Storage of Platelet Concentrates at 22°C

By Scott Murphy, Seyed N. Sayar and Frank H. Gardner

RAPID REDUCTION in platelet viability during storage has been the major obstacle preventing the widespread use of platelet transfusions to control hemorrhage in thrombocytopenic patients. Recent work has shown that storage of platelet-rich plasma (PRP) is satisfactory for as long as 96 hours if it is carried out at room temperature (22°C), rather than in the refrigerator (4°C). At the completion of storage, PRP can be further processed to prepare platelet concentrates (PC) for infusion if it is desirable to restrict the volume administered to the patient. Ultimately, this technique would be unsatisfactory for many groups since it would not allow the immediate processing of fresh platelet-poor plasma (PPP) for its Factor VIII content. To allow the recovery of this blood component, studies have been performed to evaluate the possibility of storing platelets in concentrate form. This preparation would have the added advantage that PC would be immediately available in an emergency situation when skilled technical assistance might not be on hand. The major portion of this report is to be concerned with this data.

In addition, another problem of equal importance has been studied. In the past, most groups have found it necessary to acidify PRP by the addition of extra ACD anticoagulant prior to centrifugation to prevent irreversible platelet clumping upon resuspension of the sedimented button. This technique has three important disadvantages. First, it necessitates entry of the closed, sterile system of plastic bags if the amount of ACD added is to be a constant proportion of the volume of PRP. Secondly, although there is disagreement on the point, the reduced pH of the supernatent PPP has interfered with subsequent recovery of Factor VIII by cryoprecipitation. Finally, the results to be described in this report indicate that acidification reduces the storage interval possible for PC. For all these reasons, the technique first proposed by Mourad which allows preparation of PC without acidification seemed useful. Studies of this technique form a second portion of this report.

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MATERIALS AND METHODS

PC were prepared and their viability after storage was assessed by a variation of techniques previously described. Whole blood was obtained from normal adult male volunteers. Included in this study are results obtained with blood drawn into either ACD NIH formula A or ACD supplemented with adenine (52.4 mg. free base/100 ml. ACD-A); no difference was found in the viability of fresh or stored platelets obtained after primary anticoagulation with either of these two solutions. After separation of PRP by differential centrifugation, PC were prepared by one of two techniques. In one group (acidification), extra ACD-A (without adenine) was added to PRP (5.5-11 ml. to 100 ml. PRP). The platelets were sedimented by centrifugation, and the platelet button was immediately resuspended to a final volume of approximately 25 ml. In the second group (no acidification), PRP was immediately centrifuged and the platelet button was allowed to rest undisturbed for one hour at 22°C under approximately 25 ml. of PPP. It was then resuspended. Both preparations routinely resuspended without formation of gross or microscopic platelet aggregates. Storage studies were carried out in bags constructed from either standard commercial plastic (Fenwal PL-130) or a new, investigational plastic (Fenwal PL-1461). The latter material was found to be superior for PC storage (see Results).

When PC were to be stored, they were maintained in a constant temperature incubator at 22°C and, except where noted, agitated at 20 cycles per minute on an aliquot mixer. The companion PPP was stored in the same incubator without agitation. At the end of the storage interval, the PC was mixed with its companion PPP, and the standard procedures previously described of labeling with radioactive chromium ($^{51}$Cr), reinfusion into the original volunteer, and calculation of per cent yield and lifespan ($T_{1/2}$) were carried out to assess the viability of the stored platelets. It should be noted that when the viability of fresh PC prepared without acidification was to be studied, the platelets underwent two episodes of resting undisturbed as a button for one hour. The first occurred during the initial PC preparation prior to $^{51}$Cr labeling; the second occurred after labeling when the platelets were washed in nonradioactive PPP to remove excess $^{51}$Cr.

On 12 occasions, ABO-compatible PC from normal donors prepared by one of the two techniques described were stored, pooled after storage, and infused into thrombocytopenic patients with leukemia or aplastic anemia. The maximum in vivo yield on the day of transfusion and subsequent lifespan were determined by platelet counting. Whole blood platelet counts were performed by the method of Brecher and Cronkite. Platelets in PRP and PC were counted with a Model F Coulter Counter. PC pH was measured at room temperature with a pH meter (IL Model 135A) immediately after removal of the specimen from the plastic container. Platelet morphology was examined under oil, phase-contrast microscopy after incubation of the specimen for one hour at 37°C.

