THE PRESERVATION OF THE COAGULATION SYSTEM IN STORED WHOLE BLOOD

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THE problem of hemorrhagic diathesis during massive transfusion therapy has been recognized for several years.[1-4] The studies by Stefanini and Chatterjea,[1] Krevans and Jackson,[3] and Jackson, Krevans, and Conley[4] indicated that this phenomenon is dependent on the rapid infusion of large amounts of platelet-poor whole blood. Stefanini, Mednicoff, Salomon, and Campbell[2] demonstrated that the abnormal bleeding could be corrected by the immediate infusion of fresh blood platelet concentrates or by closely repeated direct transfusions of fresh whole blood.

Procedures that require large amounts of whole blood, such as open cardiotomy, dialysis, and arterial grafting, have been widely employed at the Cleveland Clinic Hospital for several years. Hemorrhagic diathesis has rarely occurred, because all blood is drawn immediately prior to such scheduled procedures. However, in certain emergency procedures, it was necessary to utilize large quantities of banked blood immediately at hand. In several instances, a hemorrhagic syndrome ensued, similar to the cases reported by Stefanini and associates[2], which were characterized by low platelet counts, increased capillary fragility, prolonged bleeding time, prolonged clotting time, and impaired prothrombin consumption. In our cases, the surgical hemorrhage was controlled by utilizing direct blood transfusions.

As the number of such emergency procedures increased, it became apparent that it would be impossible to meet the increased demand for fresh whole blood at any time of the day or night. The urgent problem was to devise a method of administering large volumes of blood of varied storage periods in a manner that would obviate the hemorrhagic syndrome. In order to do so, it was first necessary to determine the length of time the coagulation mechanism would remain intact in blood taken into nonvacuum siliconized bottles of anticoagulant acid citrate dextrose solution (ACD) using nonwettable donor sets. At the time the investigation was undertaken, the published studies on this problem seemed inapplicable to our situation.

Tests that were developed during the recent past to study blood coagulation defects were adapted and modified for the present study to analyze the coagulation defects in stored whole blood. From this information, it was hoped to determine the storage stability of blood as it is processed in the Cleveland Clinic blood bank, and to devise a satisfactory plan for administering massive transfusion therapy.

For purposes of analysis, the coagulation mechanism can be considered as occurring in three sequential stages: stage I, evolution of thromboplastin; stage II,

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conversion of prothrombin to thrombin by thromboplastin; and stage III, conversion of fibrinogen to fibrin by thrombin. *Table 1* lists the factors known to play a role in each of these stages of coagulation.

Table 1.—*Blood factors in the three stages of coagulation*

Source		
Stage I Blood platelets *plus* Antihemophilic-globulin (AHG) Plasma thromboplastin component (PTC) Plasma thromboplastin antecedent (PTA) Hageman factor Stuart factor Factor V	Ca^{++} Wettable surface →	Thromboplastin
Stage II Prothrombin	Thromboplastin *plus* V, VII, Stuart Ca^{++} →	Thrombin
Stage III Fibrinogen	Thrombin Ca^{++} →	Fibrin

The factors that are known to be the most labile during storage are the platelets, antihemophilic-globulin (AHG), and factor V (Ac-globulin, proaccelerin, or labile factor). Hence, the primary concern was the fate of these three factors in banked blood.

Materials

The blood bank of the Cleveland Clinic utilizes three sources for the supply of blood for transfusion. The primary source is the American Red Cross Regional Center; blood from this center is drawn into nonsiliconized vacuum bottles. The second source is the professional donor, and the "repay" donor, whose blood is drawn and is processed at the Clinic blood bank. All such donor blood is collected in siliconized nonvacuum glass bottles, with nonwettable donor sets, utilizing only gravity flow during phlebotomy. The third source is the commercial blood banks of the area. Blood at these banks is drawn into nonsiliconized partial-

vacuum bottles. All three types of containers utilize the standard ACD solution as an anticoagulant preservative. All blood is stored at 4 C. until it is removed just prior to use.

Whole blood samples. Samples for testing were obtained from two sources: random bottles were sampled just prior to distribution from the blood bank; and serial samples were obtained from donor blood drawn especially for this project. All such blood was kept at 4 C. for the duration of the testing period, except during the time necessary to obtain samples for testing. Blood samples were obtained with siliconized syringes and needles; these samples were placed in siliconized glass tubes for further processing.

Low-spun (platelet-rich) plasma. Platelet-rich plasma was always obtained by spinning the whole blood samples in siliconized tubes at 500 rpm for 10 minutes in the Serval Angle table-model centrifuge with a 27-place head.

