Permanent Lesions of Stored Platelets Correlate to pH and Cell Count While Reversible Lesions Do Not (44226)

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Abstract. **The demand for stored platelet concentrates (PC) for therapeutic transfusions has been increasing for the past three decades. Loss of platelet functionality increases with the length of storage time due to a multitude of factors collectively referred to as a platelet storage lesion. As more of the causes of the storage lesion have been defined, storage conditions have improved along with the therapeutic value of the transfused platelet samples. This report addressed new aspects of the storage lesion correlated with the pH of the storage medium. Platelet function was evaluated as aggregation induced by the synergistic agonist pair, U46619 (TXA2 mimetic) plus epinephrine or the strong agonists SFLLRNP (a peptide thrombin receptor agonist) or thrombin each added alone. Stored PC were compared to freshly prepared platelets as platelet-rich plasma (PRP) or washed platelets re-suspended in hepes Tyrode's buffer. The pH of the storage plasma, was inversely proportional to the cell count with platelets stored for 6 days. Agonist-induced platelet aggregation was significantly impaired by storage for 6–7 days as PRP; however, upon washing, a significant level of activity was restored. Washed platelets more accurately reflect conditions of transfused platelets that may regain activity *in vivo*. There appeared to be two subpopulations of stored PRP samples—one that retained activity and one that lost virtually all activity with the agonists tested. However, the lack of response to agonist observed with one subpopulation was reversed to the same level obtained with the active subpopulation upon washing. The subpopulation of stored PRP samples that were inactive with U46619-plus-epinephrine did not correspond to the subpopulation of samples that were nonresponsive to SFLLRNP, indicating that loss of activity with selected samples was possibly receptor specific. Loss of agonist-induced aggregation with PRP samples did not correlate with storage pH; however, the level of aggregation with washed platelets correlated significantly with pH. Results implied that pH caused a permanent storage lesion that could only be detected with washed platelets. A partially reversible lesion was superimposed on the pH lesion and was only detectable with PRP samples. Results indicate that continued attention must be paid to regulate the pH of stored PC even in the second generation plastic bags.**

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The advent of new plastic containers in the 1970s increased the feasibility of storing platelets for transfusion. The therapeutic value of platelets diminishes with time of storage due to a multitude of factors generically referred to as the platelet lesion. Platelets are now routinely stored as platelet concentrates (PC) in approximately 50 ml of plasma for up to 5 days at 20°–24°C with constant agitation. The benefits from the transfusion of PC have been expanded in the treatment of a variety of diseases and have resulted in a 400% increase in PC usage from the early 80s (1). The transfusion of PC to thrombocytopenic patients is

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now common practice. Platelet administration to cancer patients undergoing chemotherapy-induced thrombocytopenia was shown to lower the morbidity and mortality related to serious hemorrhage (2). Studies indicate that the clinical status of the patient receiving transfused platelets influences the apparent function and survival of the platelets *in vivo* (3).

Because of the demand for quantity as well as quality of platelet preparations, recent work has centered around maximizing platelet storage conditions to minimize the progression of the platelet lesion. Temperature, storage time (4), storage bags (5–7), and plasma additives or synthetic storage mediums (8–13) have all been developed or studied with the aim of minimizing the reduction of platelet viability. Intensive research has also focused on the volume of plasma required to maintain stored platelets (14–17). The benefits of minimizing storage plasma are many-fold: (a) more plasma would be recovered from each PC prepared and, therefore, would be available for preparation of other plasma components (16); (b) recipients would be exposed to less plasma, which would lessen the risk of volume overload, a possibility with infants and patients with respiratory distress syndrome (17); and (c) alloimmunization and transfusion-related diseases could be avoided (16). However, increased platelet count due to reduced storage volumes may decrease the storage plasma pH (18–20). Even with "second generation" plastic bags, storage of PC still appear to approach hypoxic conditions that result in an increased production of lactic acid and a decreased pH (5, 6, 21).

The effects of pH on stored platelet function are well documented (21–23) and may mimic physiological conditions of tissue fluid stasis and inflammation that expose platelets to altered pHs (24). At manipulated pHs below 7.4, platelets undergo shape change, becoming spherical and devoid of pseudopodia, as detected by electron microscopy (25). However, more recent studies demonstrated that even in the presence of minimal pH changes, PC stored in plasma or a glucose-free citrate-acetate-NaCl solution underwent dramatic morphological alterations after 8 days (26). At pH 6.4, platelet aggregation has been shown to be reduced by more than 50% with selected agonists, and at pH 5.4, platelet aggregation as well as plasma coagulation are abolished (27). The reduction of the platelet medium pH from 7.35 to 6.8 causes a pH-dependent decline in agonist-induced arachidonic acid release (28). As the pH approaches 6.0, stored platelets undergo the release reaction, with the release of components from both alpha- and dense-granules (29). This can also occur during prolonged storage at pH around 6.8 (26). Ristocetin-induced platelet aggregation has been shown to be sensitive to pH (30). Some thrombin receptor activating peptides are more potent at a pH of 7.2 than 8.1 (31), and thrombin itself has also been shown to be pH sensitive (32). Other agonists that have a demonstrated pH sensitivity include thromboxane A2 mimetics (24), ADP, and collagen (33).

