Improved Storage of Platelets for Transfusion in a New Container


The storage of platelet concentrates (PC) at 22°C in polyvinylchloride containers has been limited to 3 days. Even within this time interval, fall in pH to 6.0 or less with associated loss of viability occurs in a substantial number of PC. Previous work demonstrated that the fall in pH resulted from increased production of lactic acid due to hypoxic conditions within the container and could be prevented by storage in a container constructed from material that was more permeable to oxygen than conventional polyvinylchloride plastic, PL-146. We have now studied PC storage in a new polyolefin plastic, PL-732. This material has increased permeability to oxygen, and pH fall to less than 6.0 does not occur during 5 days of storage. In autologous studies using chromium-51 labeling, mean in vivo recovery and survival T1/2 were 51% and 3.1 days, respectively, after 5 days of storage. After the same storage interval, mean corrected platelet increments in thrombocytopenic patients were 15.7, 11.1, and 6.9 x 10^11/µl after infusion. In paired studies in the same patient, PC stored for 5 days in PL-732 were consistently superior to PC stored for 5 days in PL-146. At 4-6 and 24 hr after infusion, bleeding times were shortened appropriately for the elevation of the platelet count achieved. These good results were achieved by agitating PC (50 ml) on a flat-bed agitator at 70 cycles per minute. Inconsistent results were observed in autologous studies with two other forms of agitation. We conclude that the use of PL-732 will permit the satisfactory storage of PC for 5 days at 22°C. Storage of PC at 4°C has not been evaluated in this container.

The storage of platelet concentrates (PC) at 22°C for transfusion has been less than satisfactory for three reasons. First, the storage interval has been limited to 3 days, an inadequate time period for blood centers to provide a continuous supply of platelets without excessive outdated. Second, even at 3 days, many PC will have pH less than 6.0, which is associated with a loss of viability. Finally, polyvinylchloride (PVC) containers currently in use contain a plasticizer, di-2-ethylhexyl phthalate (DEHP), which diffuses into the PC during storage.

Previous studies have shown that pH fall with storage in PVC containers is due to increased production of lactic acid stimulated by hypoxic conditions within the container. It was found that pH fall could be retarded if PC were stored in a container whose walls were more permeable to oxygen than PVC. However, these containers were experimental and not durable enough for clinical use. We have now studied platelet storage in a new container constructed of Fenwal plastic, PL-732. PL-732 is a blow-molded polyolefin, contains no plasticizer, has increased permeability to oxygen and carbon dioxide relative to currently available PVC, and is sufficiently durable to permit standard component preparation. Storage for 5 days appears to be satisfactory as judged by platelet survival measurements in normal volunteers and measurements of platelet function in thrombocytopenic patients. The pH of PC stored in this new plastic is maintained well above 6.0 throughout this storage interval.

Previous work has indicated that successful storage of PC at 22°C requires continuous, gentle agitation. In a comparison of two forms of agitation—a “ferris-wheel” apparatus (pictured in Murphy and Gardner) and a horizontal, flat-bed agitator with a to-and-fro motion—the use of the flat-bed agitator gave superior in vivo results as judged by autologous platelet survival studies in normal volunteers. For many years, it had been our concept that in vivo viability correlated with maintenance of normal platelet morphology as judged qualitatively by phase microscopy. In the study of the two forms of agitation, platelet morphology was assessed quantitatively by measuring the extent of shape change in response to adenosine diphosphate (ADP) and by calculating the dispersion (geometric standard deviation) of the size distribution of the platelet suspension as determined by Coulter Counter. In vivo viability correlated with maintenance of morphology as reflected in these two measurements. With the new PL-732 container, we find that maintenance of viability varies with the form of agitation used. Once again, superior in vivo viability has been found to correlate with superior maintenance of platelet morphology as determined by the two quantitative measurements.
MATERIALS AND METHODS

Except where noted, whole blood was collected into CPD anticoagu-ulant and PC were prepared to a final volume of 50 ml as previously described. Storage containers were constructed from either commercially available Fenwal PVC (PL-146) or the experimental material (PL-732). The dimensions and inside surface area of the two containers are similar. Four types of agitators were used: (1) a horizontal, flat-bed shaker (Eberbach, Ann Arbor, Mich.) with a to-and-fro motion of 60 cycles per minute and an excursion of 1 cm; (2) a motor-driven "ferris wheel" (rate of rotation 5 cycles per minute) as pictured in figure 3; (3) the commercially available Fenwal elliptical rotator; and (4) the commercially available Helmer "tumbler" agitator. These four agitators will be referred to hereafter as (1) flat-bed, (2) ferris wheel, (3) elliptical agitator, and (4) tumbler agitator. Unless specifically indicated, the flat-bed agitator was used throughout the study.

