ing of ventricular muscle was the outstanding action. Conduction time in ventricular muscle was prolonged and the threshold for electrical stimulation raised. The characteristic shortening of refractory period produced by digitalis glycosides did not occur. The tendency to cause spontaneous tachycardias would preclude the use of coumingine in clinical medicine.

Received September 10, 1951. P.S.E.B.M., 1951, v78.

Failure of Transfusion of Hemophilic Plasma to Induce Abnormalities of Hemostatic Mechanism in Normal Individuals. (19044)

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Most investigators agree that the fundamental defect in hemophilia is represented by the deficiency of a specific plasmatic factor(1). This factor has been variously defined as "antihemophilic globulin", "thrombocytolysin", "plasmakinin", "thromboplastinogen", etc. Much experimental evidence has accumulated in support of this theory, the most significant observation being perhaps that small amounts of "antihemophilic globulin" are able to normalize temporarily the clotting defect of hemophiliacs. On the other hand, some evidence has been presented to show that hemophilia might be due to the presence of a circulating anticoagulant; a significant finding in support of this theory is that hemophilic plasma may be made to clot normally by simple dilution with physiological saline(2).

The favorable effect of transfusion of fresh normal plasma on the clotting activity of hemophiliacs is well known. On the other hand, the effect of transfusion of hemophilic plasma on the clotting mechanism of normal subjects does not seem to have been investigated. Should a circulating anticoagulant be responsible for the clotting defect of hemophilia, the transfusion of sufficient amount of hemophilic plasma in normal subjects could be expected to be followed by detectable abnormalities of the coagulation mechanism.

Materials and methods. Two patients with classical hemophilia as confirmed by clinical and laboratory data were used as donors in this study. Both patients presented the usual response to transfusion of fresh human plasma and did not show precipitating antibodies(3) against human plasma or "antihemophilic globulin". Five hundred ml of blood were collected in Baxter vacuum bottles containing 100 ml of ACD solution, the donors fasting for at least 4 hours prior to donation. The blood was centrifuged at 2,000 r.p.m. for one hour in refrigerated centrifuge and the plasma separated with sterile technic. Within 2 hours from the time of collection the plasma was injected into compatible normal subjects. An amount of 5 ml of plasma per kilo weight was administered at a speed of 150-200 drops per minute. In the hemophiliacs prior to donation and in the normal subjects before, immediately after the transfusion and one hour later the following determinations were made: (a) bleeding time, according to the method of Ivy *et al.* (4); (b) tourniquet test, according to the method of Frugoni and Giugni(5), maintain-

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ing a pressure equal to the mean pressure of the subject for 10 minutes; (c) platelet count, according to the method of Dameshek(6); (d) clotting time of whole blood in glass and silicone-coated test tubes, according to a modification of the method of Lee and White (7); (e) prothrombin activity of plasma and serum, according to the procedures recently described by one of us(8,9); (f) activity of plasma prothrombin conversion factor (PPCF, labile factor) in plasma and serum, according to the methods of one of us (8,10); (g) accelerator effect of serum, by the method of de Vries *et al.*(11); (h) plasma fibrinogen level, by a slight modification of the method of Quick(12); and of the fibrinolytic activity of plasma, by the method of one of us(13); (i) ability of plasma collected from normal subjects after the transfusion with hemophilic plasma to correct the coagulation defect of known hemophiliac *in vitro*. Citrated (0.02 M) plasma under study and citrated plasma of known hemophiliac were mixed in various proportions to a total volume of 0.9 ml; 0.1 ml of $CaCl_2$ M were added and the clotting time of the mixture and the prothrombin activity of the serum one hour after the completion of coagulation determined; (j) effect of the addition of plasma collected from normal subjects after the transfusion with hemophilic plasma on the coagulability of normal plasma *in vitro*. Citrated plasma from normal individual was mixed with citrated plasma obtained from normal subjects after transfusion of hemophilic plasma, in various proportions to a volume of 0.9 ml. The rest of the procedure was as described under paragraph (i); (k) changes in the electrophoretic pattern of plasma from normal subjects after transfusion with hemophilic plasma. Hemophilic plasma presents a characteristic pattern(14), which was found in plasma from both hemophiliacs used in this study. The technic employed was that of Bernfeld *et al.*(15).

Results. The results obtained in these experiments are summarized in Tables I, II and III, where the findings in one case are reported. The other case gave essentially similar data. Both normal subjects received an amount of hemophilic plasma equal to approximately ⅛ of their calculated plasma volume. No abnormality of the hemostatic mechanism was detected in normal recipients following the transfusion. No bleeding manifestations of any kind were noted; bleeding time, capillary fragility and the coagulation factors examined were found unchanged after the transfusion. No alterations of the normal electrophoretic pattern were noticed. Moreover, it is significant that plasma collected from normal subjects immediately after the transfusion of hemophilic plasma was still able to correct temporarily the coagulation defect of a known hemophiliac. It also did not retard in any way the coagulation process of other normal plasma *in vitro*. To exclude the possibility of any delayed effect, one recipient was studied 24 hours after the transfusion of hemophilic plasma. Again, no abnormality of the hemostatic mechanism could be detected.

A drop in the platelet count was noticed immediately following the administration of plasma; the platelet count gradually rose and came back to normal level in 3 hours. Such a phenomenon cannot, however, be specifically attributed to the transfusion of hemophilic plasma. It has been observed by us that a temporary drop in the platelet level occurs in the majority of normal subjects receiving normal plasma(16).

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TABLE I. Effects of the Transfusion of Fresh Hemophilic Plasma in Normal Subjects.