While the pH-induced loss in activity of stored PC is a major cause of the platelet storage lesion (19, 20, 34) further investigations have also revealed evidence of irreversible changes associated with platelet activation and plasma protease activity during storage (35). Broadly, any biochemical or metabolic alteration that occurs upon storage and compromises platelet function can be considered a lesion (36). This would include changes in cytosolic calcium, assembly of filamentous actin, modification of components associated with signal transduction, change in receptor availability, an altered adenylate energy charge (36), and altered cytochrome levels (37). Apparent changes of platelet structure/function *in vitro* may be reversible *in vivo* (38).

The current study focused on new aspects of pH, platelet count, and response to various agonists with freshly prepared platelets and stored platelets as platelet-rich plasma (PRP) versus the corresponding washed samples. The apparent loss of response to agonists by stored platelets could be partially reversed upon washing. No correlation in pH or cell count to reduced agonist-induced aggregation was detected with stored platelet rich plasma. However, significant correlations were observed when these platelet preparations were washed. The results imply a reversible platelet lesion in PRP samples superimposed on a permanent pH-induced platelet storage lesion.

Materials and Methods

Fresh platelet samples were obtained by venipuncture from volunteers who donated 45 ml of whole blood. Each donor signed a consent form in accord with the University's Committee on Human Studies. Blood (45 ml) was collected into a tube with 1/10 the volume of 3.8% sodium citrate (5 ml) and centrifuged at 400*g* for 10 min to yield platelet-rich plasma (PRP). These freshly prepared samples (typically 20–25 ml) were used as Day 0 controls and were not stored; therefore, they required no added dextrose. Stored platelet concentrates (PC) were obtained from the Penn-Jersey American Red Cross. The PC provided were 6–7 days post-collection in approximately 50 ml of plasma and were used the day of receipt. The pH was determined immediately following specimen collection from the transfusion bag. Platelet counts for all samples were determined using a Coulter counter. A platelet-poor plasma (PPP) sample was obtained from a portion of the PC PRP sample by centrifugation and used to dilute the PRP sample to 300,000 platelets/μl when necessary. These diluted PRP samples were then pH-corrected to 7.4 with 1*M* NaOH prior to aggregation studies. A portion (10–15 ml) of the PRP samples was also washed as previously described (39) in three times their volume of sodium citrate (pH 6.0) and sufficient tris buffered saline (TBS; 20 m*M* Tris-HCl, 150 m*M* NaCl, pH 7.4) to make up to 50 ml, centrifuged at 500*g* for 10 min, and the platelet pellet resuspended in a hepes Tyrode's buffer (136 m*M* NaCl, 2.7 m*M* KCl, 1 m*M* $CaCl_2$ 3.3 m*M* NaH_2PO_4, 1 m*M* $MgCl_2$, 3.8 m*M* hepes, pH 7.4) with bovine serum albumin (1 mg/ml) and dextrose (1 mg/ml) to yield a final platelet count of 300,000/μL. This permitted the direct com-

parison of results with the PRP sample to their correspondingly washed sample. All samples were analyzed immediately post-preparation to ensure no alterations of the pH.

Fibrinogen (100 μg/ml final concentration) was added to washed platelet samples prior to the addition of agonists. Aggregation was monitored as percent change in light transmission using a Chronolog-lumi Aggregometer (Havertown, PA) as previously described (40). The initial rate of aggregation was not quantitated but only the extent of aggregation. The aggregation of a 500 μl PRP sample was measured in response to the thrombin-activating peptide, SFLLRNP, 7.6 μ*M* (originally kindly supplied by Drs. N. Greco & G. Jamieson, then purchased from TANA Labs, Houston, TX) in lieu of thrombin, which would also induce coagulation and overshadow aggregation. However, 0.05–0.1 U/ml thrombin (Fibrindex, Ortho Diagnostic Systems, Raritan, NJ) was employed with washed platelet samples since the interfering coagulation proteins were removed and also due to cost considerations. Both of the agonists, acting at the seven transmembrane thrombin receptor, induced equal levels of aggregation with the fresh PRP and washed samples. Also, in a limited number of experiments SFLLRNP was compared to thrombin with washed platelet samples, and both agonists induced equal levels of aggregation. Two other agonists were used in these studies, 1 μ*M* U46619 (Cayman Chemical, Ann Arbor, MI), a thromboxane A2 mimetic, and 10 μ*M* epinephrine (Sigma, St. Louis, MO) were added alone or in combination. When added together, the final concentrations of U46619 and epinephrine were 0.5 μ*M* and 5 μ*M*, respectively. All agonists were added at Time 0 and final percent aggregation was recorded at 3 min. Statistical analysis of the data was performed as described in the text with significance taken as a minimum $P < 0.05$.