The following measurements were made on PC as previously described: platelet count and size distribution as determined with a Coulter Counter, extent of shape change in response to ADP, pH at 22°C, and platelet morphology by oil-phase microscopy. In vivo recovery and life-span were determined by labeling stored platelets with chromium-51 and reinfusion into the original normal donor. Percentage recovery and T1/2 survival were calculated as previously described.

Eight patients with thrombocytopenia secondary to acute leukemia and/or cytotoxic chemotherapy received 0.15-0.2 U of stored PC/kg of body weight. PC were stored for 3 days in PL-146 containers (control) or 5 days in PL-732 containers (experimental). The choice of storage container was determined at random. Subsequent to this transfusion, 5 patients remained stable clinically so that they could receive a second infusion using PC stored in the alternate container. Three patients received only one infusion. Platelet count by phase microscopy and the template modification of the Ivy bleeding time were measured before infusion and, where possible, at 1-4, 4-6, and 24 hr after infusion. The corrected increment was calculated from the following formula: (posttransfusion count – pretransfusion count) x body surface area (sq m)/platelets transfused (x 10^11).

All normal participants and patients provided written, informed consent after approval of the study by the local human investigation committees.

RESULTS

In preliminary in vitro studies, platelet count and pH were determined after storage of PC for 24 hr. As indicated in Fig. 1, for any given platelet count, pH was higher in PL-732 than in PL-146 and remained adequate to sustain oxidative metabolism even in PC with platelet counts up to 3 x 10^6/cu mm. As previously reported, pH fell to less than 15 mm Hg when the platelet count exceeded 1.5 x 10^6/cu mm in PL-146. According to previous hypotheses, the superior oxygenation in PL-732 should prevent the pH fall characteristic of PC stored in PL-146. Indeed, Fig. 2 shows that the pH of PL-146 units was far lower than that seen in PL-732 units after 3 days of storage. In PL-732, there was no pH less than 6.8 even in PC with platelet counts as high as 3 x 10^6/cu mm. During the course of the patient studies, 101 PC were prepared in a volume of 30-60 ml and stored for 5 days in PL-732. The pH after 5 days was in the range 6.7-7.4 in 93 PC. The pH fell to 6.1 in one PC and rose to 7.5 and 7.6 in 5 and 2 PC, respectively. On the other hand, 95 PC were stored for 3 days in PL-146, and in 35 (37%) the pH fell to less than 6.0.

Fifty milliliters of PC, obtained from normal volunteers, were stored in PL-146 or PL-732 for 1, 3, or 5 days using the flat-bed agitator. After storage, the platelets were labeled with chromium-51 and infused into the original donor. Figure 3 graphs the calculated in vivo recovery and survival T1/2. There is no significant change in recovery or T1/2 during 5 days of storage in PL-732. After 3 days in PL-146, mean recovery was substantially reduced, predominantly because there was a pH fall to less than 6.0 in 4 of 9 PC studied. To provide a point of reference for comparison with PL-732, PC were also stored for 5 days in PL-146. Prior to storage, the platelet counts of PL-146 units but not PL-732 were adjusted to 10^6/cu mm so that pH fall would not occur. With such an adjustment, the mean recovery value for PL-146 was lower than for PL-732, but the difference was not statistically significant.

Table 1 lists the results of the patient studies.

Figures 4 and 5 express these results graphically. In Fig. 4 it can be seen that in all but one of the paired studies, the increments at all intervals after infusion were greater when platelets were stored in PL-732. This difference was statistically significant at 4-6 hr (p < 0.01). Mean increments (x 10^3/cu mm) for PL-
732 were 15.7, 11.1, and 6.9 at 1–3, 4–6, and 24 hr, whereas in PL-146, the corresponding values were 7.7, 7.3, and 3.5. Figure 5 shows the relationship between the bleeding time and platelet count before and after infusion. The enclosed area in the figure represents the expected correlation. Before infusion, all but one bleeding time was greater than 20 min. Beyond 4 hr after infusion of PC stored in PL-146 or PL-732, the bleeding times were shortened to at least the level expected for the corresponding rise in platelet count. In fact, in many instances, the bleeding time was even shorter than would be predicted.

