levels of hemoantibodies than controls; these findings, however, are not yet conclusive.

All experiments described in this report were terminated shortly after the last PVP administration. For this reason no statement can be made as to the duration of the storage of PVP. It still remains to be established whether morphologic and functional changes attributable to the administration of PVP are wholly or partially reversible, or permanent.

Summary. 1. Storage of PVP, or of a derivative of PVP, was observed in mice after 12-24 injections of a 3.5% solution. The extent of storage appeared to depend on the mouse strain. 2. Simultaneous administration of PVP and sulfonated azo dyes led to renal excretion of dye, and to a decrease in reticulo-endothelial dye storage. 3. PVP administered after dye was given resulted in elution of dye from reticulo-endothelial tissues. 4. PVP administered prior to dye injection prevented storage of dye. 5. On the basis of these findings, an attempt was made to evaluate biologic properties of PVP.

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Studies on Platelets. IV. A Thrombocytopenic Factor in Normal Human Blood, Plasma, or Serum.* (19466)

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In abnormal conditions, individuals may develop sensitivity to normal plasma and respond to its administration with a series of symptoms which have been grouped under the definition of "plasma transfusion reaction" (1). This has been found to occur consistently in paroxysmal nocturnal hemoglobinuria and, occasionally, in some types of blood dyscrasia and extensive carcinomatosis. Most of these patients exhibit some degree of thrombocytopenia.

Marked, but transitory thrombocytopenia appeared, in our earlier observations(2), to be one of the characteristic features of plasma transfusion reaction. Further study, directed to investigate the specificity of this finding revealed that temporary but significant reduction in blood platelets usually followed the transfusion of compatible blood, plasma or serum of normal donors into normal recipients

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TABLE I. Thrombocytopenic Effect of the Transfusion of Blood, Plasma and Serum in Non-Thrombocytopenic Recipients (23 of 36 Cases).

Material admin.	Type and group		Diagnosis		Percentage and time of max platelet drop	
	Donor	Recipient	Donor	Recipient	%	Hr
1. Blood	O,cde	O,cde	Polycythemia vera	Normal	46 in	½
2. "	A,Cde	A,Cde	Normal	"	40	1
3. " *	O,CDe	O,CDE	"	Idiopathic hypothrombinemia	63	1
4. Plasma	O,cDe	O,CDe	"	Normal	43	1
5. "	A,CDe	A,CDe	"	"	51	2
6. "	O,CDe	O,CDe	"	Lobar pneumonia (convalescent)	54	1
7. "	"	"	"	"	48	1
8. "	"	O,CDe	"	"	51	1
9. "	"	B,CDe	"	Normal	9	½‡
10. "	O,cdE	O,Cde	"	"	45	3
11. "	O,cDE	O,CDe	"	Adenoma of prostate	50	1
12. "	O,cde	"	"	Chronic glomerulonephritis	58	1
13. "	O,Cde	O,Cde	"	Obesity	44	1
14. "	A,CDe	A,cDe	"	Cataract	40	1
15. "	AB,cDE	AB,cDe	"	Neuroasthenia	56	1
16. "	B,Cde	B,Cde	"	Congenital spherocytosis§	63	½
17. "	A,Cde	A,Cde	Idiopathic hypoprothrombinemia	Peptic ulcer	60	½
18. "	"	"	Normal	Idiopathic hypoprothrombinemia	62	1
19. "	O,CDe	O,CDe	Liver cirrhosis	Iron deficiency	43	1
20. "	B,cdE	B,cDe	Disseminated lupus	Normal	30	1‡
21. "	A,cdE	A,Cde	Normal	Liver cirrhosis	48	1
22. Serum†	A,Cde	A,CDe	"	Idiopathic hypoprothrombinemia	76	6
23. "	AB,CDe	AB,CDE	"	Normal	58	3

* Transfusion followed by urticarial rash. † Transfusion followed by moderate chill and fever. ‡ Considered a negative result. § Splenectomized one year previously.

or nonthrombocytopenic patients(3). In practically all instances, no evidence of clinical reaction occurred at the time of the induced thrombocytopenia.