Results

We initiated a series of studies to determine the extent to which the platelet count of stored platelet concentrates (PC) and the resulting pH contribute to the platelet storage lesion commonly observed with these preparations. The potential relationship of pH versus platelet count for samples stored for 6 or 7 days was determined by graphing pH relative to cell count. The data were analyzed *via* linear regression and plotted with 95% confidence intervals (Fig. 1). A significant decrease of pH was observed in the Day 6 PC ($P = 0.0027$) relative to increasing platelet count; however, no significant correlation was observed in the Day 7 samples (the pH distribution of samples is shown in Fig. 3C, $P = 0.1544$).

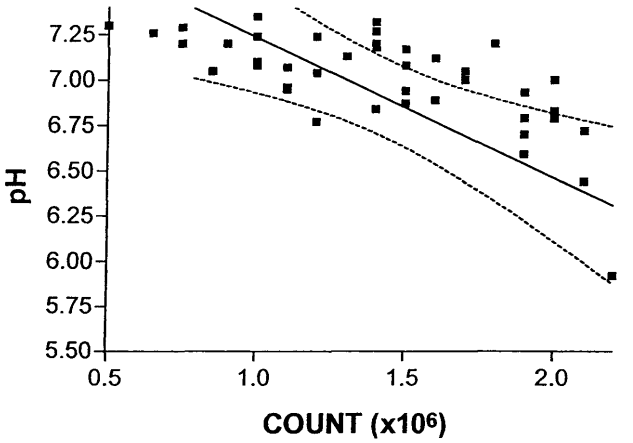

Figure 1. Correlation of storage plasma pH to platelet count of platelet concentrates stored for 6 days. Data were analyzed by linear regression with 95% confidence intervals depicted as dashed lines. The correlation of pH to cell count was significant with a *P* value < .003.

Based upon these findings further experiments were conducted to determine if there was a correlation between count and/or pH versus agonist-induced aggregation of platelet-rich plasma (PRP). Agonists employed for these studies were epinephrine (10 μ*M*), U46619 (1 μ*M*), and the thrombin receptor activating peptide, SFLLRNP (7.6 μ*M*). Whereas freshly prepared PRP samples showed significant aggregation responses to epinephrine or U46619 alone, the stored PC showed little, or, no activity, presumably due to the storage lesion (Table I). Due to this lack of activity with the stored PC, it was decided to determine if both agonists added together would work in synergy. Stored samples showed significant synergistic activity with the combined

Table I. Agonist-Induced Platelet Aggregations with Freshly Prepared (Day 0) and Stored (Day 6–7) Platelet Concentrates in Both PRP and Washed Samples

Agonist	Days of incubation		
PRP	Day 0	Day 6	Day 7
Epi	23.8 ± 17.8 (12)	1.6 ± 2.5 (31)*	2.2 ± 1.8 (10)*
U4	35.1 ± 35.9 (11)	0 ± 0 (30)*	0.3 ± 0.8 (10)*
U4 + Epi	72.9 ± 19.9 (10)	22.7 ± 22.4 (24)*	20.2 ± 18.3 (19)*
SFLLRNP	73.8 ± 14.4 (12)	9.4 ± 13.3 (44)*	17.1 ± 17.3 (20)*
Washed	Day 0	Day 6	Day 7
U4 + Epi	84.8 ± 10.5 (10)	55.0 ± 14.1 (24)*	44.5 ± 22.9 (19)*
Thrombin	76.3 ± 20.6 (12)	51.2 ± 21.4 (43)*	51.8 ± 24.7 (20)*

Note. Data are reported as means ± standard deviations (number of samples). Data were analyzed by ANOVA and Newman-Keuls multiple comparisons test. The (*) represents deviations from Day 0 preparations with a *P* value < 0.05. U4, U46619; and Epi, epinephrine.

agonists; however, the response was less than that seen with freshly prepared platelets (Table I). The reduced activity observed with the stored PRP (Days 6 and 7) was significantly less than the Day 0 samples using the Newman-Keuls multiple comparisons test with a P value < 0.05. No significant difference was noted between the Day 6 and Day 7 samples.

The aggregation response for each Day 6 and 7 PRP sample to U46619-plus-epinephrine was graphed against the corresponding sample's storage pH and cell count. The data were analyzed by linear regression and tested for significance. Aggregation did not correlate with either pH ($P >$ 0.39) or count ($P >$ 0.62) in the 6-day or 7-day stored samples.

