrombin consumption and serum prothrombic activity. Sera from hemophilic, thrombocytopenic and silicone blood frequently exhibit more prothrombic activity by the one-stage method than the parent plasmas. By the two-stage procedure prothrombic activity is lower, but still greater than normal.

2. A prothrombin conversion accelerator (spca) can be separated from these sera. The high one-stage prothrombic activity reflects unconsumed prothrombin plus spca which evolves during coagulation.

3. The addition of spca accelerated the coagulation of hemophilic and silicone blood. In hemophilia the one-stage serum prothrombic activity was not reduced, but by the two-stage technique substantial prothrombin consumption could be demonstrated.

4. Since spca is inert in the two-stage system, the modified two-stage method provides a more reliable measure of serum prothrombin than the one-stage procedure.

5. The clotting defect of one subject with idiopathic thrombocytopenic purpura disappeared with clinical improvement following splenectomy.

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