Materials and methods. Blood, plasma or serum were transfused into 36 compatible recipients, both normal individuals and patients suffering from various diseases but with normal platelet count. The recipients used in this series were selected from individuals who had never been previously transfused, since substances with agglutinating or lysing activity against platelets are demonstrable occasionally in the serum of individuals who had received multiple blood or platelet transfusions(4). The donors, normal subjects and a few patients with various pathological conditions but with normal platelet level, were fasting for at least 4 hours prior to donation. Four hundred ml of blood were collected in vacuum bottles containing 100 ml of ACD solution.§ The plasma was separated by centrifugation at 2,000 r.p.m. for one hour and aspirated into vacuum containers. Serum was obtained by separation from clotted blood collected in vacuum bottles; it was then kept at room temperature for 5 hours to allow complete neutralization of thrombin and stored at 4°C until used. Blood, plasma and serum were typed, grouped and cultured. They were administered to compatible recipients within 5 days of collection, when not otherwise stated, in an amount of approximately 10 (blood) or 5 (plasma or serum) ml/kg weight for adults and 16 (blood) or 8 (plasma or serum) ml/kg weight in children at a speed of 80 to 100

§ 100 ml of ACD solution (Baxter) contain: sodium citrate, 1:37 g; citric acid, 0.5 g; dextrose, 2.45 g.

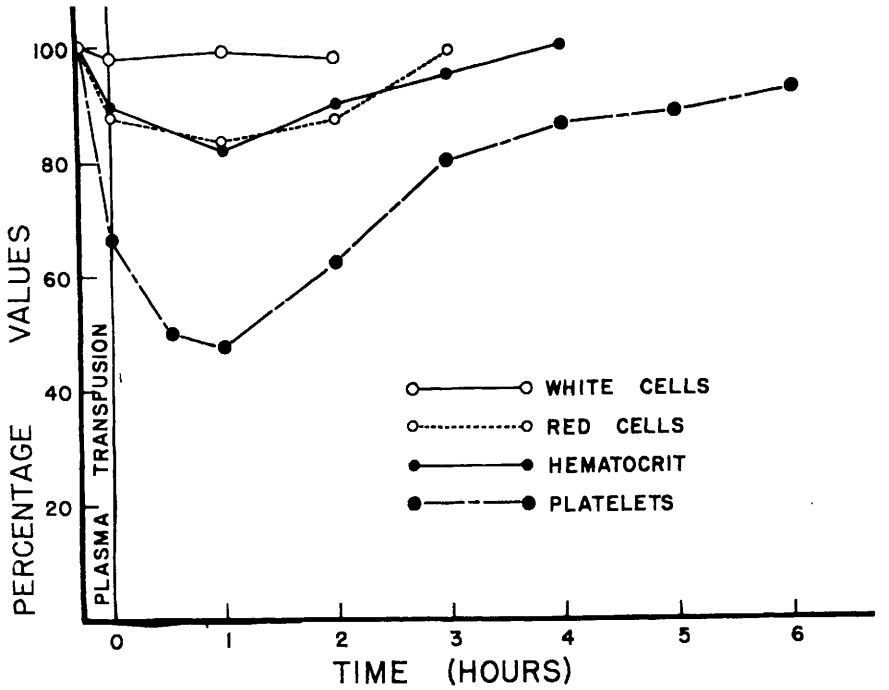

FIG. 1. Changes in hematocrit, red and white blood cells, and platelet count in normal recipients following transfusion of normal plasma (average values of 31 experiments).

drops per minute. Nineteen additional recipients received blood or variously treated plasma or plasma products, for a total of 55 observations in the course of this study. Mild clinical reactions were observed in two cases only: one recipient receiving serum (No. 16, Table I) complained of chill shortly after the termination of the transfusion and had fever for a few hours afterward; another presented urticarial rash at the end of the transfusion. Before, immediately after and at various intervals of time following the transfusion (30 minutes, 1, 2, 3, 6, 12, 24, 48 hours) several determinations were performed in all recipients. Hematocrit, red and white cell counts and differential count were obtained by standard technics(5); platelets were counted in duplicate by the indirect method of Dameshek(6). This method is accurate to ± 6.5%. The effect of the induced thrombocytopenia on the various factors and mechanisms of hemostasis was studied by means of a series of technics which have been outlined previously(7).

Results. a) *Effect of blood, plasma, and serum transfusion on the formed elements of the blood.* Moderate drop in the hematocrit reading (2 to 4 mm) for approximately 1 to 3 hours followed the transfusion of plasma (Fig. 1). At the same time red cell count and hemoglobin content decreased slightly (0.3 to 0.7 million mm^3 and 0.6 to 1.4 g per 100 ml respectively), in rough correlation with the hematocrit reading. Equivocal changes in the white cell count were noted, probably not significant in view of the daily fluctuations of this value. The number of platelets, on the other hand, dropped significantly (more than 30%) and consistently in 32 of the 36 cases studied within 3 hours of the administration of blood, plasma or serum. The percentage and time of maximum platelet drop are given in Table I which includes 23 representative cases. The lowest value was reached in most cases after 1 hour, the platelet level being back to normal as early as 1 hour and as late as 48 hours after the transfusion, with an average of approximately 4 hours (Fig. 1). The transfusion of whole blood in 3 recipients caused comparable effects, that of serum was associated with more marked and more persistent thrombocytopenia than in the case of