High-spun (platelet-poor) plasma. Platelet-poor plasma was obtained by spinning platelet-rich plasma in siliconized tubes at 3000 rpm for 20 minutes in the Serval Angle centrifuge.

Platelet suspension. Platelet suspensions were prepared by washing the sediment obtained from high-spun plasma twice in 0.85 per cent saline solution, and, after the final washing, thoroughly resuspending the platelets by vigorous agitation with a wooden applicator stick. The final volume of saline solution was one third the original volume of platelet-rich plasma.

Methods

Platelet count. All platelet counts were made on samples of whole blood after a standard two-minute mixing period. The direct method of Rees-Ecker, as described by Wintrobe,[5] was used. The counts were made in duplicate by one person* in order to standardize the method as much as possible. The direct method was chosen as a result of the report of Wilson, Eisemann, and Chance,[6] which indicated that the direct method of platelet counting was unreliable when applied to their similar studies. Platelet morphology was studied on all samples from blood smears made on coverslips and stained with Wright's stain. All smears were evaluated by one observer (D.A.S.).

Thromboplastin generation test (TGT). The thromboplastin generation tests were performed according to the method of Biggs and Douglas,[7] utilizing a more concentrated platelet suspension, as described above. It was determined by a series of pilot experiments that the use of ACD solution, as the anticoagulant, did not affect the test. In addition, several modifications of the original method were developed in order to test for thromboplastic activity in the stored plasma. These are described later in this report.

Recalcified clotting time. To 0.1 ml. of platelet-rich plasma was added 0.28 per cent of calcium chloride ($CaCl_2$), and the mixture was incubated in a 37 C. water

**Miss Mary Margaret Potter, B.A., M.T. (A.S.C.P.), made all the counts, and rendered other invaluable technical assistance throughout this study.*

bath. After the first minute of incubation, the tube was tipped every 15 seconds until the fibrin clot appeared. Each test was run in duplicate or triplicate, with fresh low-spun plasma as a control.

Clot retraction, percentage. The clot retraction was determined by placing a measured amount of low-spun (platelet-rich) plasma into a 12-mm. conical, graduated, Pyrex centrifuge tube. A volume of 2.5 per cent $CaCl_2$ equal to one tenth the volume of plasma was added, and a wooden applicator stick was inserted into the tube. The mixture was incubated for one hour in a 37 C. water bath. The wooden applicator stick and adherent clot were then removed; the remaining serum-$CaCl_2$ mixture was measured, and is expressed as the percentage of the original volume. For each test, a similar sample of fresh low-spun plasma was used as a control system.

Prothrombin time. The one-stage prothrombin time was determined by the standard method of Quick,[8] using a commercial preparation of brain thromboplastin. In the correction studies, barium sulfate ($BaSO_4$)-adsorbed, fresh-frozen, Seitz-filtered beef plasma, as well as a commercial preparation, was used as the source of factor V. These methods are qualitative; no quantitative measurements were attempted for assay of prothrombin or factor V.

Russell's viper venom (Stypven) coagulation time.* Stypven is a powerful anticoagulant that accelerates the conversion of prothrombin to thrombin in the presence of a platelet lipoid factor and calcium ions. Its action is independent of factor VII, thereby differing from brain thromboplastin[9]. Stypven coagulation time was determined using a procedure based on O'Brien's[10] method and modified for the present study. One tenth of one milliliter of 0.28 per cent $CaCl_2$ was added to an incubation tube in a 37 C. water bath, which contained 0.1 ml. of the test plasma, 0.1 ml. of platelet suspension, and 0.1 ml. of 1:10,000 Stypven. The clotting time of the resultant mixture is recorded in seconds. One-tenth milliliter of 0.85 per cent saline solution was substituted for the platelet suspension in the incubation tube, when necessary, to keep the total volume constant.

Results

Platelet count. Platelet counts were performed on random samples obtained from 60 nonvacuum siliconized bottles stored in the blood bank. Almost all of these bottles contained 200,000 platelets per cubic millimeter during the first three days of storage. The number of platelets declined in gradual fashion until the seventh day, when most bottles contained about 150,000 platelets per cubic millimeter; after this time there was a rapid and erratic decrease in platelet numbers in the stored blood.