RESULTS

Events Occurring During Storage

There was no difference in initial platelet counts between PC prepared by the two techniques (mean = 2,010,000 per mm.$^3$). The initial mean pH's of PC prepared with and without acidification were 6.5 and 6.8, respectively (PC acidified with 5.5 ml. ACD to 100 ml. PRP). During the first 24 hours of stor-

In initial studies, the larger volume (11 ml.) was used, but subsequently the smaller (5.5 ml.) was found to be sufficient to allow easy PC resuspension. There was no difference in the results obtained with these two volumes.

Fenwal has submitted Investigational New Drug applications and New Drug Applications for many parenteral solutions and anticoagulant systems in PL-146 to Food and Drug Administration, and approval for commercial use is expected in the near future.

Ames Lab-Tek, Inc., Westmont, Ill.
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The pH of acidified and unacidified PC fell at similar rates although, at any point in time, the pH of the former would be lower due to the lower starting point. The rate of fall in pH varied inversely with PC volume; after 24 hours of storage, PC with volume of 10 ml. had a mean pH 0.4 units less than those with volume of 25 ml. This was the rationale for using a relatively high PC volume (25 ml.) throughout these studies. After 48 hours of storage, pH fell below 6.0 in approximately 20 per cent of PC prepared without acidification and resuspended to a final volume of 25 ml.; the pH of 35 per cent of these concentrates fell below 6.0 in 72 hours.

When the pH remained above 6.0, platelets in most instances maintained their normal discrete, discoid configuration by phase microscopy; if the pH eventually fell below 6.0, the cells became spiny spheres with dendritic projections and tended to aggregate. This morphologic change could be reproduced if the pH of fresh PC was artificially lowered with isotonic hydrochloric acid. On the other hand, even after 24 hours, small clumps of as yet unidentified material were frequently visible grossly. This material is probably not formed from platelets since microscopically platelet aggregates were not seen to accompany its formation. Furthermore, platelet counts enumerated either by phase microscopy or Coulter Counter fell by a mean of only 10 per cent during three days of storage suggesting that the platelets were remaining discrete. This was true for both acidified and unacidified PC. To date, we have been unable to solubilize this precipitated material for further study and it has reacted apparently nonspecifically with both fluorescein-labeled antifibrinogen and antigammaglobulin.

Comparison of Two Plastics

When acidified PC were stored for 24 hours, the results (Table 1) were significantly better for units stored in the investigational plastic (PL-146) when compared with units stored in the standard, commercial material (PL-130). These results should be interpreted cautiously since they may reflect a subtle, random variability of plastic within PL-130 or PL-146, rather than an overall superiority for PL-146. They do emphasize the necessity for considering plastic type as a significant variable in studies of this sort. All the data to follow were obtained with bags prepared from PL-146.

Viability After Storage – PC vs PRP

Figure 1 compares yields and lifespan T %’s for platelets stored for one to three days either as acidified PC or PRP. PC storage is slightly inferior but

<table>
<thead>
<tr>
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<th>Investigational (PL-146)</th>
<th>Commercial (PL-130)</th>
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</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>47 ± 3*</td>
<td>35 ± 2 p &lt; 0.005</td>
</tr>
<tr>
<td>T % (Days)</td>
<td>3.9 ± 0.3*</td>
<td>3.1 ± 0.2 p &lt; 0.02</td>
</tr>
<tr>
<td>Number Studies</td>
<td>13</td>
<td>22</td>
</tr>
</tbody>
</table>

* ± S.E.M.
the differences are not great. Only PC in which pH remained above 6.0 are included in these figures. If pH fell below 6.0, the morphologic observations reported above were associated with an abrupt loss of viability. Figure 2 summarizes these results for acidified PC stored for three days.

**PC Viability Before and After Storage—Acidified vs Nonacidified**

With very rare exceptions, PC prepared without acidification by Mourad's technique resuspended without difficulty. When such concentrates were labeled with $^{51}$Cr and reinfused into the original donors, a mean initial yield of 56 per cent was found followed by a normal lifespan mean ($T\frac{1}{2} = 4.1$ days). These results do not differ significantly from concurrent results obtained with PC prepared after acidification (Fig. 3). When PC prepared without prior acidification were stored one through three days, the results were the same as those achieved with PC prepared with prior acidification. This method of preparation offers the additional advantage that at any point during storage, PC pH is higher than that of a companion PC prepared with acidification.
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Fig. 3.—Comparison of $^{51}$Cr yield and lifespan for PC prepared with and without acidification. Results essentially equivalent for both fresh platelets and platelets stored for one to three days. At least five studies done for each group. No differences statistically significant.

Therefore, a longer period of useful storage is achieved before the critical pH of six is reached.

