Effect of Coagulase-Globulin on Residual Prothrombin Activity (Prothrombin Consumption) of Hemophilic Blood. (19528)

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It has been demonstrated(1) that coagulase-globulin produces a marked reduction of the coagulation time of hemophilic blood *in vitro* and *in vivo*, and that in hemophilic blood the titer of this factor is reduced. Since Brinkhous(2) has presented evidence that the lack of a plasma factor results in slow or incomplete prothrombin conversion, and Quick (3) has demonstrated that after hemophilic blood clots there is little reduction in serum prothrombin activity ("prothrombin-consumption"), the present study was carried out to investigate the changes in residual prothrombin activity after clotting of hemophilic blood was accelerated *in vitro* by the addition of coagulase-globulin.

Materials and methods. 1. *Normal whole blood* (N.W.B.), drawn from a normal adult subject using siliconized apparatus. 2. *Hemophilic whole blood* (Hem. B.), drawn from 2 hemophiliacs (No. 1 and No. 2) using siliconized apparatus. 3. *Saline*, 0.85%. 4. *Coagulase-globulin* (CG) prepared from fresh normal plasma as previously described(1) and redissolved in a volume of saline equal to the original volume of plasma from which it was prepared. 5. *Coagulation time* (C.T.): Blood from the siliconized syringe added to 3 glass tubes, 10 x 75 mm, for each determination, the coagulation time at 37°C being determined by the third tube after preliminary observation of the first two. 6. *Prothrombin activity*, according to the method of Quick (4, p. 142) by determining clotting time at 37°C of a mixture of 0.1 ml deprothrombinized rabbit plasma, 0.1 ml thromboplastin, 0.1 ml, 0.02 M $CaCl_2$, and 0.1 ml of serum. Serum obtained by centrifugation (1 min. at 3,000 r.p.m.) of each tube at 15-minute intervals after initial formation of solid clot.

Results. As shown in Table I, the pattern of serially determined residual prothrombin in normal serum(1) is significantly different from that in hemophilic serum(2,7). The addition of 0.2 ml of CG(4,9) produces a striking reduction in the coagulation time and also alters the pattern of residual prothrombin in the direction of normal. When the volume of CG is increased to 0.4 ml(5,10) the effect is even more pronounced. The addition of normal blood to hemophilic blood(6,11) has a similar but somewhat less striking corrective effect. Of interest are the observations(3,8) that the addition of saline to hemophilic blood not only reduces slightly the coagulation time but also significantly alters the pattern of residual prothrombin.

Discussion. The previous demonstration (1) that hemophilic blood is partially deficient in coagulase-globulin and that this plasma fraction is able to remedy the clotting defect *in vitro* and *in vivo* may be, at least in part, explained by the present study. Since residual serum prothrombin in hemophilic blood indicates little or no consumption, and since the addition of coagulase-globulin fraction alters this pattern in the direction of normal to the same degree as an equivalent amount of whole blood, then it seems justified to conclude that the factor supplied by coagulase-globulin as well as by whole blood is intimately associated with prothrombin conversion. Brinkhous(2) has concluded that the factor deficient in hemophilia is needed for platelet utilization. Quick(3) has reached essentially the same conclusion, and has suggested that the plasma factor (thromboplastinogen) reacts with the platelet factor (thromboplastinogenase) to form active thromboplastin. Although this reaction between platelets and coagulase-globulin is still under investigation, preliminary observations indicate that the plasma factor which reacts with platelets is probably coagulase-globulin. Although the purity of the coagulase-globulin has been previously defined(1) and leaves much to be desired, nevertheless, it has been shown to be free of calcium, prothrombin, thrombin, and fibrino-

TABLE I. Effect of Coagulase-Globulin on Residual Prothrombin Activity (Prothrombin Consumption) of Hemophilic Blood.

	No.	ml		ml	C.T., min	Min after formation of solid clot 15	30	45	60
						—Prothrombin activity, sec—			
1. N.W.B.		2			8	8	24	25	26
2. Hem. B.	1,	2			24	10	9	9	8
3.	1,	2	+ Saline,	.2	19	9	12	12.1	13.1
4.	1,	2	+ CG,	.2	7	9.8	16	16	16.6
5.	1,	1.8	+ CG,	.4	6	8.9	20	21.1	22
6.	1,	2	+ N.W.B.,	.2	14	10	13	13.5	14.5
7.	2,	2			135	12	11	11	11
8.	2,	2	+ Saline,	.2	110	10	12.9	15	15
9.	2,	2	+ CG,	.2	12	9.5	15	16	18
10.	2,	2	+ CG,	.4	9	10	17.5	18.5	20.1
11.	2,	2	+ N.W.B.,	.2	13	10	14.1	15.1	15.2

gen, and its deficiency in hemophilic blood suggests a specific deficiency of this plasma factor.

The observations that simple dilution of hemophilic blood with saline not only reduces its coagulation time in glass and in silicone tubes(1) but also alters the pattern of residual serum prothrombin activity (Table I, 3,8) have at the present time no ready explanation. Tocantins(5) has supposed that dilution disrupts an "accelerator-inhibitor complex" and that in hemophilia there is an excess of inhibitor, but the evidence for such an increase in inhibitor is not generally accepted. In any case, whether or not dilution disrupts normal or abnormal physico-chemical relationships, its effect is considerable, and it must be emphasized that great caution must be used in interpreting the results obtained from artificial systems, particularly in the *in vitro* assay of antihemophilic substances.

Summary. 1. The coagulase-globulin fraction of normal human plasma not only reduces the coagulation time of hemophilic blood but also alters the pattern of residual serum prothrombin activity in the direction of normal. 2. An equivalent amount of normal blood produces the same effect. 3. Simple dilution of hemophilic blood with saline by itself produces significant alterations in coagulation time and residual prothrombin.

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Experimental Hypersensitivity. Relationship of Dosage to Serological and Pathological Responses Following Injection of Heterologous Protein.* (19529)

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Although the histological response in the rabbit to injections of heterologous protein has been repeatedly described(1-5), the fre-

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