The curves in *Figure 1* represent the combined results of serial platelet counts on stored blood obtained by two methods of phlebotomy. The platelet counts of the blood taken into vacuum nonsiliconized bottles showed a notable platelet loss immediately after phlebotomy. In these bottles, the number of platelets

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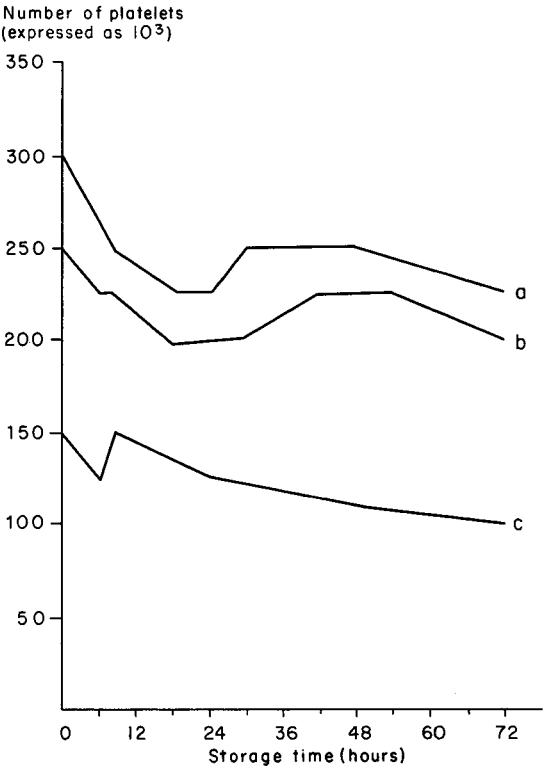

Fig. 1. Graph showing serial platelet counts of blood stored in various containers: a and b, range of serial platelet counts in several nonvacuum siliconized bottles; c, average of serial platelet counts in several vacuum nonsiliconized bottles.

decreased rapidly during the first three days of storage. By contrast, blood taken into nonvacuum siliconized bottles showed little or no immediate decrease in number of platelets, and the platelet counts declined only slightly during three days of storage. The serial nature of these determinations, each necessitating a period of mixing, probably resulted in values lower than if the bottles had been undisturbed. With each mixing there was unavoidable foaming, which is a major factor in platelet destruction.[11] These results are in good agreement with the report of Mustard and Walker,[12] who utilized similar technics.

Clot retraction, percentage. In control experiments using fresh low-spun plasma, clot retraction began promptly and was complete at the end of one hour. The fibrin clot was tightly adherent to the wooden applicator stick, and virtually no serum remained entrapped. The clot was firm, rubbery, and glistening white. As the plasma aged, especially after 48 hours, the speed of retraction decreased, and retraction became variably incomplete. The fibrin clot became soft, gelatinous, and grayish-white.

Clot retraction was universally good during the first 48 hours of storage in nonvacuum siliconized bottles. After this time, in many bottles there was a rapid and erratic decline in clot retraction, although in many bottles tested it was still 80 per cent complete at 72 hours. After 72 hours, the speed and effectiveness of clot retraction declined rapidly.

In nonsiliconized vacuum bottles, there was great variability in clot retraction from sample to sample of blood stored for the same period of time. In general, the clot retraction was much slower and incomplete in such samples, and it was not possible to predict the efficacy of clot retraction in the various samples of the same age stored in this type of container.

Platelet morphology. The platelets passed through several definite morphologic phases during storage. The times at which these changes were noted varied from sample to sample, but were consistently present in each.

During the first four to six hours, the platelets resembled those seen in a fresh direct preparation. From 6 to 18 hours, they appeared swollen, with rounded or discoid forms predominating; the hyalomeres were granular and of the usual size and color. After 18 hours, the hyaloplasm of increasing numbers of platelets had pseudopodia or mycelial-like projections. Clumping was a prominent feature during this period. Few rounded forms remained at 24 to 30 hours, and increasing numbers of platelets appeared which consisted of a dark, basophilic, finely granular hyalomere surrounded by scanty amounts of basophilic cytoplasm. During the next 18 hours, these forms became increasingly numerous, until at 48 hours, and thereafter, the dominant form was the small basophilic hyalomere with scanty, deeply basophilic cytoplasm. This platelet form gave the smears a characteristic "peppered" appearance when viewed at a magnification of 430 x.

Thromboplastin generation test. The results of the TGT are summarized in *Table 2*. The thromboplastic activity of the platelets collected and stored in nonvacuum siliconized bottles remained entirely normal up to 72 hours, when tested with normal $BaSO_4$-adsorbed plasma and normal serum. It then decreased slightly, but the platelet suspensions remained remarkably active up to seven days.