The third agonist studied with PRP, SFLLRNP, again showed significantly less aggregation with the stored PC as compared to the freshly prepared samples (Table I). The level of SFLLRNP-induced platelet aggregation with Day 6 PC was not significantly different from the levels detected with the Day 7 PC. Again, the aggregation responses to SFLLRNP were plotted against both pH and count. Analysis by linear regression demonstrated that there was no significant correlation to either pH or count in Day 6 (P = 0.4724, 0.8356) or Day 7 (P = 0.6414, 0.3986) stored platelet preparations. More than 50% of the Day 6 samples and 25% of the Day 7 samples did not aggregate at all in response to SFLLRNP. It was reasoned that these nonresponsive samples might skew the analysis and were, therefore, omitted with the remaining data points analyzed *via* linear regression. However, without the zeros, SFLLRNP-induced aggregations still showed no correlation with pH or count.

Platelets were washed and re-suspended in hepes Tyrodes' buffer as described in Methods. This presumably removed toxic compounds that accumulated in the storage plasma. These conditions only approximate what may happen upon transfusion of PC. SFLLRNP-induced aggregations with PRP was repeatedly demonstrated to induce approximately equivalent thrombin-induced aggregation responses with the corresponding washed platelet sample (Table I). Therefore, thrombin was employed with all washed platelet samples and compared to results with SFLLNRP added to PRP samples. The response of the fresh, Day 0 samples as PRP did not increase significantly with U46619-plus-epinephrine or thrombin/SFLLRNP upon washing (Table I). The response to both U46619-plus-epinephrine and thrombin decreased with storage time in the washed samples (Table I). Day 6 and 7 samples showed significantly less reactivity to both agonists compared to Day 0 according to the Newman-Keuls multiple comparisons test at the 0.05 significance level. Day 6 reactivity was not found to be significantly different from Day 7 with either set of agonists. With both the Day 6 and Day 7 stored platelet preparations, the response of the washed samples to U46619-plus-epinephrine and thrombin was significantly greater than that observed with the corresponding U46619-plus-epinephrine and SFLLRNP-activated PRP samples as analyzed by a paired Student's t test ($P <$ 0.001) (Table II).

Since many samples were nonresponsive to the agonists, we analyzed the data further by separating the samples into two independent sets based on activity to the agonists. Data of samples with a minimal response (0%–12.5%) to U46619-plus-epinephrine or SFLLRNP are presented as the A groups, and samples with a significant response (>12.5%) to the agonists are presented in the B groups (Table II). When analyzed separately, Group A samples showed significant increases in reactivity upon washing with all agonists employed for both Day 6 and Day 7 samples. Group B samples showed no significant increase upon washing in reactivity to U46619-plus-epinephrine for either Day 6 (P = 0.1) or Day 7 (P = 0.068). However, Group B samples showed a significant increase in activity upon washing for both Day 6 and 7 samples reacted with thrombin as compared to PRP samples reacted with SFLLRNP (Table II). When the aggregation response was compared between the two populations of platelet samples after washing, Group A compared to B, the apparent distinction between the two groups observed in PRP samples disappeared (Table II). This distinction between the two PRP groups did not correlate to pH (Day 6 P = 0.967, Day

Table II. Variations in PRP Activity to U46619 + Epinephrine (U4 + Epi) or SFLLRNP and the Corresponding Response of Washed Platelets to U4 + Epi or Thrombin

	PRP		Washed	
	Day 6	Day 7	Day 6	Day 7
	U4 + Epi		U4 + Epi	
total samples	22.7 ± 22.4 (24)	20.2 ± 18.3 (19)	55.0 ± 14.1 (24)*	44.5 ± 22.9 (19)*
Group A	3.2 ± 4.7 (11)	4.5 ± 5.5 (9)	57.5 ± 13.3 (11)*	39.3 ± 25.5 (9)*
Group B	42.7 ± 14.0 (13)	34.4 ± 12.9 (10)	52.8 ± 15.0 (13)	49.1 ± 20.4 (10)
	SFLLRNP		Thrombin	
total samples	9.4 ± 13.3 (43)	17.1 ± 17.3 (20)	51.2 ± 21.4 (43)*	51.8 ± 24.7 (20)*
Group A	1.6 ± 3.5 (31)	3.2 ± 4.4 (10)	50.7 ± 20.4 (31)*	50.8 ± 31.4 (10)*
Group B	28.7 ± 8.9 (12)	31.0 ± 13.3 (10)	52.6 ± 24.7 (12)*	52.9 ± 17.3 (10)*

Data are reported as means ± standard deviations (number of samples). Data were analyzed by a paired Student's t-test. The (*) represents deviations from corresponding PRP preparations with P value < 0.001. Group A represents PRP samples with % aggregation ≤ 12.5%, and Group B represents samples > 12.5%.

