THE NEUTRALIZATION OF HEPARIN WITH POLYBRENE[1]

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IN 1953 Preston and Parker (1) reported that polybrene neutralizes heparin *in vivo* and *in vitro*, the ratio necessary for neutralization *in vivo* in the dog being approximately 0.75 mg. of polybrene to 1.0 mg. of heparin.

In tests using dogs' blood a prolongation of the coagulation time occurred upon the addition of polybrene to normal blood *in vitro* (fig. 1). In a series of test tubes containing various amounts of polybrene and heparin the coagulation time of whole blood was shortest when the two drugs exactly neutralized each other. If either occurred in excess, coagulation times were prolonged. Because of these properties it was found possible to titrate for the presence of unknown quantities of heparin in the blood using known amounts of polybrene. This heparin-polybrene titration test is similar in principle to the heparin-protamine titration tests described by Allen and associates (3) and by LeRoy, Halpern and Dolkart (4). By means of this titration, circulating heparin was consistently detected in dogs after the injection of commercial heparin and during peptone shock. No heparin could be detected in normal dogs' blood or in dogs with radiation syndrome. Although this method of titration was useful in detecting circulating heparin in dogs' blood, subsequent attempts to apply the method to patients gave unreliable results (2).

These studies also showed that intravenous polybrene in dogs rapidly neutralized the hypocoagulability of blood caused by the injection of commercial heparin and the hypocoagulability of peptone shock (fig. 2 and 3) but had no effect on the coagulation defect of radiation syndrome. In the doses used (0.5 to 1.5 mg. per kg.), no alteration of coagulation of normal dogs' blood *in vivo* and no toxic effects were observed (1).

Polybrene is a quaternary ammonium salt having an empirical formula as follows: $(C_{13}\ H_{30}\ Br_2\ N_2)x$. It is a polymer, probably linear, of N,N,N'N'-tetramethylhexamethylene-diamine and trimethylene bromide (5). Its structural formula and chemical name are given in Figure 4. The drug was supplied in ampules as a colorless solution containin 15 mg. per cc.

The purpose of this paper is to report the effect of intravenous administration of the drug to heparinized and non-heparinized patients.

Clinical Studies

Dosage and administration. Polybrene was given intravenously in doses of 0.5 to 1.0 mg. per kg. to 33 patients hospitalized for a variety of unrelated conditions. Whole blood coagulation times were measured by a three tube Lee and White method before and at intervals after the injection in 31 patients. An effort was made to control several factors known to affect the coagulation time of blood using a technic previously employed by Preston and Parker (1). Silicone coated syringes were used. The coagulation time was considered to be the longest time taken for blood to coagulate in any of the three tubes. The coagulation time for normal human blood by this method is 10 to 25 minutes. All patients had normal blood coagulation times prior to injections of polybrene or heparin.

Sixteen patients received a single intravenous injection of 0.5 to 1.0 mg. per kg. of polybrene. In most cases the dose was given slowly in 10 cc. of physiologic saline solution. Coagulation times done at intervals up to 90 minutes after injection showed no deviation from normal.