In three studies represented by connected measurements, two with PL-146 and one with PL-732, the bleeding time at 1–3 hr after infusion was longer than expected in relation to the platelet count. In all three cases, the bleeding time corresponded to the platelet count 24 hr after infusion. These results suggest that the in vivo function of platelets stored in the two containers is similar when compared to the increments achieved and that the increments in PL-732 are at least equivalent if not superior to those observed in PL-146.

We emphasize that the success of storage in PL-732 varies with the conditions used for storage. In Fig. 6, in vivo recovery using chromium-51 labeling in normal volunteers is shown for studies in which the form of agitation and the volume of the PC were varied. Results are satisfactory if PC are stored in 30 ml for 3 days, but in vivo recovery is variable and the mean is significantly reduced for this volume after 5 days of storage \((p < 0.05, \text{relative to } 50 \text{ ml})\). An additional 20 ml of plasma in the unit permits satisfactory storage for 5 days. When 50 ml PC are stored for 7 days, however, there is a significant reduction in recovery \((p < 0.05, \text{relative to } 1 \text{ day of storage})\) so that we would not recommend this storage interval at this time. Much of the variability with 5-day, 30-ml and 7-day, 50-ml storage was due to pH fall. As shown in Fig. 6, the 3 poorest recoveries were found in PC in which pH had fallen to 6.0 or below.
Recovery is also variable and the mean is significantly reduced when PC are agitated either for 3 days on the ferris wheel or for 5 days on the elliptical agitator ($p < 0.01$, relative to same time interval with flat-bed agitation). However, storage for 5 days on the tumbler agitator yielded results that were comparable to storage on the flat-bed agitator.

We examined the results of the 5-day study on the elliptical agitator in more detail in an attempt to understand the variability in recovery that was observed. As we have observed previously with the ferris wheel, loss of viability was associated with alterations in platelet morphology. By oil-phase microscopy, disc-to-sphere transformation was the most prominent change. This was reflected in a decreased extent of shape change following ADP stimulation and an increase in the dispersion of the Coulter counter size distribution. The correlation between these two measurements and the reduction in in vivo recovery is shown in Fig. 7. Thus, loss of viability was associated with a deterioration of morphology. Furthermore, there was also a correlation between loss of viability and a rise in pH from initial values of 7.1–7.2 (Fig. 8).

**DISCUSSION**

Containers constructed of PL-732 plastic apparently offer a solution to the three problems associated...
with storage in PL-146. Fall in pH to less than 6.0 was not observed during a 5-day storage interval in PL-732. It is well known that pH fall to less than 6.0 results in loss of viability and that this phenomenon imposes a limit on the effectiveness of PC stored in containers such as PL-146. Previous work suggested that the fall in pH could be retarded if the walls of the container had increased permeability to oxygen, and the results of this study (Figs. 1 and 2) support this concept.

The autologous studies using chromium-51 labeling in normal volunteers suggest that viability is well maintained after 5 days of storage in PL-732 (Fig. 3). Posttransfusion increments in thrombocytopenic patients confirmed this observation. Mean increments of 15.7, 11.1, and 6.9 x 10^11 platelets/µM sq m/10^11 platelets infused at 1−3, 4−6, and 24 hr posttransfusion, are compatible with successful storage techniques reported in the literature. Furthermore, in 5 paired studies in which patients received platelets stored in both containers, PC stored for 5 days in PL-732 produced increments that were at least as good if not better than increments produced by PC stored for 3 days in PL-146 (Fig. 4). In addition, the function of platelets stored for 5 days in PL-732 was equivalent to the function of platelets stored for 3 days in PL-146 as judged by bleeding time corrections posttransfusion in thrombocytopenic patients (Fig. 5). Since PC stored for 3 days in PL-146 are currently considered acceptable for clinical use, PC stored for 5 days in PL-732 should be equally acceptable.

Results of storage of PC for 5 days in containers constructed from PL-732 varied with the conditions used for storage. As indicated in Fig. 6, in vivo recovery was variable and significantly reduced for 5 days of storage if the PC volume was 30 ml rather than 50 ml. Some of this variability was related to pH fall. In addition, the use of two alternate forms of agitation, either the ferris wheel or the elliptical agitator, resulted in variable and significantly reduced in vivo recovery even when the volume was 50 ml. However, storage on the tumbler agitator produced very good results (Fig. 6). The elliptical and tumbler agitators both provide rotary forms of agitation, but the modes of rotation are different. With the elliptical agitator, the container rotates about its midpoint, staying within

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**Fig. 5.** Bleeding times and platelet counts in thrombocytopenic patients before (x) and after transfusion of platelets stored for 5 days in PL-732 (open symbols) and for 3 days in PL-146 (closed symbols). Triangles, circles, and squares represent measurements made 1−3, 4−6, and 24 hr after infusion, respectively. The enclosed area represents the 95% confidence limits of this relationship in untransfused aplastic patients. The arrows connect sequential measurements obtained in the same patient. Bleeding time correction by platelets stored in the two plastics is equivalent relative to the platelet count achieved.