TABLE II. Effect of Transfusion of Normal Plasma and Serum on Platelet Level of a Normal Recipient.*

	Platelet level (per mm³)					
	Before	After	1 hr	3 hr	6 hr	18 hr
Plasma (5 ml/kilo wt)	734640	563200	374740	448000	536675	778900
Serum (" ")	751830	337740	290560	135600	293400	694400

* Plasma was injected first; patient allowed to recover completely from effect of transfusion. 48 hr later serum was injected.

blood or plasma (Table II). No hemorrhagic manifestations were observed in the course of this study. Clotting time of whole blood and native plasma in glass and Silicone-coated test tubes, clot retraction, prothrombin activity of plasma and serum were never significantly altered during the phase of induced thrombocytopenia. Bleeding time and tourniquet test remained normal. Platelets collected from the recipients at the peak of the induced thrombocytopenia appeared functionally active, since they were able to: a) restore to normal clot retraction, and b) accelerate normally the conversion of prothrombin to thrombin when added to normal native platelet-poor human plasma.

b) *Demonstration that thrombocytopenia induced by normal plasma is due to activity of a plasma component.* The administration of 100 ml of ACD solution (4 cases) and of red cells twice washed with saline solution (4 cases) was not followed by significant thrombocytopenia in normal recipients. These results indicated that the thrombocytopenia induced by transfusion was due to the activity of a factor present in plasma (and serum). *In 4 cases, however, there was no significant drop in platelet level after transfusion of the recipient's own plasma.*

c) *Thrombocytopenic effect of normal plasma after various treatments.* Five hundred ml of blood were collected from the same donors twice at an interval of 3 days. The plasma was separated, pooled and divided into 2 aliquots. The first was administered to a recipient within 2 days and the second one was given three days later, after appropriate treatment. These observations were run in duplicate, and in the second series the order of administration of the treated and untreated plasma was reversed. These experiments demonstrated that the thrombocytopenic activity of normal plasma was unaffected by storage at 4°C for 4 months, heating at 56°C for 30 minutes, decalcification by passage over a cation-exchange resin (IRC-50) (1 g per 10 ml of blood), collection in plastic bags‖ through Silicone-coated needles (which limits to a minimum the disintegration of platelets), passage through a Seitz filter. In the last experiment, since the presence of sodium citrate interferes with the removal of prothrombin by Seitz filters(8), plasma was obtained by centrifugation from blood decalcified by passage through a column of cation-exchanger IRC-50, and was subsequently filtered through Seitz under vacuum. Finally, administration of adequate amounts of heparin sodium to 4 recipients either before or during and after the plasma transfusion failed to modify the thrombocytopenic effect.

d) *Lack of thrombocytopenic activity of plasma after treatment with $Ca_3(PO_4)_2$ gel.* Under sterile conditions, 12 ml of $Ca_3(PO_4)_2$ gel 0.2 M per 250 ml of plasma to be used were centrifuged in 250 ml glass bottles at 2000 r.p.m. for 10 minutes to precipitate the solid gel and the supernatant water decanted. Since the presence of sodium citrate prevents the absorption of certain plasma proteins by gels(9), the blood was decalcified by passage through a column of ion-exchange resin IRC-50. Plasma was obtained by centrifugation, added to the gel, gel and plasma well mixed by agitation, and incubated at 37°C for 15 minutes. Plasma was then separated from the gel by centrifugation at 3000 r.p.m. for 1 hour in the cold and finally aspirated into a new 250 ml bottle. When injected into three normal recipients, the plasma so treated did not demonstrate appreciable thrombocytopenic activity (Fig. 2).

‖ Bought from Fenwalt Company, Ashland, Mass.