Qualitative assays of AHG were also determined. There was a rapid decrease in AHG activity in the stored plasma, which was rather notable by 24 hours, when tested with normal platelets and normal serum. AHG activity declined only slightly thereafter.

Thromboplastin generation improved when stored platelets and stored plasma were tested together. This observation, in addition to the apparent thromboplastic activity of AHG-deficient 48-hour plasma, seemed to indicate the presence of a thromboplastic factor in the plasma itself. Stefanini[13] had previously noted this effect. As shown in *Table 2,* stored high-spun (platelet-poor) $BaSO_4$-adsorbed plasma and normal serum were used alone as a source of thromboplastin. The 48-hour, 72-hour, and 168-hour-old plasma showed remarkable thromboplastic activity. In

contrast, fresh plasma that was similarly treated showed practically no such activity.

Table 2.—*Results of thromboplastin generation tests (TGT) on stored whole blood in nonvacuum siliconized bottles*

Incubation mixtures	Substrate clotting time, seconds						
	Intervals of incubation, minutes						
	One	Two	Three	Four	Five	Six	Seven
Normal plasma, serum, platelets	22″	7″	7″	7″	—	—	—
Normal plasma, serum, 1-hr. platelets	25	8	7	7	7	—	—
Normal plasma, serum, 4-hr. platelets	10	7	7	7	—	—	—
Normal plasma, serum, 24-hr. platelets	17	8	8	8	8	—	—
Normal plasma, serum, 48-hr. platelets	—	7	7	7.5	—	—	—
Normal plasma, serum, 72-hr. platelets	45	14	10	10	10.5	—	—
1-hr. plasma, normal serum, platelets	25	8	7	7	7	—	—
4-hr. plasma, normal serum, platelets	19	8	9	8.5	9	—	—
24-hr. plasma, normal serum, platelets	44	12.5	12.5	11	10	—	—
48-hr. plasma, normal serum, platelets	—	9	9	8.5	9.5	—	—
72-hr. plasma, normal serum, platelets	—	26	10	10	9.5	10	—
24-hr. plasma, normal serum, 24-hr. platelets	29	11	10	9	—	—	—
48-hr. plasma, normal serum, 48-hr. platelets	—	9.5	8	8	—	—	—
72-hr. plasma, normal serum, 72-hr. platelets	41	17	9.5	11.5	12	—	—
$BaSO_4$-high-spun plasma, normal serum and saline solution	>90	>90	>90	>90	75	58	—
$BaSO_4$-high-spun 48-hr. plasma, normal serum, saline solution	>90	17.5	16	16	16.5	—	—
$BaSO_4$-high-spun 72-hr. plasma, normal serum, saline solution	>90	88	59	35	25	18	—
$BaSO_4$-high-spun 168-hr. plasma, normal serum, saline solution	>90	>90	>90	45	17	—	—

Stypven coagulation time. *Table 3* summarizes data of a typical mixing experiment utilizing the Stypven coagulation time. The Stypven coagulation time was 18 seconds for fresh low-spun (platelet-rich) plasma. When the same plasma was high-spun, and the platelet-poor sample was tested, the Stypven coagulation time had lengthened to 25 seconds. When the thrice-washed platelet suspension was then returned to the plasma, the clotting time was shortened to 11 or 12

Table 3.—*Results of mixing experiments using Stypven coagulation time**

Fresh plasma, ml.			19-hr. plasma,† ml.			3-day plasma,† ml.			Stypven time, seconds
Low-spun	High-spun	Platelets‡	Low-spun	High-spun	Platelets‡	Low-spun	High-spun	Platelets‡	
0.1	—	—	—	—	—	—	—	—	18
—	0.1	—	—	—	—	—	—	—	25
—	0.1	0.1	—	—	—	—	—	—	11
—	—	—	0.1	—	—	—	—	—	13
—	—	—	—	0.1	—	—	—	—	13
—	—	—	—	0.1	0.1	—	—	—	9.5
—	—	—	—	—	—	0.1	—	—	13.5
—	—	—	—	—	—	—	0.1	—	14
—	—	—	—	—	—	—	0.1	0.1	9
—	0.1	—	—	—	0.1	—	—	—	10
—	0.1	—	—	—	—	—	—	0.1	9.5
—	0.1	—	—	—	—	—	0.1	—	15
—	—	—	—	—	—	—	0.1	0.1†	9.5
—	—	—	—	—	—	—	0.1†	0.1	8.0
—	—	0.1	—	—	—	—	0.1	—	10

*The methods of preparing the plasma and platelet suspensions are given in the text, page 127. In each case, 0.1 ml. of 1:10,000 Stypven and 0.1 ml. of $CaCl_2$ were added to the reaction mixture.