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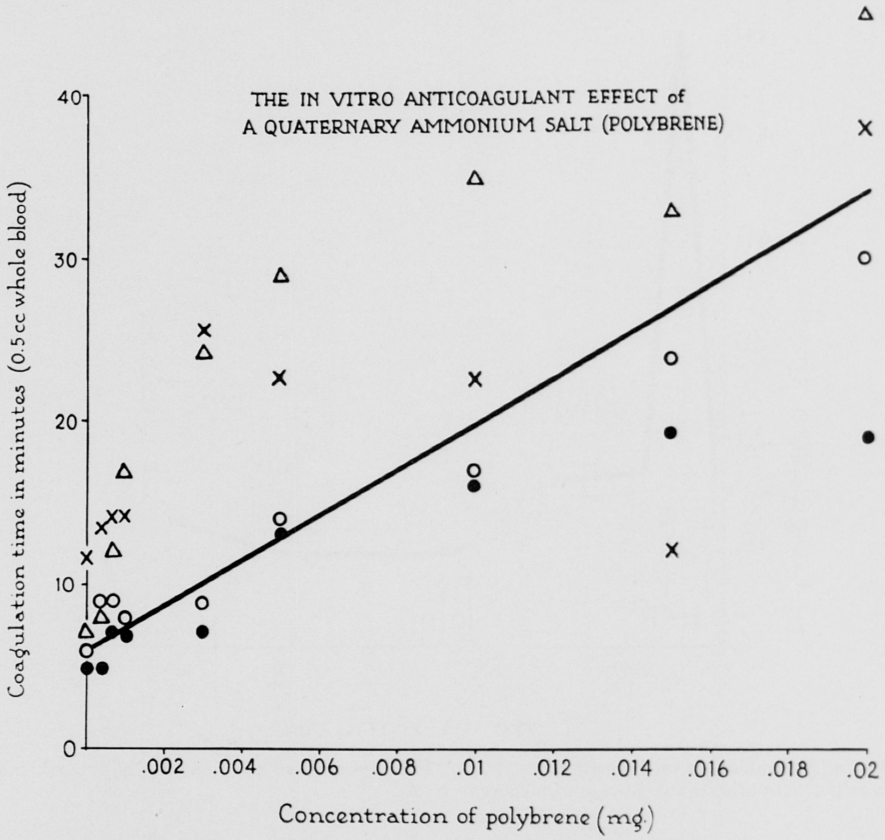

Fig. 1. Prolongation of the coagulation time of dog's blood by the addition of polybrene. Titrations using the blood of four dogs, indicated by the symbols ●, °, Δ, and x.

Ten patients received intravenous heparin followed a few minutes later by polybrene. Doses of heparin were 0.5 to 1.0 mg. per kg. and polybrene 0.4 to 1.0 mg. per kg. Blood was drawn for a second coagulation time approximately 5 minutes after completion of the injection of heparin. Through the needle still in place polybrene was injected in amounts varying from 0.4 to 1.0 mg. per kg. The exact doses of heparin and polybrene and the effect of the injections on the blood coagulation times are recorded in Table I. In most cases there was a prolongation of the coagulation time to 60 minutes or more following the injection of heparin. Polybrene produced a prompt neutralization of this anticoagulant effect.

One patient was given 50 mg. of heparin (0.62 mg. per kg.) 20 minutes after a similar dose of polybrene. The anticoagulant action of heparin was blocked, and coagulation times 30 and 180 minutes later were within normal limits.

Four patients were given concentrated aqueous heparin[5] subcutaneously in doses of 4 mg. per kg. This dose is sufficient to prolong blood coagulation time for approximately 16 hours in normal individuals. Figure 5 shows the result of administration of polybrene to 2 of these patients several hours after receiving the aqueous heparin and at a time when their coagulation times were prolonged. Elevated coagulation times were also returned promptly to normal in the other two patients but a repeated dose of polybrene was required in each one in order to maintain coagulation at normal levels until the effect of the heparin had worn off.

Four patients in the series of 33 received two or more injections of polybrene.

Toxic effects. Three of the earlier patients injected with polybrene complained of pain in the back or neck after completion of the injection (one patient

[5]Concentrated aqueous heparin (Abbott) contained 10,000 units per cc. Heparin used elsewhere in these experiments contained 1000 units per cc.