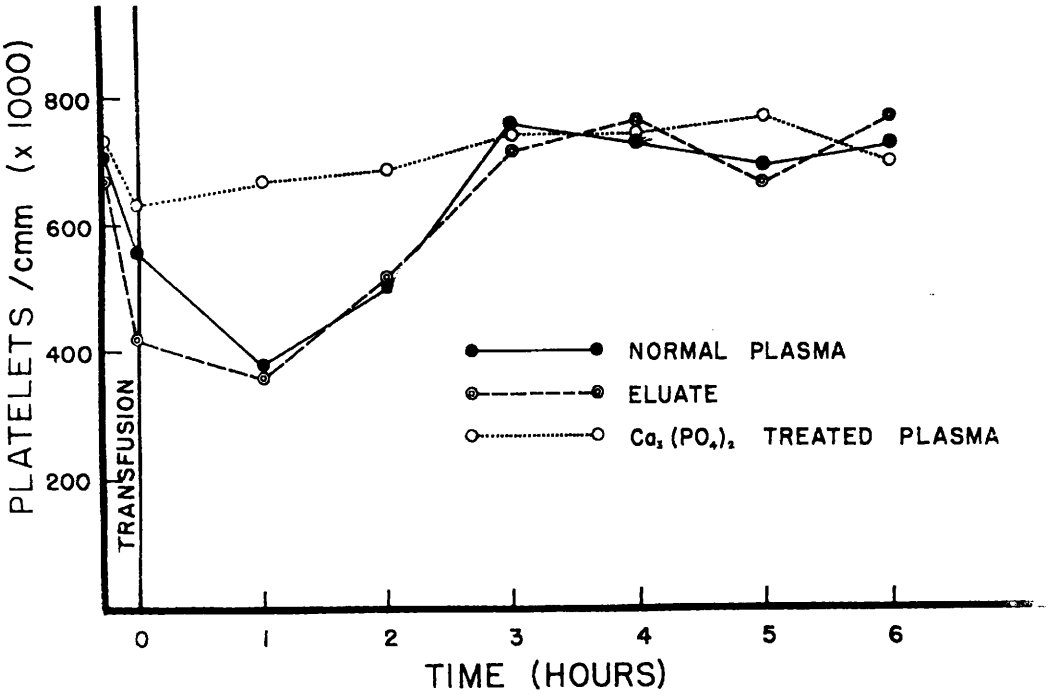

FIG. 2. Thrombocytopenic effect in normal recipients of untreated plasma, plasma treated with tricalcium phosphate gel and the eluate with sodium citrate of the material absorbed on the tricalcium phosphate gel.

e) *Elution of the thrombocytopenic factor from the $Ca_3(PO_4)_2$ gel.* The solid part of the $Ca_3(PO_4)_2$ gel was washed twice with cold distilled water, and finally treated with 15 ml of sodium citrate solution 0.2 M per 250 ml of plasma absorbed. The gel was suspended in the citrate by shaking and the bottle centrifuged in the cold at 3000 r.p.m. for 1 hour. The supernatant fluid (eluate) was separated, controlled for sterility, added to 50 ml of saline solution and injected intravenously in a normal recipient. This experiment was repeated 3 times and in every case a thrombocytopenic response practically identical to that induced by the original plasma was observed (Fig. 2).

f) *Miscellaneous observations.* No appreciable changes in number and "activity" of bone marrow megakaryocytes were observed in 2 recipients during the period of induced thrombocytopenia. Serum collected from the recipients at the peak of the induced thrombocytopenia failed to exhibit agglutinating or lytic properties against platelets, when tested with published technics(4). Samples of capillary (finger) blood and of citrated venous blood were collected from several recipients at the peak of the induced thrombocytopenia. Smears of capillary blood were prepared with Wright's stain. Plasma containing most of the platelets was obtained by centrifugation of the citrated blood at 500 r.p.m. for 5 minutes, diluted 1/20 with cold buffered saline solution and observed directly in the chamber of a hematocytometer. (All glassware, syringes and needles were coated with Silicone.) In neither case was presence of platelet clumps observed.

Discussion. The etiology of the phenomenon here described is open to many speculations. The platelet fall was clearly in excess of variations of hemodilution or of daily variations in the platelet count. Since no thrombocytopenia followed transfusion of the recipient's own plasma it is possible that the drop in platelet level might have been due to mild, sub-clinical transfusion reaction induced by a constituent present in plasma or released from the formed elements of the blood. Transfusion reactions either hemolytic

(10) or due to plasma reaction(2) are accompanied by thrombocytopenia, at times severe enough to induce hemorrhagic manifestations(10). Only 2 of the recipients included in this study, however, showed evidence of reaction, and the thrombocytopenia was, in these cases, not greater than that usually observed. As no recipient in this series had previously received blood or plasma, the possibility that thrombocytopenia might be due to sensitization to blood or plasma products, although not disproved, is rather remote. The thrombocytopenic activity of normal plasma could also have been due to incompatibility of the platelet products injected into the recipient. Although suspected, the existence of platelet groups has not been firmly established thus far. The relative uniformity of the thrombocytopenic effect (this failed to occur in only 4 of 36 recipients) seems to limit the role of platelet incompatibility in the phenomenon, but this aspect deserves further investigation.