†These samples were from whole blood that was stored at 4 C. for the time indicated.

‡When platelet suspensions were omitted, 0.1 ml. of 0.85 per cent saline solution was added to the reaction mixture to make up the volume.

seconds. In contrast, using plasma samples from stored blood, there was no difference in the Stypven coagulation times of platelet-rich and of platelet-poor plasma. When the suspensions of stored platelets were returned to their respective stored plasmas, in each case the Stypven coagulation time of the mixture was again shortened. The activity of stored platelets as measured by adding suspensions of stored platelets to fresh high-spun plasma, was equal to or greater than the fresh-platelet suspensions. Of great significance is the observation that when high-spun, three-day old plasma was used as a source of platelet factor, using high-spun fresh plasma as the test sample, the Stypven coagulation time was shortened from 25 seconds to 15 seconds.

From the results of this series of experiments, one may state that: (1) the platelet factor necessary to produce plasma coagulation with Stypven is present and active in stored as well as in fresh platelets; (2) the processing of platelets (e.g., making platelet suspensions) in such a fashion as to cause lysis and disinte-

gration, accelerates the conversion of prothrombin to thrombin by Stypven; and (3) the platelet factor remains active after diffusion into the plasma, and appears to be storage stable in such plasma, at least as long as 72 hours at 4 C.

Prothrombin time. The prothrombin time was normal (14 seconds) in all samples during the first 12 hours of storage. After this time, there was an abrupt prolongation of the prothrombin time in more than one half of the samples tested. By the end of 24 hours, all samples showed prolonged prothrombin time. This prolongation continued in a gradually progressive fashion through 96 hours. Thereafter, the prothrombin time stabilized, and in all samples tested between the fifth and eleventh days of storage the range of prothrombin time was 18 to 20 seconds.

During the first 96 hours, the addition of 1 part of factor V to 10 parts of stored plasma, returned the prothrombin time to 14 seconds. The addition of factor V to 5-to-14 day old plasma shortened the prothrombin time to less than 15 seconds in all cases.

Recalcified clotting time. Twenty of thirty plasma samples had normal recalcified clotting times during the first 24 hours of storage. Only one sample had a clotting time longer than 130 seconds during the first 12 hours, and four samples were longer than 130 seconds during the second 12 hours. Of 42 plasma samples, 29 had a clotting time within normal limits during the first 72 hours, and of the 13 in which it was longer than 120 seconds, in only five was it longer than 130 seconds. In each case, the prolonged clotting time could be corrected by the addition of factor V during the first 48 hours of storage.

In general, the over-all clotting mechanism of the stored plasma was intact, as measured by this in vitro test. This was true despite demonstrable deficiencies of one part or another of the coagulation mechanism.

Discussion

Since Lewisohn[14] described a procedure to preserve blood for transfusion in sodium citrate solution, efforts have been made to prolong the period during which blood could be safely stored. Most of this work has emphasized the preservation of the erythrocytes during storage. The occurrence of hemorrhagic diathesis during or immediately after the use of banked blood for massive transfusion therapy resulted in increased interest in the effect of storage on the coagulation system of drawn blood.

Thrombocytopenia after multiple transfusions of banked blood has been well demonstrated.[1-4] A great decrease in the number of platelets usually occurs only after a massive transfusion (5000 ml. or more in adults).[3] The occurrence of hemorrhagic diathesis has been related directly to the thrombocytopenia, with relatively minor changes noted in amounts of factor V and AHG in normal persons.[1] Finkbiner, McGovern, Goldstein, and Bunker[15] have recently shown that those two factors may be of importance in patients with severe hepatic disease.

In the past, it has been observed that other factors influence the platelet count in patients who have had severe hemorrhage, or after surgical procedures.[16] Desforges, Bigelow, and Chalmers[17] studied the decrease in number of circulating platelets during gastrointestinal hemorrhage, and concluded that the decrease was not related to the amount of blood loss, or to the presence or absence of shock. Warren, Lauridsen, and Belko [18] postulated that the stress of major surgery, acting through the adrenal cortex, caused the decrease in platelets in their patients. They concluded that this decrease was independent of such factors as severity of hemorrhage, transfusion, or hemodilution. They could produce the same effect through administration of corticotropin.