	Hemophiliac	Normal		
		Before transfusion	After transfusion	1 hr later
Red blood cells, millions/cmm	2.95	4.01	3.86	3.79
White blood cells/cmm	8700	7100	7500	9000
Polymorphs, %	65	60	58	61
Lymphocytes, %	29	27	32	34
Monocytes, %	5	9	8	5
Eosinophiles, %	1	2	1	1
Basophiles, %	0	2	1	0
Platelets/cmm (× 10,000)	64.8	44.1	27.02	35.24
Hematocrit, %	30	39.5	38	37
Bleeding time, min	8	4½	5	3½
Tourniquet test, petechiae*	12	6	8	5
Clotting time of whole blood glass test tubes, min	59	8	7½	7
Silicone-coated test tubes, min	470	25	18	22
Prothrombin activity, plasma, %	95	100	100	100
" " serum, %	90	2.4	2	3.5
PPCF, plasma, %	80	95	100	90
" serum, %	65	4.5	12	0
Accelerator effect of serum, %	14.5	75.6	92.6	70.8
Plasma fibrinogen level, mg %	372.8	342.6	297.5	354.9
Plasma fibrinolytic activity, %	4	8	17	9.5

* No. of petechiae on volar surface of forearm in area 1 inch diam., after application of mean arterial pressure for 10 min.

TABLE II. Ability of Plasma Collected from Normal Individual Before and After Transfusion of Hemophilic Plasma to Correct Coagulation Defect of Known Hemophilic Plasma.

(a) Hemophilic plasma, vol	10	9	5	1	0
Plasma from normal individual, vol	0	1	5	9	10
Clotting time of recalcified plasma, min	22	5	3½	3½	3
Prothrombin activity of serum, %	90	72.5	33.7	18.2	3
(b) Hemophilic plasma, vol	10	9	5	1	0
Plasma from normal individual, inj. with hemophilic plasma, vol	0	1	5	9	10
Clotting time of recalcified plasma, min	23½	4½	3½	2½	2½
Prothrombin activity of serum, %	87.5	68.4	40.2	12.5	4

TABLE III. Clotting Ability of a Normal Plasma *in vitro* After Addition of Various Volumes of Plasma Collected from a Normal Individual Receiving a Transfusion of Hemophilic Plasma.

(a) Normal plasma, vol	10	9	5	1	0
Plasma from normal individual, before transfusion of hemophilic plasma, vol	0	1	5	9	10
Clotting time of recalcified plasma, min	2¼	2½	2½	2½	3
Prothrombin activity of serum, %	.9	2.3	2	1.8	3
(b) Normal plasma, vol	10	9	5	1	0
Plasma from normal individual, after inj. of hemophilic plasma, vol	0	1	5	9	10
Clotting time of recalcified plasma, min	3	2¼	2½	2½	2½
Prothrombin activity of serum, %	2.1	1.3	.9	2	1

It is realized that in experiments of this kind a circulating anticoagulant may fail to reveal its presence through excessive dilution. With this possible limitation, our findings fail to support the view that an inhibitory or anticoagulant factor represents the fundamental defect in hemophilia.

Summary. In an attempt to demonstrate the presence of a circulating anticoagulant in hemophilia, normal human subjects were

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transfused with compatible hemophilic plasma. No effect on the hemostatic mechanism of normal subjects was demonstrated following the transfusion, nor did the ability of the recipient's plasma to correct the clotting defect of known hemophilic plasma change following this procedure.

Received September 10, 1951. P.S.E.B.M., 1951, v78.

Anticonvulsant Activity of 2,2-Disubstituted-1,3-Propanediols in Electroshock Seizures. (19045)

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It has been recently shown that certain 2,2-disubstituted-1,3-propanediols possess marked anticonvulsant properties against convulsions produced by pentylenetetrazole (metrazol) (1,2). One compound of this series, 2,2-diethyl-1,3-propanediol (DEP) which received a more thorough pharmacological investigation(1-4) proved very effective against convulsions produced by chemical agents such as strychnine, pentylenetetrazole and picrotoxin, but was relatively weak in preventing electroshock seizures(1,4). It was of interest to investigate whether all 2,2-disubstituted-1,3-propanediols would show stronger anticonvulsant activity in chemoshock convulsions than in electroshock convulsions or whether this property was peculiar to DEP. In view of the close chemical relationship of certain 2,2-disubstituted-1,3-propanediols to barbiturates, the possibility that each of these compounds may be converted in the body to the same anticonvulsant substance was considered.

Experimental. White male mice of the CF-1 strain weighing between 18-24 g were used. Seizures were produced by applying through corneal electrodes 60 cycle current from a Hans-Tech electroshock apparatus with a current intensity of 50 milliamperes and 0.2 second duration. With the machine used the current delivered was independent of the external resistance. Experiments have shown that with a stimulus of 12.5 milliamperes and duration of 0.2 second about 50% of mice convulse; thus 50 milliamperes represents about 4 times the convulsive threshold dose. Most mice survived repeated shocks of 50 milliamperes administered at hourly intervals without showing marked changes in sensitivity to electroshock seizures. Drugs were administered orally in a 5% suspension of gum acacia. Groups of 10 mice were treated at dose levels increasing in geometrical progression by a factor of approximately 1.5 and shocked 30, 90 and 150 minutes after the administration of the drug. The number of animals protected at each dose level was noted. A mouse was considered to convulse if it showed the extensor tonic phase of the convulsion as described by Toman *et al.*(5). Mice showing only clonic seizures are considered non-reactors. The results were obtained graphically(6) and are expressed as the dose protecting 50% of the animals from the tonic extensor phase of electroshock convulsions. The compounds used in these studies were prepared by Dr. B. J. Ludwig and his associates of these Laboratories.

Results. The table gives the doses in mM/kg protecting 50% of animals from the extensor tonic phase of electroshock seizures, applied 30 minutes after administration of

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