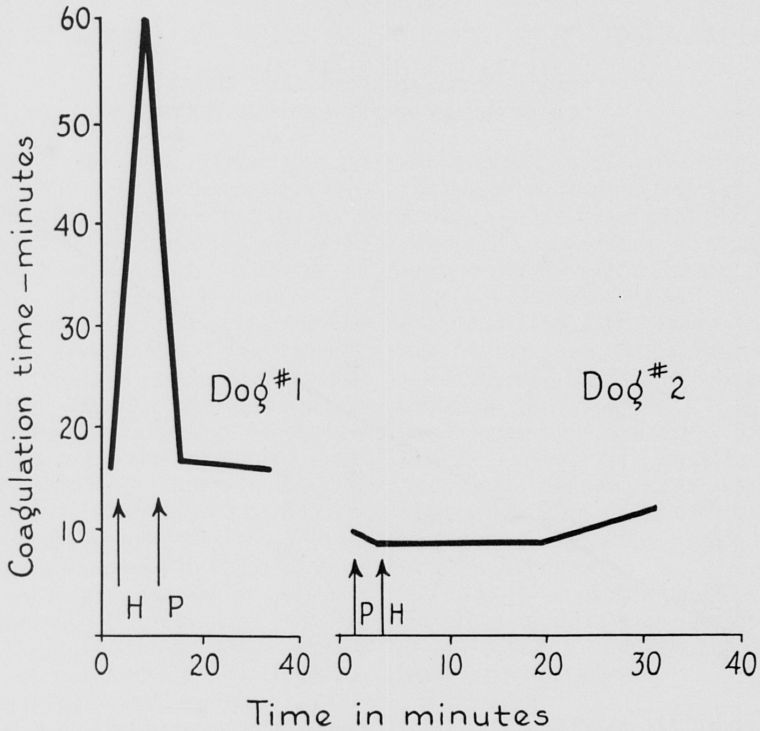

Fig. 2. Neutralization by heparin of polybrene. The dogs received 1 mg. per kg. of heparin intravenously at H and 0.75 mg. per kg. of polybrene at P.

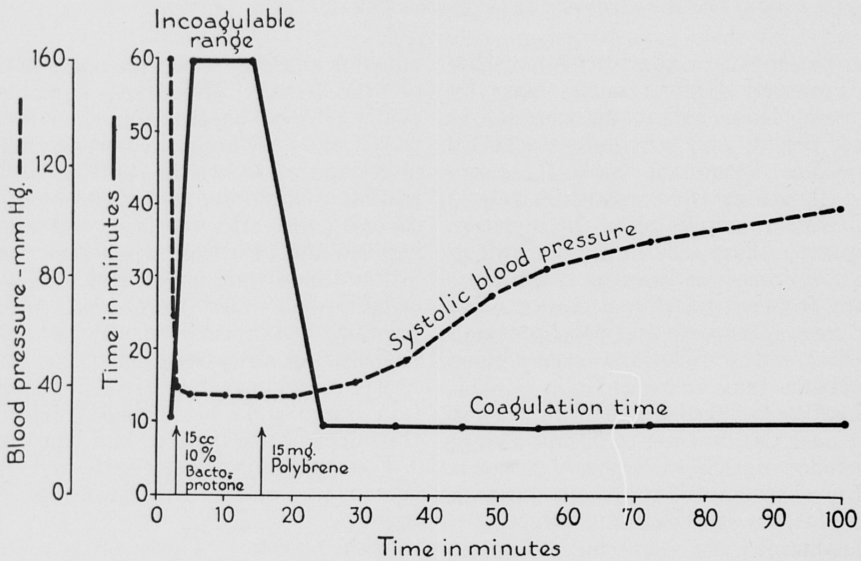

Fig. 3. Effect of 15 mg. of polybrene injected intravenously in a dog in peptone shock. The shock was induced by 15 cc. of 10 per cent bactoprotone. The coagulation defect was immediately neutralized but no effect on the blood pressure occurred.

TABLE I

EFFECT OF POLYBRENE ON THE HYPOCOAGULABILITY OF BLOOD PRODUCED BY HEPARIN INJECTED INTRAVENOUSLY

	Heparin followed by Polybrene, mg. per kg.						
	0.5 followed by 0.4—one patient	0.6 followed by 0.6—one patient	0.7 followed by 0.5—one patient	0.8 followed by 0.7—one patient	1.0 followed by 0.6—one patient	1.0 followed by 0.7—aver. 4 patients	1.0 followed by 1.0—one patient
	Lee and White Coagulation times in minutes						
Before Heparin	18	17	10	19	19	13	10
2-5 minutes after Heparin	71	130	65	101	125	83	30*
5-10 minutes after Polybrene		21	16	13	34	11	10
30 minutes after Polybrene	14	11	18	11	35		

*This coagulation time was made 2 minutes after the intravenous injection of 1.0 mg. per kg. of heparin. In other patients the coagulation time was determined 4-5 minutes after the injection.