However, the effect of surgical trauma and stress on platelet count seems to have been unclear in the past. The division of opinion is noted in a review of the subject by Mustard.[19] In his own series of 21 nontransfused patients who underwent surgery, he noted about a 10 per cent increase in the platelet count in the immediately postoperative period. In contrast, in 62 patients who underwent surgical procedures and who received transfusions, Mustard[20] found that one half demonstrated a decrease in circulating-platelet count. Similarly, Stefanini and Chatterjea,[1] noted that of 36 patients receiving platelet-poor whole blood from normal donors, 32 showed a transient but significant thrombocytopenia. Krevans and Jackson[3] reported on 27 adults, 14 of whom received more than 5000 ml. of stored blood, and in all of them thrombocytopenia developed; 11 of them had clinically abnormal bleeding. In the other 13, who received less than 5000 ml. of stored blood, thrombocytopenia developed in a few, but none of these had abnormal bleeding.

In the light of these reports, it would seem that the primary cause of hemorrhagic diathesis in massive transfusion therapy is the transfusion of stored whole blood, and that stress, adrenocortical hormones, and shock, play secondary roles in this syndrome.

The clotting mechanism in vitro. I. *Platelets. Platelet counts.* The studies presented in this report illustrate that blood drawn into nonvacuum siliconized bottles, utilizing nonwettable donor sets, generally shows a high platelet count immediately after phlebotomy, in contrast to the counts for the other methods tested. Further, the decline in platelet numbers during storage was slower and more gradual in nonvacuum siliconized bottles than in nonsiliconized vacuum bottles. These results are in accord with those of Mustard and Walker,[12] who showed that platelet preservation during storage is dependent upon the numbers of platelets lost during phlebotomy. The probable mechanism of this rapid decrease in platelet numbers is the activation of thrombin that, in turn, acts as a catalyst on the platelets, causing increased lysis of those remaining after phlebotomy. After these results had been determined, further tests of the clotting mechanism were largely confined to blood stored in siliconized nonvacuum bottles.

Platelet survival. Platelet survival can be estimated in a variety of ways, accord-

ing to: platelet morphology, thromboplastic activity, and clot retraction.

Platelet morphology. There seems to be little agreement in the literature regarding correlation of form and function of platelets, despite recent advances in electron and phase microscopy.[21-24] However, there is general agreement that the normal shape is discoid, and this proved to be true in this study with the light microscope. The progressive morphologic changes that were observed, were the same as those reported by others,[23] although the speed of change varied with the technics used. Despite the apparent regularity of the changes noted in the stored platelets, it was not possible to judge accurately their activity or viability from these morphologic forms. Tullis[21] stated that intactness does not imply normal survival.

Thromboplastic activity. The TGT can be used to measure relative or qualitative thromboplastic activity, although it is not satisfactory as a quantitative test. The results here reported show that storage has little effect on the thromboplastic activity of the platelets. Mustard,[23] Tullis,[21] and Minor and Burnett[25] found that platelets stored by various methods retained their ability to assist in the generation of thromboplastin in vitro.

The $BaSO_4$-adsorbed high-spun plasma obtained from stored whole blood had a distinct ability to generate thromboplastin when used as a platelet substitute in the TGT. This confirms the observation of Stefanini and co-workers[11,26] that the hemostatic effect of platelets must be due, at least in part, to certain relatively stable constituents that are liberated when these bodies disintegrate. They further concluded that this substance must be storage stable. O'Brien[27] found a thromboplastic factor in serum and, from a series of isolation experiments, concluded that it must originate from lysed platelets.

The Stypven coagulation time studies also demonstrate this platelet-like activity in the stored plasma. O'Brien[10] showed that the active platelet factor that caused plasma to clot when reacted with viper venom, was in the lipid fraction of the platelet extract. He believed it to be phosphatidyl-ethanolamine, but later work showed that the active principle is phosphatidyl-serine.

It is apparent from the studies reported here that this platelet thromboplastic factor is stable, and acts independently of the presence of viable or even of intact platelets.

Clot retraction. Clot retraction is generally believed to be a platelet function. The exact mechanism by which the platelets affect the fibrin strands to produce retraction is not well understood. One theory holds that the fibrin strands shorten when acted upon by an enzyme (retractozyme) that is released during platelet lysis. Another hypothesis states that clot retraction is the result of a direct physical action of the platelet processes on the fibrin mesh, and this action requires the presence of viable platelets.