Occasional severe prolonged thrombocytopenia, at times with occurrence of spontaneous bleeding manifestations, has been reported in normal individuals receiving plasma of patients with idiopathic thrombocytopenic purpura(11). This could conceivably be due to an excess of the thrombocytopenic factor present in normal plasma. Since platelets are destroyed at greatly increased rate in idiopathic thrombocytopenic purpura(12), one may ask whether the thrombocytopenic factor is not related to products of platelet disintegration. Serum, where most products of platelet disintegration are probably found, is in fact able to produce more sustained and pronounced thrombocytopenia (Table II). This aspect is being investigated at present, together with the possibility that the thrombocytopenic factor might be related to agents active in the process of blood coagulation, some of which exhibit similar physico-chemical properties.

The pathogenetic mechanisms through which thrombocytopenia is produced remains unsolved. Platelet production by the bone marrow megakaryocytes was not inhibited during the period of induced thrombocytopenia. The thrombocytopenic effect was of similar magnitude in 2 previously splenectomized recipients. There was no evidence of agglutination of platelets in the circulation of the recipient, nor was thrombocytopenia prevented by the administration of heparin, which decreases the agglutinability of platelets *in vivo* and *in vitro*. Platelets obtained from recipients at the acme of the induced thrombocytopenia appeared functionally active and sera, collected at the same time, did not agglutinate or lyse normal human platelets. Further investigation is in progress.

Summary. 1. Temporary, but significant thrombocytopenia was noted in 32 out of 36 individuals receiving compatible blood, plasma or serum from non-thrombocytopenic donors. No spontaneous bleeding manifestations, alterations of the various hemostatic mechanisms, changes in number or activity of the bone marrow megakaryocytes, alterations of function or morphology of the remaining platelets, agglutinating or lysing activity of the recipient's serum against normal platelets were noted. Heparinization of the recipient did not affect the thrombocytopenic effect of the transfusion of compatible plasma. 2. The thrombocytopenic effect was apparently due to a component of plasma, stable at 56°C, not absorbed by Seitz filters nor by ion-exchange resins, but absorbed on $Ca_3(PO_4)_2$ gel from which it could be eluted with sodium citrate solution. Work is in progress to establish the nature, the mechanism of action of this agent, and its relation to products of platelet destruction.

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Direct Titrimetric Method for Determination of Carbonates in Small Amounts of Serum.* (19467)

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This paper presents a simple, direct acidimetric titration procedure for the estimation of carbonates, employing small volumes of serum or plasma. A simple method employing small volumes has often posed a critical problem in the management of newborns and prematures. The principle of the new method is: 1) $HCO_3^- + Ba(OH)_2 \rightarrow BaCO_3$ (ppt.); 2) Ash to remove organic material (not necessary for pure inorganic solutions); 3) $BaCO_3 + H_3BO_3$ (hot 10% sol. in excess) $\rightarrow Ba(H_2BO_3)_2$; 4) $Ba(H_2BO_3)_2 + 2HCl \rightarrow BaCl_2 + 2H_3BO_3$; bicarbonate ion is precipitated as the barium salt, by the addition of barium hydroxide directly to the solution to be analyzed (*i.e.*, serum or plasma). The barium carbonate is dissolved in a hot solution of 10% boric acid in a manner similar to that described for dissolving calcium carbonate in the ultramicro estimation of serum calcium(1-3). This solution is diluted and titrated directly with standard hydrochloric acid to the pH of pure boric acid solution of similar strength. This titration represents the amount of carbonate in equivalents. A correction is made for the bicarbonate present in the reagents. The new method has a number of desirable features. The procedure is simple and accurate in spite of the small amounts involved. The direct titration as employed has a sharp end point. Only the stable standard acid is used (in contrast to the hitherto available titrimetric methods where serum carbonate is converted to carbon dioxide gas which is distilled into an excess of barium hydroxide, and the excess is then back titrated)(4,5). It is possible to set up simultaneously any number of determinations and complete all of them in about 1½ to 2 hours, the overall working time (with large numbers of determinations) being distinctly less than in the gasometric procedures of Van Slyke (6). Moreover, the hazard of handling large amounts of mercury is avoided and the cleaning problem is eliminated. The equipment is less expensive, and on the scale described in this paper, the method is not hinged to an ultramicro burette, which was employed, since with 0.1 N standard acid instead of 1.0 N acid one can use the usual type of two milliliter microburette. Conversely, in employing more dilute standard acids, such as 0.1 or 0.01 N, it is possible to modify this method from 0.01 ml to 0.001 ml of sample. The drawback of the new method is that it requires about 1½ hours for a single determination, which prevents it from being used in emergencies. Another drawback is that a modicum of experience must be acquired in determining the reagent blank, which in this

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