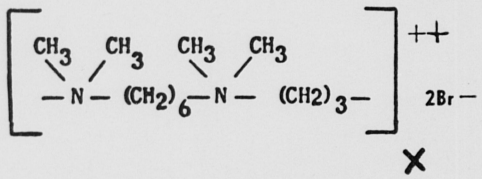

Fig. 4. "Polybrene" (1,5-dimethyl-1, 5-d diazaundecamethylene polymethobromide.)

received 0.5 mg. per kg. and two 0.7 mg. kg.). In two patients the pain was severe, but in all it was of short duration and subsided spontaneously. One of these patients complained of a flushing sensation, and another complained of a metallic taste in her mouth. In one patient who complained of severe back pain, blood pressure recordings were made at 5 minute intervals for 20 minutes after injection. No change in pressure was noted.

For subsequent patients the doses of polybrene was diluted with 0.9 per cent saline solution to a volume of 10 to 15 cc. Three to five minutes were taken for the injection. With these precautions no toxic effects were observed even though larger doses of polybrene were used, except for one anaphylactic reaction.

This patient received three doses of polybrene of 1.0, 0.7 and 0.35 mg. per kg. Times between doses were 30 weeks between the first and second and 5 weeks between the second and third. The third injection was discontinued when the patient suddenly developed nausea, chest pain, severe spasms of coughing, and generalized erythema interpreted as a mild anaphylactic reaction. The symptoms subsided within 4 minutes.

Following one intravenous injection, a small amount of polybrene was inadvertently allowed to escape outside a cubital vein. There were pain and redness which persisted for 48 hours. This was expected since the solution used was alkaline in reaction.

Thrombocytes were counted in 10 patients. In 7 there was a decrease in circulating thrombocytes to about 50 per cent of pre-injection levels, apparent 5 to 30 minutes after the injection of polybrene. Thirty to 60 minutes after the injection the thrombocyte counts returned to normal. In 3 patients, one who

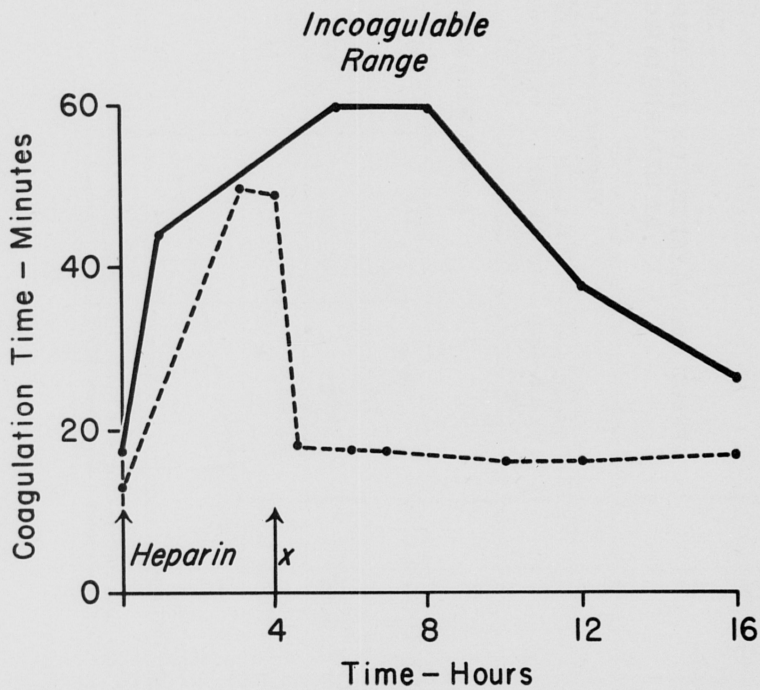

Fig. 5. Effect of polybrene following the subcutaneous injection of concentrated heparin. The solid line is a control experiment showing the prolongation of coagulation time following the injection of 400 mg. of heparin (average 6 patients.) The broken line shows the effect of injection of 400 mg. of heparin followed by the intravenous injection of 50 mg. of polybrene at X (average 2 patients).

received 0.5 mg. per kg. and two 0.7 mg. per kg., no significant change in thrombocyte counts were noted.

Observations of blood pressure, pulse, and numbers of erythrocytes and leucocytes were made and urinalyses were run before and during the first 24 hours after the injection of polybrene in most of these patients. No abnormalities attributable to polybrene were observed.