The dissociation of the various platelet functions has been noted previously.[11,21] In contrast to the storage-stable thromboplastic factor, most authors agree that clot

retraction decreases greatly within 10 to 24 hours after phlebotomy. However, results reported here show that clot retraction remained excellent through 48 hours. After 72 hours, clot retraction decreased notably, even though good thromboplastic activity was retained by the same platelets. This loss of platelet function coincided with the appearance in the stained smears of the small, basophilic platelet forms previously described.

The life span of normal platelets appears to be about three days, as measured by various radiophosphorus technics.[28,29] Hirsch, Gardner, and Thomas[30] reported in vitro life span of platelets as being from 36 to 72 hours, as measured by glycolytic activity. The decrease in oxygen consumption is roughly correlated with a decrease in clot retraction. Stefanini and Dameshek[11] noted correlation between clot retraction and platelet survival in vivo. In all these studies, if the platelets were washed, concentrated, or otherwise were disrupted, there was a significant decrease in viability, which was roughly correlated to a decline in clot retraction.

The reports of Krevans and Jackson,[3] and Jackson, Krevans, and Conley,[4] state that most platelets remain viable only about four hours in ACD solution, and that after this time, if they are transfused they are rapidly removed from the circulation. However, in their studies,[3,4] the blood was taken into nonsiliconized equipment. Finkbiner and associates[15] support this observation, and state further that in surgical procedures requiring multiple transfusions, each alternate bottle of blood should be less than 24 hours old.

In contrast, Stefanini and Dameshek[11] noted that if platelets were stored in siliconized nonvacuum bottles, more than one half remained in the circulation at 12 hours. If the platelets were washed, or otherwise subjected to trauma, there was poor survival in vivo. Adelson, Rheingold, and Crosby,[28] utilizing radiophosphorus-tagged platelets, showed that excess trauma to the platelets led to a rapid decrease in the half-life in vivo. Hirsch, Gardner, and Thomas[30] noted that the oxygen consumption of platelet suspensions in ACD solution did not reach zero until after three days of storage. Mustard[20] stated that trauma to the platelets during phlebotomy greatly shortened their survival during storage in ACD solution. Of 62 patients, those in whom there was a decrease in circulating platelets had received blood that showed the greatest decline in platelet numbers during storage. In contrast, when such patients were transfused with blood that had been collected by gravity into siliconized bottles, no thrombocytopenia was noted.

From the study here reported, as well as the other evidence cited, it is apparent that blood carefully drawn by gravity, through nonwettable donor sets into siliconized, glass containers, has the majority of the platelets preserved, and that these platelets remain viable 48 hours after phlebotomy. Further, clot retraction appears for the most part to correspond to viability of platelets in vitro, under the experimental conditions. That is, clot retraction measured on platelet-rich plasma roughly corresponds to platelet viability.

The question of in vivo survival of stored platelets, however, remains controversial. Independent studies by several authors have demonstrated that in vivo survival ranges from almost immediate disappearance to survival for as long as 24 hours. A further source of controversy is whether or not the transfused platelets, even if they survive, are able to function normally in the clotting mechanism. Many of the results described in the literature and reported at recent meetings seem to hinge on the conditions under which the platelet infusions are carried out. This has led to some disagreement in this area. Certainly the platelet survival in thrombocytopenic patients is greatly shortened, especially if they have undergone previous blood transfusions.

In a series of patients at the Cleveland Clinic Hospital who received more than 15,000 ml. of blood in an exceedingly short period of time at operation, those who received one unit of "fresh" blood from a siliconized nonvacuum bottle (0 to 48 hours old) for every three to four units of stored blood, demonstrated no clinical evidence of hypocoagulability, and clotting times (both Lee-White and ground glass) were within normal limits at the end of the procedure. Unfortunately, because of the emergency situation pertaining at surgery, no other detailed studies were carried out in these patients. When such a system was not utilized, similar patients almost invariably showed the typical hemorrhagic diathesis, which was not corrected by infusion of bottled blood, but only by direct blood transfusion utilizing the siliconized syringe technic.

It is, therefore, most important that spacing of fresh and older stored blood be scheduled for any procedure in which massive exsanguinating hemorrhage might be anticipated. If the hemorrhagic syndrome does develop, immediate direct blood transfusion is indicated.

II. *Other clotting factors. AHG (antihemophilic-globulin).* These studies showed a rapid decline in AHG activity during the first 24 hours of storage. Pitney and Dacie[31] stated that this loss may be as much as 50 per cent in 24 hours; a recent report[15] stated that 50 per cent loss does not occur until blood has been stored for one week. Mustard[32] showed that loss of AHG activity is proportional to platelet loss during collection, and related this loss to activation of the clotting mechanism.

