Interaction of Factor VIII (Antihemophilic Globulin) with Hexadimethrine Bromide (Polybrene®)* (28554)

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It was shown(1) that intravenous injection of Polybrene into dogs resulted in a coagulation defect due to a marked depression of the blood level of coagulation Factor VIII. No evidence could be obtained to prove that loss of Factor VIII was due to direct interaction with Polybrene; the data, in fact suggested the opposite: a second injection of Polybrene failed to lower Factor VIII levels further, and no evidence of interference with Factor VIII activity could be demonstrated on addition of Polybrene to plasma *in vitro*.

Recently, during experiments designed to permit assay of Factor VIII activity in heparinized blood(2), evidence has been obtained suggesting that interaction between Polybrene and Factor VIII does occur, and that under certain conditions the presence of Polybrene in plasma leads to answers in the Factor VIII assay which are falsely low.

Methods. Plasma samples. Blood was collected from normal donors with a 2-syringe technic; all glassware was siliconed. Nine parts of blood were added to one part of 0.1 M acid citrate anticoagulant(3); and, after thorough mixing, the tubes were placed in a bath of melting ice. Within an hour of collection, plasmas were separated by centrifugation for 10 minutes at 10,000 *g* at 4°C. Plasmas from 6 to 12 donors were pooled and used within the hour or stored at a temperature of —25°C for a maximum of 2 weeks.

Blood from heparinized patients was collected by a similar technic. In some instances the citrate solution contained Polybrene (hexadimethrine bromide, Abbott) in a concentration of 500 μg/ml.

In vitro tests. One-tenth volume of Polybrene was added to normal plasma to provide a final concentration of 100 μg/ml. The plasma was then absorbed with one-tenth volume of aluminum hydroxide suspension (Cutter Laboratories, Berkeley, Calif.) at 37°C for 3 minutes. Next it was centrifuged at room temperature at 1500 *g* for 10 minutes, the supernate being removed and saved for testing.

The same amount of Polybrene was added to a second sample of plasma *after* it had been absorbed with aluminum hydroxide. A third aliquot of the same plasma had one-tenth volume of saline added after absorption and served as a control.

Factor VIII assays. Factor VIII assays were performed by the method of Bergna, modified slightly as described elsewhere(4). In brief, the technic consisted of incubating together a platelet substitute (cephalin), diluted beef serum, and plasma to be tested, and calcium. After 8 minutes the thromboplastin generated in this mixture was assayed by adding it with calcium to plasma containing a normal amount of prothrombin and fibrinogen. The beef serum supplied an excess of all plasma factors needed for thromboplastin generation except Factor VIII. It had been maximally contact-activated by addition of a Celite suspension (Celite, Analytical Filter-Aid, Johns Manville). The plasma to be tested was absorbed first with an aluminum hydroxide suspension (*vide supra*) and was added to the mixture in very high dilution, usually 1:200.

Measurement of Polybrene levels. No simple and accurate assay for Polybrene is available. It is best measured in terms of the amount of heparin it can neutralize. Heparin, in turn, is most accurately quantitated by a technic employing the thrombin time. In the method developed here, heparin was added to plasma containing Polybrene until slight excesses of unneutralized heparin remained. These were quantitated by the thrombin time.

Various concentrations of heparin (Heparin, sodium, Abbott, 1000 U/ml) were added to aliquots of the control plasma and to aliquots of the same plasma containing Polybrene. One-tenth ml thrombin was added to a tube

* This investigation was supported by a PHS Research Grant from Nat. Heart Inst.

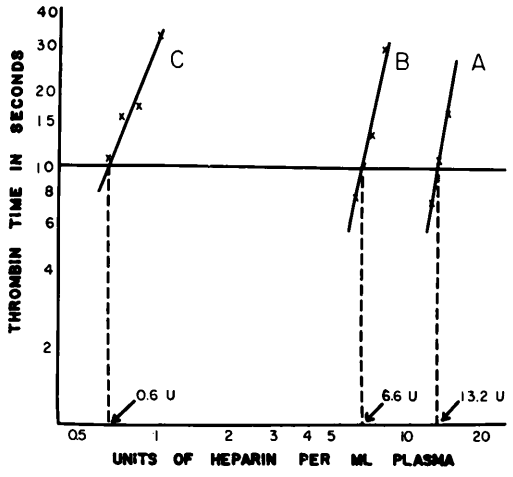

FIG. 1. Polybrene assay method. A. Polybrene added to plasma *after* absorption with aluminum hydroxide. B. Polybrene added to plasma *before* absorption with aluminum hydroxide. C. Control plasma without Polybrene.

containing 0.1 ml heparin and 0.1 ml plasma. The thrombin concentration used was that which would clot the control plasma in the absence of heparin in 5 to 10 seconds. Heparin concentrations were chosen for each plasma sample which provided clotting times between 5 and 60 seconds. It proved simpler and more accurate to run a series of such concentrations and to determine graphically the amount of added heparin which would produce the same clotting time in all plasmas, rather than to try to establish the exact amount by trial and error. The results were plotted on log log paper as illustrated in Fig. 1. For each sample of plasma the clotting times grouped closely around a straight line, and the lines for all samples were approximately parallel. The points at which these lines crossed the 10-second line on the graph were convenient to use to indicate an amount of heparin for each sample which resulted in an identical clotting time, indicating an identical amount of unneutralized heparin. The value of the control sample was then subtracted from the values obtained for the samples containing Polybrene, the remainders indicating the exact amount of heparin neutralized.