7 $P = 0.723$) or platelet count (Day 6 $P = 0.429$, Day 7 $P = 0.522$) for U46619-plus-epinephrine or SFLLRNP (pH Day 6 $P = 0.425$, Day 7 $P = 0.636$) (count Day 6 $P = 0.639$, Day 7 $P = 0.823$). These two apparent subpopulations of PRP samples (Groups A and B) were also analyzed to determine if the U46619-plus-epinephrine responsive and nonresponsive groups reacted similarly with SFLLRNP. Figure 2 demonstrates that in PRP samples the nonresponsive and responsive groups with U46619-plus-epinephrine did not correlate with the same groups responding to SFLLRNP. However, after washing, each platelet sample's responsiveness to U46619-plus-epinephrine and thrombin showed significant correlation based on linear regression analysis (Day 6 $P < 0.0001$, Day 7 $P = 0.0176$).

Thrombin- and U46619-plus-epinephrine-induced aggregation levels were plotted against pH and count for the washed platelet samples. Unlike the results obtained with PRP samples the r value determined by linear regression analysis was significant for pH versus thrombin-induced aggregation in Day 6 washed platelets ($P = 0.0098$) (Fig. 3A) but not with the 7-day sample ($P = 0.6119$). The pH also correlated to U46619-plus-epinephrine-induced aggregation for both Day 6 ($P = 0.0416$) and Day 7 ($P = 0.0098$) washed platelet preparations (Fig. 3B and 3C). Finally, platelet count correlated with the thrombin response with both Day 6 ($P = 0.0006$) and Day 7 ($P = 0.0299$) washed platelet preparations but not with the U46619-plus-epinephrine combination (Fig. 4).

Discussion

Metabolic products that are normally removed from the circulation *in vivo* may accumulate in the plasma of stored platelet concentrates (PC) leading to a storage lesion associated with reduced platelet function. Understanding the