There is a definite decline in AHG during storage. From this study it would seem difficult to evaluate the exact amount of AHG lost, or the rate of decline in its activity, because of the concomitant increase in plasma thromboplastic activity that has been herein demonstrated. As noted before, this seems to be secondary to platelet lysis. It is probable that through 72 hours of storage, the loss of AHG activity is not enough to become a factor in the hemorrhagic diathesis that may occur during massive blood transfusion therapy.

Prothrombin. Fahey, Ware, and Seegers,[33] and Bell,[34] showed that prothrombin is storage stable in ACD solution. Finkbiner and associates[15] state that the average prothrombin concentration is 72 per cent of normal in blood stored from 11 to

18 days. This study showed only minimal loss of prothrombin activity during 14 days of storage at 4 C. Hypoprothrombinemia is not a factor in the hemorrhagic syndrome secondary to transfusion.

Factor V. Factor V was extremely storage labile, producing a prolonged prothrombin time in more than one half the samples tested after 12 hours of storage. This lability seemed independent of the platelet count, platelet activity, or plasma thromboplastic activity. Mustard[32] related the rate of factor V loss directly to the numbers of platelets lost during phlebotomy. Fahey, Ware, and Seegers[33] found that the addition of platelet extract or decalcified human plasma decreased the storage stability of factor V, and that factor V is more storage stable in platelet-free than in platelet-rich plasma. Finkbiner and associates[15] noted only a 10 per cent decrease in the concentration of factor V after 12 hours of storage, with a steady decline thereafter. He also noted that in patients with hepatic disease (who already have a factor V deficiency) transfusion of stored blood decreased the factor V value of these patients even further. It is probable that in patients with normal hepatic function, the use of blood stored as long as 96 hours would not cause any serious factor V deficiency.

Recalcified clotting time. Despite the deficiencies of individual factors in the clotting mechanism caused by storage, the recalcified clotting time in many instances remained within normal limits. During the first 72 hours it was rarely prolonged more than 10 seconds. Mustard[23] also commented on this phenomenon; but whether or not it is due to the insensitivity of the test, to the presence of platelet or of plasma thromboplastic factor in the stored blood, or to the formation of highly active intermediate products during storage, is in the realm of speculation at this time. However, it seems that the test for recalcified clotting time is not an adequate guide to the status of the coagulation system of stored blood in vitro.

Conclusions

The clotting mechanism of blood carefully drawn through siliconized equipment into a nonvacuum siliconized ACD solution bottle, and stored at 4 C., remains essentially intact during a period of 48 hours, despite a decrease in some coagulation factors, as tested in vitro. This is especially true of the platelets, which appear to be the critical element in the production of hemorrhagic diathesis during massive blood transfusion therapy. This is in contrast to blood drawn into nonsiliconized vacuum bottles, which showed a great decrease in platelets immediately postphlebotomy, and a rapid fall in platelet numbers thereafter.

This increased preservation of the clotting factors is probably due to the prevention of platelet lysis and the activation of Stuart factor against wettable surfaces. This, in turn, means that the activation of the clotting mechanism is retarded, and less thrombin is produced. Since thrombin, in turn, increases platelet lysis, less thrombin production means less platelet destruction. When coagulation

is retarded, such substances as antihemophilic-globulin (AHG) and factor V are spared.

Blood drawn and processed by the technic described in this paper may be considered as fresh blood through 48 hours of storage at 4 C. When an emergency need arises for massive transfusion therapy (4000 ml. or more of whole blood) it is recommended that one unit of such blood be utilized for every three units that have been stored more than 48 hours, or which have been drawn into vacuum nonsiliconized containers. If such a technic is followed, hemorrhagic diathesis will generally be avoided.

Patients who received more than 15,000 ml. of blood in an extremely short time, have been transfused in this fashion here at the Cleveland Clinic. No hemorrhagic phenomena due to hypocoagulability have been observed, and the clotting time at the end of the procedures has been normal in these patients.

This system of transfusion for emergency procedures has the advantage of giving the blood bank personnel time to locate and carefully to type and to cross-match donors in an orderly fashion, thus insuring a high quality of work at a time of extreme pressure.

The procedures and equipment recommended in this report can be easily adapted to most blood banks already using glass vacuum containers, with relatively little increase in unit cost. It is especially adaptable to the medium-sized or smaller blood banks where blood from volunteer repay donors and professional donors is collected and processed.

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