Necessity for Agitation During Storage

We have routinely agitated PC during storage to prevent platelet packing. Eight concentrates were stored without agitation for 48 hours resulting in a mean yield of 22.6 ± 2.3 per cent (S.E.M.) and a mean T½ of 2.6 ± 0.2 (S.E.M.) days. Comparable results for 13 PC agitated for 48 hours were 36 ± 2.4 per cent and 3.6 ± 0.2 days, respectively. The differences between both yields and T½’s are statistically significant (p <0.05).

Effect of Contact with Aged Platelets on Viability of Fresh Platelets

On nine occasions, an unacidified PC was prepared and stored at 22°C. Four days later, a fresh PC from the same donor was prepared, labeled with $^{51}$Cr, washed once, and mixed for one half hour at 22°C with the stored unit. The technique was such that only minute amounts of free $^{51}$Cr would remain to label the stored platelets. The combined units were then reinfused to evaluate the viability of the fresh platelets which had been exposed to stored PC. The results (Table 2) indicate a significant but surprisingly slight injury. PC with final pH less than six were no more damaging than PC with pH above six. Probably for practical purposes in the blood bank, stored and fresh units can be pooled for infusion without prejudicing the viability of the fresh cells.

Table 2.—Effect of Exposing Fresh PC to PC Stored for Four Days

<table>
<thead>
<tr>
<th></th>
<th>Fresh-Exposed</th>
<th>Fresh-Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%  )</td>
<td>47 ± 3*</td>
<td>59 ± 4</td>
<td>0.025 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>T½ (Days)</td>
<td>3.9 ± 0.2*</td>
<td>4.2 ± 0.2</td>
<td>p &gt; 0.25</td>
</tr>
<tr>
<td>Number Studies</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* ± S.E.M.
Patient Studies

On 12 occasions, 10 patients with thrombocytopenia secondary to either aplastic anemia or leukemia were transfused with multiple units of PC which had been stored for 24 hours as described above. Enough platelets were administered so that if a circulating yield of 40-50 per cent were obtained, the platelet count would be elevated to over 100,000. Therefore, an accurate estimate of platelet lifespan could be obtained by platelet counting. The results are summarized in Table 1. In some patients, rapid platelet destruction occurred either on the day of transfusion (A.H., F.T., R.A.) or during the first 24 hours (D.S., R.W.) All of the patients studied had received whole blood transfusions prior to the platelet transfusion so that such results would not be unexpected even with fresh platelets. In fact, F.T. and R.A. were subsequently transfused with fresh platelets and immediate destruction was again observed.

In the remaining patients, yields within the range predicted by the autologous studies in normal volunteers were obtained. Of greater importance is the observation that significant numbers of the transfused platelets continued to circulate 24 and 48 hours after the infusion. This stands in contrast to previous data obtained after infusion of platelets stored at 4°C in which none were found circulating 24 hours after infusion. On one occasion, A.C. was transfused with platelets stored as PC for 48 hours. An initial yield of 32 per cent was obtained with 76 per cent and 40 per cent circulating on day one and two, respectively.

With the exception of a brief, uncomplicated fever and chill response in one patient (S.B.), we have observed no untoward reactions during any of our transfusion studies. The small clumps of unidentified material which were visible grossly after storage were trapped in the filter of the infusion apparatus and did not enter the recipient's circulation.

Discussion

The data presented suggest a simple and safe technique by which the routine blood bank using standard, commercial plastic equipment can prepare PC suitable for storage at 22°C and still obtain a maximum harvest of other blood components, most importantly packed red cells and Factor VIII. The technique first described by Mourad which allows the preparation of PC without prior acidification of PRP yields platelets whose viability is not significantly different from those prepared from acidified PRP. This procedure simplifies PC preparation by eliminating the necessity for entering the closed system to introduce extra ACD or having a fixed quantity of extra ACD present in the satellite bag prior to phlebotomy. The hazards of inadvertent bacterial contamination are consequently reduced. Furthermore, the higher pH of the final PC increases the time interval that the pH remains above 6.0, the minimum pH below which platelets become nonviable; the potential

*The studies of Aster indicate that the low yield (10%) obtained in F.W. who had massive splenomegaly probably represents the effect of splenic pooling and not destruction of the transfused platelets.
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storage interval is prolonged as a consequence. The data of two investigations\textsuperscript{7,8} indicate a reduction of Factor VIII activity in cryoprecipitates obtained from acidified plasma. This problem will not exist with the technique proposed here. Pool\textsuperscript{14} has shown that Factor VIII is stable in PPP at 22°C for at least one hour. This time interval is adequate for the complete processing at 22°C of a unit of blood into its components without deterioration of Factor VIII activity.