**Fig. 6.** In vivo recovery in autologous studies in normal volunteers with variation of PC volume, storage interval, and method of agitation for storage in PL-732. The symbols represent individual studies. Numbers in parentheses represent final PC pH. The horizontal line represents the lower limit of the results when 50 ml PC are stored for 5 days with flatbed agitation (see Fig. 3). Use of 30 ml volume for 5 days, storage for 7 days, or the use of ferris wheel or elliptical agitation frequently produce poor results.
IN VIVO RECOVERY

Fig. 7. Correlation between in vitro measurements and in vivo recovery after storage on elliptical agitator. PC were stored for 5 days in PL-732 containers. In vivo recovery varied widely for the 9 donors. There was a strong correlation between recovery and the two in vitro measurements that reflect platelet morphology (r = 0.95 for extent of shape change and -0.86 for dispersion). Normal ranges for extent of shape change and dispersion are 1.15-1.25 and 1.65-1.80, respectively, using fresh platelets.

IN VIVO RECOVERY

Fig. 8. Correlation between in vivo recovery and pH after storage in PL-732 for 5 days on the elliptical agitator. Recovery was inversely proportional to pH (r = -0.94).

a constant plane that makes a 70° angle with the bench top. With the tumbler agitator, the plane occupied by the container constantly changes as it rotates through a full 360° with each cycle. We have no explanation for the differences in results produced by these two modes. However, there is precedence for our finding that some forms of rotory agitation produce poor results. In a previous study in which PC were stored for 3 days in experimental, polyolefin containers using the ferris wheel agitator, we observed that some PC had a marked rise in pH during storage that was associated with morphological deterioration and loss of viability. Most of the pH rise appeared to be due to CO₂ escape through the walls of the container. The rise in pH, morphological deterioration, and loss of viability could all be prevented if the containers were agitated in an external atmosphere of 10% CO₂, which prevented the rise in pH. Thus, the rise in pH was implicated as a damaging factor. The data in Figs. 7 and 8 suggest that similar events are occurring in PC stored on the elliptical agitator in PL-732.

The bleeding time measurements in the thrombocytopenic patients raise several important theoretical and practical questions. At 4–6 and 24 hr after infusion, the bleeding times were even shorter than predicted by the relationship derived from aplastic patients. This finding is quite similar to results reported for 3-day storage in PL-146 by Slichter and Harker (see Fig. 6 in reference 4). These investigators did not comment on this phenomenon and we have no explanation for it. In three patients, a bleeding time measurement was performed 1–3 hr after infusion, and the expected shortening was not observed, although in all 3 instances function was normal 24 hr after infusion. This phenomenon was observed after storage in both plastics and suggests that the function of stored platelets is impaired immediately after infusion but becomes normal over several hours. A similar observation was made by Slichter and Harker in approximately half of their patients who were infused with stored platelets. Similarly, when Filip and Aster stored PC for 72 hr at 22°C in PL-146, the bleeding time correction in thrombocytopenic recipients was better at 4 hr than it was at 1 hr, although the difference was not statistically significant. Clearly, further studies should be carried out to clarify the nature of this storage lesion and the mechanism of its repair. It is important to note that the lesion was just as prominent with PC stored for 3 days in PL-146 as it was for PC stored for 5 days in PL-732.

The possibility of storing PC for 5 days is logistically very attractive, since it will unquestionably increase the availability of platelets for transfusion. Of equal importance is the fact that the use of PL-732 will improve the quality of PC stored for shorter periods of
time. In this study, 37% of PC stored in PL-146 for 3 days had a pH less than 6.0, whereas virtually all PC stored in PL-732 had a pH greater than 6.6. Furthermore, the volume of PC stored in PL-732 can be 30 ml if storage is limited to 3 days (Fig. 6). Thus, the overall effect of using this new container will be to markedly improve the availability and quality of platelets administered to thrombocytopenic patients.

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REFERENCES

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