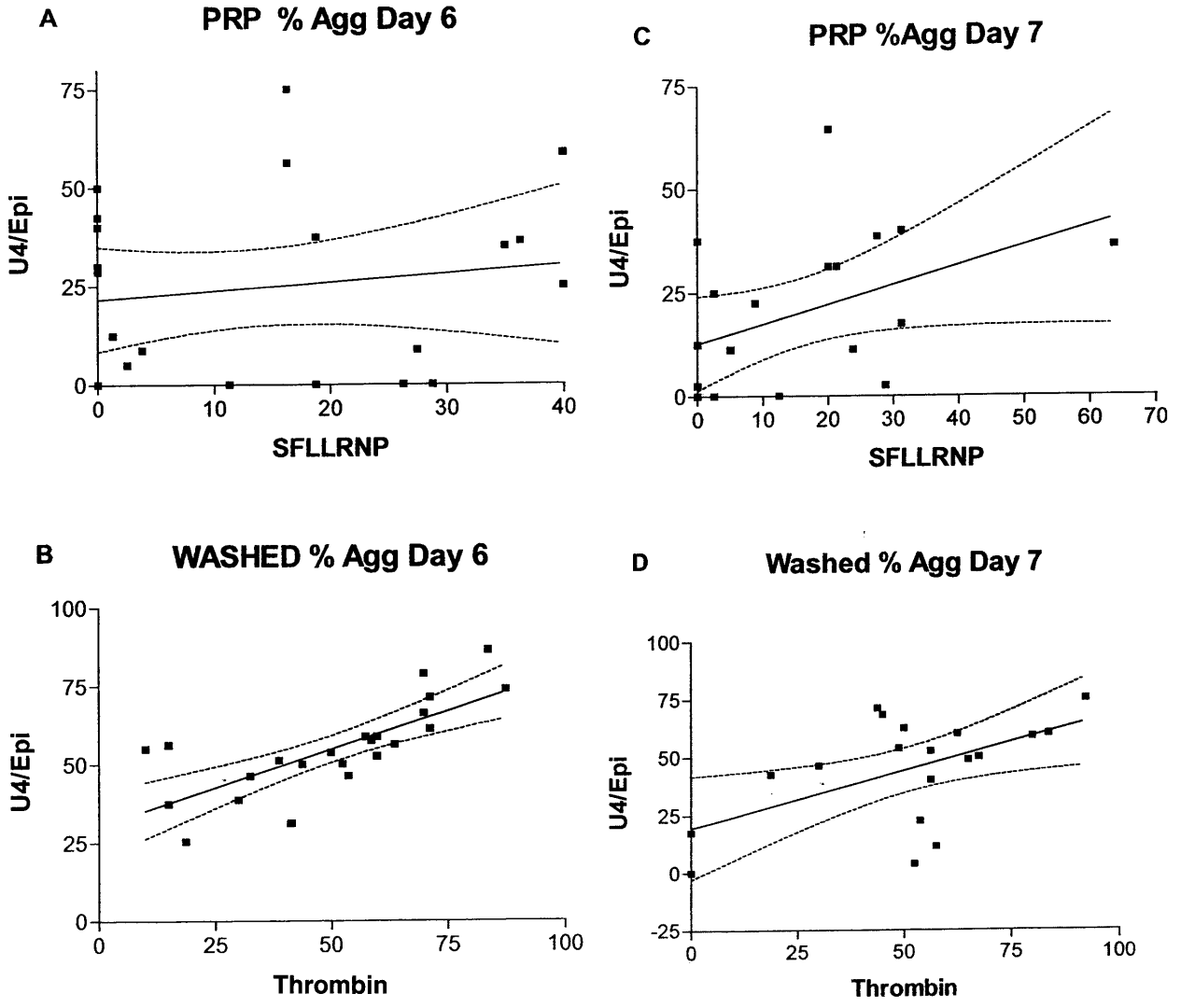

Figure 2. Potential correlation of two different agonist-induced platelet aggregations with responsive and nonresponsive stored platelet concentrates. Data were analyzed by linear regressions with 95% confidence intervals depicted as dashed lines. The U46619 + epinephrine-induced platelet aggregations with PRP samples that were responsive (> 12.5%) or nonresponsive (0–12.5%) did not correlate to SFLLRNP-induced PRP responsive and nonresponsive samples that had been stored for 6(A) or 7(C) days. Correlations were detected with these same samples that were washed and exposed to thrombin in lieu of SFLLRNP. Agonist-induced aggregations with Day 6 samples (B) correlated with a P value < .001 whereas the P value for Day 7 (D) was < .02.

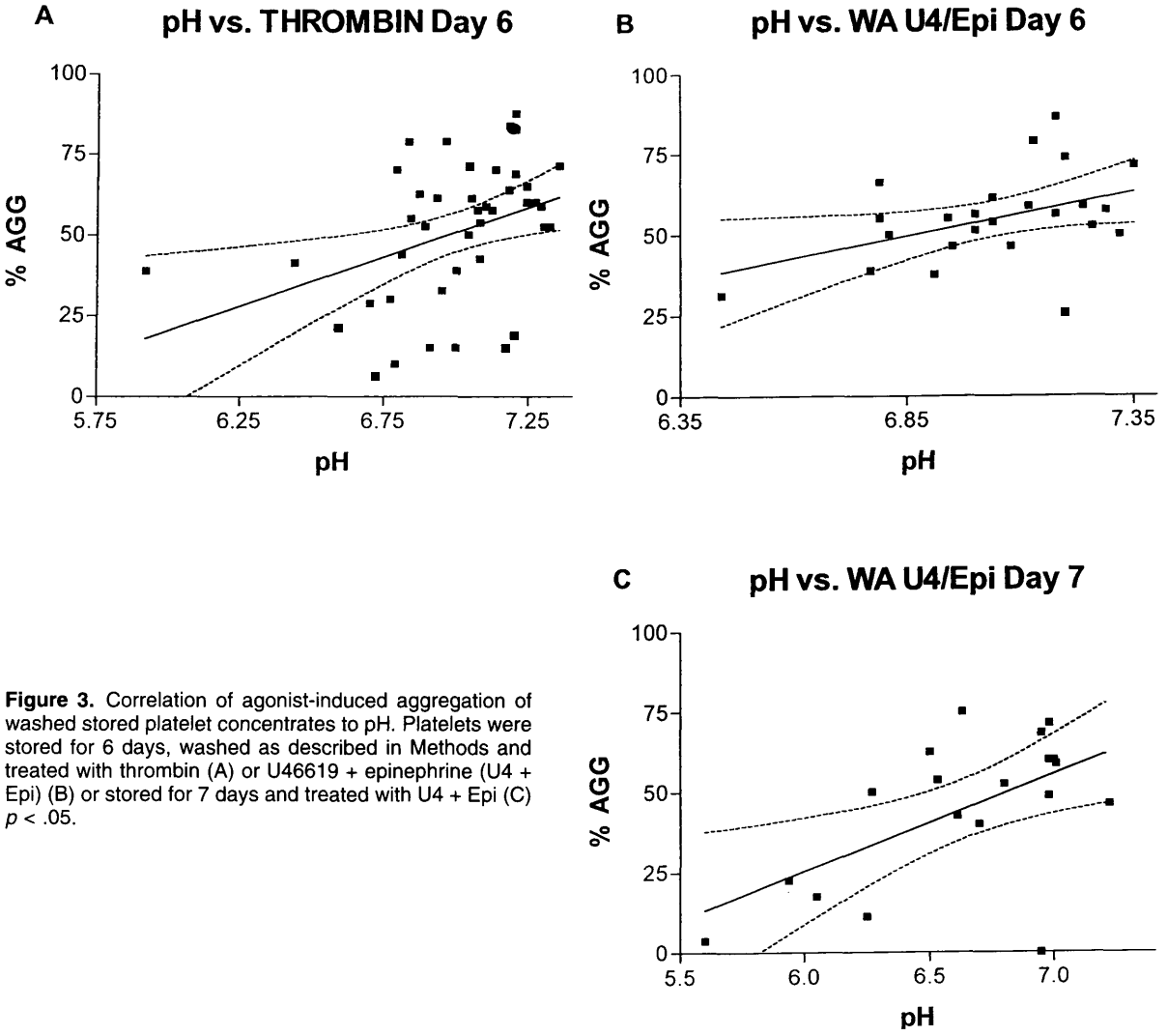

Figure 3. Correlation of agonist-induced aggregation of washed stored platelet concentrates to pH. Platelets were stored for 6 days, washed as described in Methods and treated with thrombin (A) or U46619 + epinephrine (U4 + Epi) (B) or stored for 7 days and treated with U4 + Epi (C) $p < .05$.