Results. Interaction between Polybrene and Factor VIII was first suggested by results of Factor VIII assays on samples of blood of patients undergoing open heart surgery. To eliminate the inhibitory effect of heparin, blood was collected in citrate containing Polybrene. The values obtained were markedly lower than those found in samples collected simultaneously in citrate, with Polybrene added after the plasma had been absorbed with aluminum hydroxide.

It seemed likely that Factor VIII had been lost by adsorption on the aluminum hydroxide suspension. It is well established (and we have confirmed the fact) that Factor VIII is not adsorbed on aluminum hydroxide from citrated plasma; it was therefore supposed that the presence of Polybrene might have altered this situation. Experiments were thus designed in which Polybrene was added to aliquots of the same plasma either before or after exposure to aluminum hydroxide.

Table I shows that addition of Polybrene to absorbed plasma causes a definite but relatively minor loss of Factor VIII. When Polybrene is added to plasma which is subsequently absorbed with aluminum hydroxide, the loss of Factor VIII is very striking. In the same table it can be seen that Polybrene is also lost in the adsorption step and that the average per cent loss is identical to the amount of Factor VIII lost.

Discussion. The assay of Factor VIII in blood containing heparin may provide fictitiously low results if the blood contains an amount of heparin sufficient to prolong the clotting times in the system used. This artifact can be avoided by the use of the heparin antagonist, Polybrene, in concentrations sufficient to neutralize the heparin present in the sample. Polybrene has no significant influence on our Factor VIII assay in the quantities used if it is added to the plasma after the step of absorption of plasma with aluminum hydroxide.

During the present investigation it became apparent that addition of Polybrene to the heparinized blood or plasma before the assay was begun resulted in Factor VIII levels much lower than those obtained in a sample simultaneously collected in citrate alone, even though citrate was present in both samples. The loss of Factor VIII occurred during the step in the assay procedure in which the plasma was absorbed by a suspension of aluminum hydroxide. Polybrene was also lost from the

TABLE I. Effect of Aluminum Hydroxide on Polybrene and Factor VIII Levels.

	Polybrene (units of heparin neutralized per ml plasma)				Factor VIII (% of mean normal activity)			
		Polybrene				Polybrene		
Exp No.	Saline control	Before*	After*	% loss	Saline control	Before*	After	% loss
1	.5	5.8	13.0	58	52	16	49	67
2	.6	6.6	13.2	52	75	26	57	54
3	.9	8.6	13.8	40	140	58	127	54
4	.7	6.8	14.4	56	95	36	69	48
5	.8	6.4	17.2	66	79	31	67	54
6	.8	5.4	12.2	60	92	37	76	51
Mean	.7	6.6	13.9	55	89	34	74	55

* Added to plasma *before* or *after* absorption of plasma with aluminum hydroxide.

plasma at this stage. Since Factor VIII is not lost from citrated plasma by adsorption on aluminum hydroxide when Polybrene is not present in the plasma, it appears likely that some interaction between Polybrene and Factor VIII is responsible for the different results. Most likely, Polybrene is adsorbed to aluminum hydroxide and along with it some of the Factor VIII present in the sample.

Evidence that Factor VIII interacted with Polybrene was sought by us in a previous investigation(1) in which it was shown that intravenous administration of Polybrene to dogs resulted in a profound drop in the plasma Factor VIII levels. (This drop was not an artifact due to adsorption of Factor VIII along with Polybrene during the assay procedure because Factor VIII levels remained low long after Polybrene had disappeared from the circulation.) Several aspects of the results were believed to make direct interaction of Factor VIII and Polybrene unlikely: a second injection of Polybrene given shortly after the first did not lower the Factor VIII levels further, and Polybrene had no effect on Factor VIII assay results when added to plasma *in vitro*. On the other hand, Polybrene was added to the plasma after it had been absorbed with aluminum hydroxide, and the results presented above demonstrate a striking loss of Factor VIII activity if Polybrene is added to the plasma before it is absorbed, Polybrene being adsorbed to an identical extent. Other data from the previous study were more consistent with direct interaction between the two compounds: Factor VIII levels dropped lower and faster the larger the Polybrene dose and the faster it was given. The evidence at the moment, thus, seems to be in favor of some sort of direct interaction between Factor VIII and Polybrene.

These results suggest some very interesting speculations: Polybrene has a high positive charge on its molecule. O'Brien(5) has presented suggestive evidence that heparin, a highly negatively charged compound, reacts with Factor IX. It is generally accepted that Factor VIII reacts directly with activated Factor IX in the sequence of thromboplastin generation(6). Since these 2 coagulation factors appear to have affinity for oppositely charged compounds, this may be a clue to the mechanism by which Factor VIII and Factor IX interact.

Summary. Coagulation Factor VIII and the heparin antagonist, Polybrene, are adsorbed together on an aluminum hydroxide suspension. Since Factor VIII is not adsorbed in the absence of Polybrene, this suggests that there has been a direct interaction between Factor VIII and Polybrene. The practical and theoretical consequences of this reaction have been discussed.

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Received June 14, 1963. P.S.E.B.M., 1963, v113.