Discussion

Antiheparin agents are useful clinically to reverse the anticoagulant effect should bleeding occur or surgical intervention become necessary in a heparinized patient. In addition, there are a variety of conditions characterized by abnormal bleeding in which therapy with antiheparin agents have been reported to be effective (4, 6, 7, 8). These include selected cases of post partum hemorrhage, menorrhagia, acute and chronic leukemia, irradiation hemorrhage, primary and secondary thrombocytopenic purpura, and others. Some of these patients have had prolonged coagulation times and positive protamine titration tests which Allen has interpreted as being the result of a heparinoid disturbance in the clotting mechanism (6).

Of particular interest to surgeons is the profuse ooze from wounds or mucous membranes which may occur in patients in shock, or during surgical operations in association with blood transfusion reactions. A circulating anticoagulant neutralizable with antiheparin drugs is the responsible mechanism in some of these patients, whereas increased fibrinolysis may be the chief mechanism in others (8, 9). Toluidine blue has been used successfully as treatment in the former group of patients by Friesen (8), and human fibrinogen intravenously has been recommended by Coon and Hodgson (9) for the latter.

The antiheparin effect of polybrene is similar in most respects to that of protamine sulfate and toluidine blue. Polybrene is more potent than either. It is stable and may be stored for long periods of time. The recommended dose for clinical use is 0.5 to 0.7 mg. per kg. It neutralizes heparin in the ratio of approximately 0.7 mg. of polybrene to 1 mg. of heparin. One dose of polybrene is sufficient to neutralize a dose of intravenously injected heparin. The neutralization effect wears off in several hours and therefore two or three doses of 0.5 mg. per kg. may be necessary to neutralize subcutaneously administered or depot heparin. Polybrene appears to be a satisfactory antiheparin drug for clinical use.

Summary

1. A new antiheparin drug, polybrene, was administered to 33 hospitalized patients with normal clotting mechanism.

2. No effect on the coagulability of blood, pulse, blood pressure, erythocyte, count, leucocyte count or urinalyses was noted.

3. A transient decrease in circulating thrombocytes occurred in 7 of 10 patients studied.

4. Polybrene promptly neutralized the anticoagulant effect of heparin.

5. Polybrene is a suitable antiheparin drug for clinical use. The recommended dose is 0.5 to 0.7 mg. per kg. It must be given intravenously, slowly and in dilute solution to prevent toxic effects.

References

1. Preston, F. W. and Parker, R. P.: New Antiheparin Agent ("Polybrene"), Arch. Surg., 66: 545-550, 1953.
2. Preston, F. W. and Parker, R. P.: Unpublished data.
3. Allen, J. G., Moulder, P. V., Elgehammer, R. M., Grossman, B. J., McKeen, C. L., Sanderson, M., Egner, W. and Crosbie, J. M.: A Protamine Titration as an Indication of a Clotting Defect in certain Hemorrhagic States, J. Lab. & Clin. Med., 34:473-476, 1949.
4. LeRoy, G. V., Halpern, B. and Dolkart, R. E.: An Indirect, Quantitative Method for the Estimation of Heparin Activity *in vitro*. The Heparin-protamine Titration Test. J. Lab. & Clin. Med., 35:446-458, 1950.
5. Moore, M. B.: Abbott Laboratories, North Chicago, Ill. Personal communication to the authors.
6. Allen, J. G. and others. Abnormal Bleeding. Response to Treatment with Toluidine Blue and Protamine Sulfate, J.A.M.A. 139:1251-1254, 1949.
7. Holoubek, J. E., Hendrick, J. V. and Hollis, W. J.: Toluidine Blue in Bleeding Associated with Thrombopenia, J.A.M.A., 139:214-216, 1949.
8. Friesen, S. R., Harsha, W. N. and McCroskey, C. H.: Massive Generalized Wound Bleeding during Operation with Clinical and Experimental Evidence of Blood Transfusion Reaction, Surgery, 32:620-629, 1952.
9. Coon, Wm. W. and Hodgson, P. E.: Fibrinolysis in Surgery Patients. I. Possible Relationship to a Hemorrhagic Diathesis, Surg., Gynec. & Obst., 95:717-724, 1953.