complexities of the platelet storage lesion can ultimately lead to modifications of storage procedures that may abrogate these deleterious effects. It has been well documented that a fall in the pH of PC contributes to the storage lesion (19, 20, 34). The major contributor to a reduced pH is an increased lactate production coupled to bicarbonate consumption (20). Other products may accumulate to nonphysiological levels in this closed system as a result of platelet activation during storage and may also contribute to the storage lesion. Platelet activation may be induced by interleukins that are released from contaminating white blood cells present in the PC (41). Platelet activation not only involves shape change but a burst of metabolic activity and release of storage contents (36). Our studies demonstrated, for the first time, that some lesions are reversible and independent of pH whereas others appear to be pH-dependent and permanent in nature. These observations were made employing unique washing conditions that may more accurately reflect the slow reversal process of the storage lesion *in vivo* (20, 38). Correlations were evident even when the pH remained above 6.5.

We demonstrated, as have others (18), that there was a significant correlation between high platelet counts of stored PC in second generation containers with lower storage pH. Similar results were also obtained with first generation containers (19). The exposure to acidic conditions resulting from high platelet concentrations resulted in a demonstrable loss of reactivity of stored PC to SFLLRNP/thrombin and U46619-plus-epinephrine with both PRP and washed platelets. By adjusting the pH to 7.4 for all aggregation reactions, we excluded the contribution of the existing pH's impact on aggregation and only observed the impact of platelet alterations and/or toxic storage components on the reaction. Adjusting the pH should also reverse any pH-induced change in platelet shape that might impact on their response to agonists (20). The loss in activity was shown to correlate with pH or cell count only with the washed platelets and not the PRP samples. Moroff and Chang (42) also showed that platelets stored as PRP lost activity in a pH-independent fashion. Presumably toxic compounds that accumulate in the storage plasma account for variable levels of additional inhibition of agonist-induced aggregations observed with PRP as compared to washed platelet preparations. This inhibition appears to ob-

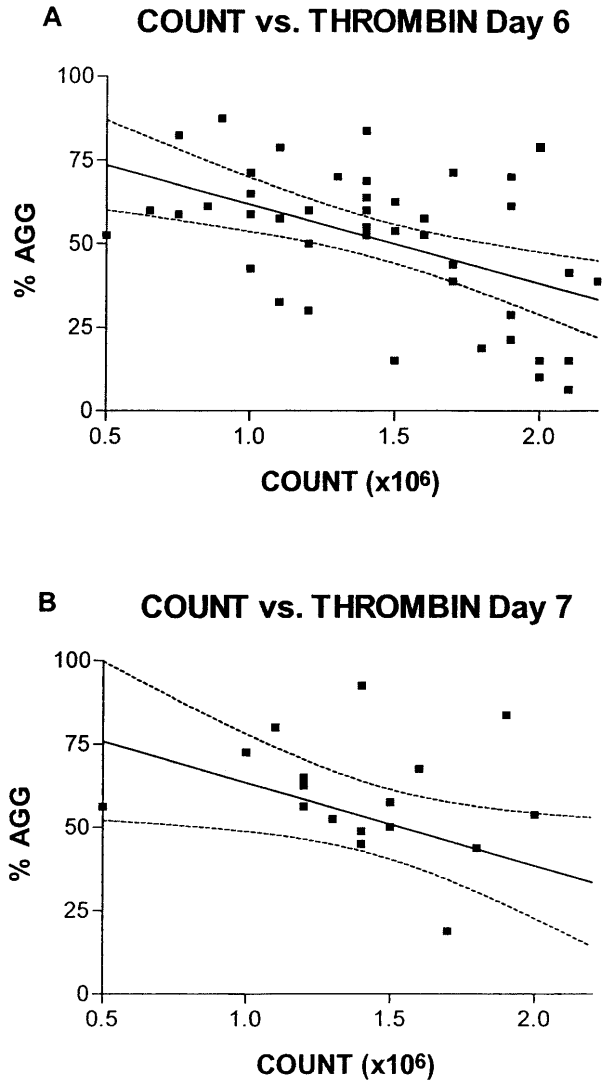

Figure 4. Correlation of thrombin-induced aggregation of washed stored platelet concentrates to cell count. Platelets were stored for 6 days (A) or 7 days (B). $P < 0.3$.

scure the correlation of activity with pH in the PRP samples. The reduced activity of washed, stored platelet samples relative to freshly prepared, washed platelet samples and the correlation of activity to reduced pH would indicate an irreversible nature to the pH-induced lesion. Whether it is truly irreversible as demonstrated *in vitro,* or slowly recovered after time *in vivo,* post-transfusion, as described by others (20, 38), is unknown and would require further investigation.