The ultimate therapeutic value of PC stored at 22°C awaits final definition. We have approached the problem of platelet preservation with a primary goal of achieving maximum maintenance of viability during storage. It is clear from previous data\textsuperscript{1} that 22°C is the optimal temperature for this purpose and from the current data that PC can be stored with similar success if attention is paid to technical details. Most important among these is the recognition that the rate of fall in pH varies inversely with initial PC volume. We have, therefore, employed a relatively high initial volume of 25 ml. Our data also show that agitation of the stored concentrate provides significant benefit. Lastly, we would emphasize that there have been significant variations in our results which could only be ascribed to changes in the lot of plastic from which the storage containers (transfer packs) have been prepared. It will be necessary to rigidly control this variable in future comparative studies.

While it is clear that this technique allows satisfactory maintenance of cell viability, only a large experience in a clinical setting will demonstrate its safety and efficacy. In our own small experience described here with PC stored for 24 and 48 hours, there have been no reactions to suggest that the plasma and cellular components of stored PC carry any greater risk than that of fresh materials. The potential hazards of inadvertent bacterial contamination, of course, are not yet defined. Certainly, one's ability to perform all the necessary manipulations in a completely closed plastic system encourages one to believe that these hazards will be minimal.

It has been an assumption in the past that maintenance of platelet viability during storage implied that functional efficacy would be maintained as well. This assumption now requires demonstration in long-range clinical trials. Recent observations\textsuperscript{15,16} which imply that young platelets are more potent functionally than old platelets suggest the possibility that the platelets may age during in vitro storage resulting in reduced functional capacity. Only multiple correlations of platelet count achieved through transfusion with shortening of bleeding time and clinical estimates of hemostatic effect will answer this question.

The technique described in this report greatly increases the ease with which a blood bank can have platelet transfusions available for thrombocytopenic patients. Critical for their effectiveness, however, is the unpredictable variability of the recipient's response as is apparent from Table 3. In some cases, fever or splenomegaly provided a partial explanation for suboptimal yield or survival, but, more frequently, one must assume that isoimmunity developing after previous whole blood transfusion is responsible for rapid platelet destruction in the recipient.\textsuperscript{11} In our experience, this phenomenon has correlated only very roughly with the number of previous transfusions and
Table 3.—Transfusions in Thrombocytopenic Patients—24-Hour Storage Interval

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical History</th>
<th>Max. Yield</th>
<th>% Circulating Day 1</th>
<th>% Circulating Day 2</th>
<th>Number Previous Transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACIDIFIED PC</td>
<td>A.C. Aplastic Anemia</td>
<td>56</td>
<td>82</td>
<td>61</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>A.C. Aplastic Anemia</td>
<td>54</td>
<td>82</td>
<td>68</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>A.C. Aplastic Anemia</td>
<td>35</td>
<td>92</td>
<td>83</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>K.C. Aplastic Anemia</td>
<td>34</td>
<td>72</td>
<td>70</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>D.S. Aplastic Anemia</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>A.H. Acute Leukemia</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>F.T. Chronic myelogenous Leukemia &amp; splenomegaly</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>R.A. Chronic myelogenous Leukemia &amp; splenomegaly</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>NONACIDIFIED PC</td>
<td>S.B. Acute Leukemia, fever</td>
<td>42</td>
<td>36</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>B.Z. Chronic Lymphatic Leukemia, fever</td>
<td>25</td>
<td>72</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>F.W. Chronic myelogenous Leukemia, splenomegaly</td>
<td>10</td>
<td>80</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>R.W. Aplastic Anemia</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Means of isotope studies in normal volunteers</td>
<td>50</td>
<td>100</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of platelets circulating on the day of transfusion which are still circulating on days 1 and 2 after transfusion.

many striking exceptions have been observed as exemplified by A.C., A.H. and R.W. in this report. Little is known about this presumably immunologic process, and only recently has there been any indication\(^\text{17}\) that progress will be made in dealing with its clinical consequences. Until such progress is made, the impact of advances in techniques of platelet preservation will be blunted.

**SUMMARY**

A technique has been proposed which allows easy preparation of platelet concentrates within a closed, sterile system and maximal utilization of other blood components. Lifespan studies have been performed to demonstrate the viability of platelets prepared in this manner. Such concentrates can be stored for as long as three days with adequate maintenance of viability for transfusion purposes if certain technical requirements are met. Important among these are attention to concentrate volume and pH; agitation of the concentrate is helpful for maintenance of viability. Twelve studies in thrombocytopenic recipients suggest that stored concentrates will be safe and effective in transfusion practice.

**ACKNOWLEDGMENTS**

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REFERENCES


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