We detected two subpopulations of PC with regard to agonist-induced aggregation of PRP; one was responsive, and a second was nonresponsive. Most of the stored platelet-rich plasma samples that were nonresponsive to U46619-plus-epinephrine aggregated in response to SFLLRNP. The converse was true with samples that were nonresponsive to SFLLRNP. This implies that the activation pathways and/or receptors of the two agonists are differentially affected by the metabolite(s)/conditions present in the storage plasma. In each case, the inhibitory effect was reversible upon washing. This indicates that this apparent storage lesion may have little impact on platelet function when PC are transfused into a patient. The inhibitor(s), however, interfered with PRP activity, and makes *in vitro* investigations into platelet activity difficult or misleading. Further inquiry into this phenomenon requires the isolation and characterization of the metabolite products that differentially modulate platelet function. It is also necessary to determine if they contribute to the irreversible component of the platelet lesion described in these studies. The demonstration of physiological significance of these *in vitro* studies must await future work where stored platelets are transfused into normal hosts and their function and survival reassessed at varying times post-transfusion.

The fact that stored PC regained a significant amount of their lost ability to respond to agonists upon washing and re-suspension in hepes Tyrode's buffer may have physiological relevance. We (data not shown), and others, (34, 42) found that adding fresh plasma to stored PC or resuspending stored platelets in fresh plasma did not restore lost aggregation responses. While inhibited platelet functions could be reversed by fresh plasma with 1-day stored platelets, this reversal no longer occurred with 3-day stored platelets (43). Similarly, adding stored platelet-poor plasma to fresh platelets did not inhibit their aggregation (34). This would indicate that the reversible component of storage-induced inhibition of platelet aggregation may be due to a toxic factor(s) that strongly associates with the surface of the platelet. Re-suspension of platelets, stored for more than 1 day, in fresh plasma may not allow for the ready dissociation of a surface associated toxic factor(s) due to the high plasma protein content. This apparent delayed dissociation may account for the several-hour time frame required *in vivo* to see hemostatic improvement in patients receiving platelet transfusions (20) or improved aggregation (*ex vivo*) of these transfused platelets (38). The rapid reversal of inhibition seen upon re-suspension of washed stored platelets in buffer instead of plasma may allow for the future identification of this inhibitory factor and its mode of action. Other potential causes of a reversible inhibition of platelet aggregation may include platelet/protein conformational changes and modification of intracellular pathways. However, it is difficult to rationalize why these factors would be differentially reversed upon suspension in buffer versus plasma.

As platelets are stored they rapidly lose responsiveness to U46619 (44) or epinephrine when the agonists are added individually. The use of agonists in combination has been demonstrated to yield synergistic activity with several other agonist pairs: ADP and epinephrine; ADP and collagen; as well as ADP and arachidonic acid (45). Here, we demonstrated that synergistic activity also exists with the TXA2 mimetic, U46619, plus epinephrine. However, unlike Di-Minno *et al.* (45), we found a significant reduction in the response to agonist pairs for 6- to 7-day stored PC as com-

pared to fresh PRP. This difference is likely due to the fact that in their study, the platelet count was kept low to prevent a fall in pH, and their samples were only stored for 5 days. The synergistic activity not only allows for analysis of stored PC *in vitro* but may also more closely approximate the conditions to which platelets are exposed *in vivo*. Therefore, viability of stored PC after infusion should be based more on the ability of this synergy to overcome "damage" done by the storage lesion, rather than the responsiveness to single agonists.

The correlation between pH and/or count and the aggregation response, while well demonstrated in the Day 6 preparations, became more variable in the Day 7 samples. This is probably an indication that at longer storage times, pH and/or count are no longer the main cause of the platelet storage lesion. Possibly the lesion becomes too severe for correlations to be made. Other stresses such as cellular breakdown, enzyme degradation, loss of structural integrity (26), depletion of necessary substrates or a change in the conformation of receptors may all be responsible for this loss in viability of platelets stored for extended periods. Two other environmental factors could also account for the apparent loss of correlation between pH/count and aggregation. These are variations in the platelet count of the stored PC (more Day 6 samples had $\geq 2 \times 10^6$ platelets/μl than Day 7 samples) and/or Day 7 samples may have had, on average, different levels of contaminating white blood cells than the Day 6 samples. It would appear that the Day 7 time point is a critical time frame at which platelet deterioration, in many samples, exceeded the orderly loss of activity resulting in the disruption of the linear relationship between pH/count and aggregation. It is not clear, at this time, what specific biological properties account for this change.

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