Platelet Storage at 22°C: Role of Gas Transport Across Plastic Containers in Maintenance of Viability

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Containers constructed of polyvinylchloride (PVC) are used for the storage of platelet concentrates (PC) for transfusion. At 22°C, pH often falls to such low levels (pH < 6.0) that viability is lost. Far lesser degrees of pH fall are observed in bags constructed of polyethylene (PE). In this study, pH, P02, Pco2, platelet count, lactate concentration, microscopic morphology, and viability after 51chromium labeling were evaluated during storage at 22°C under a variety of circumstances. The results indicate that (1) pH falls because of the generation of lactic acid by platelet glycolysis and, under some circumstances, the retention of CO2. (2) Rate of pH fall is, therefore, roughly proportional to the platelet count. (3) PE is more permeable to gases, thereby allowing CO2 escape from and easier O2 entry into the stored PC; the higher O2 tensions suppress glycolysis by the Pasteur effect. (4) Adequate agitation and container size are critical if the beneficial effect of PE is to be obtained. (5) In general, platelets stored in PE containers have excellent viability in vivo although CO2 escape can result in elevations in pH which are deleterious. (6) Storage in a 10% CO2 atmosphere prevents these deleterious pH elevations without otherwise impairing platelet viability. (7) Results similar to those achieved with PE can be achieved with PVC if this material is made thinner to allow easier penetration of gases.

There is currently an active debate concerning the best temperature for the liquid storage of platelets, 4°C or 22°C. It seems certain that viability, that is, the capacity to circulate in vivo, is best maintained at 22°C. However, platelets stored at 4°C are alleged to have a superior capacity for hemostatic function, and 4°C would be a safer temperature if inadvertent bacterial contamination occurs. As this debate continues, it is imperative that the optimal conditions for storage at both temperatures be determined. The current work has been directed towards the solution of a significant problem with storage at 22°C. As we have previously described, the pH commonly falls during storage of platelet concentrates (PC) at 22°C. When pH reaches 6.0, a dramatic morphologic change occurs, and viability is lost. Poor results are predictable if a significant number of units within a transfusion pool have undergone this change.

PC are currently stored in containers constructed of polyvinylchloride (PVC). There has been recent concern about plasticizers in this material which leave the plastic, dissolve in the stored biologic materials, and are then transfused into patients. Because of this concern, we initiated studies of PC stored in...
polyethylene (PE) containers. We were surprised to find that very little pH fall was seen in these PC. These studies were directed towards the understanding of this phenomenon and application of the findings to the practical problem of platelet storage for transfusion.

MATERIALS AND METHODS

Platelet-rich plasma (PRP) was obtained from normal volunteers by double plasmapheresis using ACD-A as the primary anticoagulant. Platelet concentrates (PC) were prepared in PVC transfer packs to a final volume of 30 ml by the method of Mourad.\textsuperscript{1} Frequently, 2 U of PRP were mixed prior to centrifugation to create PC with high platelet counts. During storage, unless otherwise noted, PC were agitated at 5 cpm within a constant-temperature apparatus which maintained the temperature at 22°C (range, 21°C–23°C). The agitator is illustrated and described in Fig. 1. In some studies, a 10% CO\textsubscript{2} atmosphere was maintained within the constant-temperature apparatus by introducing 100% CO\textsubscript{2} at a rate controlled by an automatic CO\textsubscript{2} control system (Forma Scientific, Blue Bell, Pa.).

The PC were stored in four different types of containers: (1) Standard polyvinyl chloride containers (standard PVC), 12 x 15 cm, constructed of plastic PL-146 (Fenwal Laboratories, Morton Grove, Ill.). PL-146 is 0.015 inches in thickness. Transfer packs and satellite bags, commercially available from Fenwal, are constructed of this material. (2) Thin polyvinylchloride containers (thin PVC), 12 x 17 cm. They are constructed of PL-146 with a thickness of 0.008 inches. (3) Large polyethylene containers (large PE), either the 9 x 22 cm Hemoflex bag, 0.003 inches in thickness, (Union Carbide Corporation, Chicago, Ill.) or an experimental 12 x 15 cm bag supplied by Fenwal, 0.005 inches in thickness. We found no difference between the Hemoflex bag and the experimental Fenwal bag. (4) Small polyethylene containers (small PE), 5 x 10 cm. They are constructed by Fenwal from the same polyethylene as their larger experimental container.

pH measurement at 22°C and lactate concentrations were determined, and phase-contrast-microscopic (100 x) observations were made as previously described.\textsuperscript{1} Platelet counts on PC were determined either electronically with a Celloscope (Particle Data Corporation, Elmhurst, Ill.) or by phase-contrast microscopy.\textsuperscript{10} After storage, PC were labeled with radioactive chromium (\textsuperscript{51}Cr) and reinfused into their original donors; in vivo radioactive recovery and survival \textsuperscript{1} were calculated as previously described.\textsuperscript{1} The rate of oxygen transport across standard PVC and PE was measured with an Ox Tran-100
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Fig. 2. Relationship between initial platelet count and final pH after 1, 2, and 3 days of storage. In standard PVC, pH remains at 7.0-7.2 if the initial platelet count is low, but falls at a progressively faster rate at higher counts. In large PE, pH actually rises at low counts and falls only slightly at higher counts.

(Modern Controls, Inc., Minneapolis, Minn.). $P_O_2$ and $P_C_O_2$ of samples of PC, whole blood, and platelet-poor plasma (PPP) were obtained by injecting them into a microelectrode unit with pH meter attachment (Radiometer, Copenhagen, Denmark). The instrument was prefilled with a solution depleted of $O_2$ and $CO_2$. After injection of the sample, $P_O_2$ rose to a peak and then gradually fell. The peak value was recorded as the $P_O_2$.

RESULTS

Correlation of PC pH With Platelet Count

When PC were stored in containers constructed of Standard PVC, pH fell at a rate roughly proportional to the platelet count (Fig. 2). The relationship between pH and platelet count was not strictly linear but rather sigmoid. For example, after 3 days of storage, pH would always be 6.8 or above for any platelet count less than $1 \times 10^{12}$/liter and 6.0 or below for any platelet count greater than $3 \times 10^{12}$/liter. In the interval between these extremes, the relationship was roughly linear, although for any given platelet count there was a sizable range of pH values.

Effect of Containers Constructed of PE

Figure 2 also demonstrates that the pH of PC stored in large containers constructed of PE fell at a much slower rate. Even with PC with platelet counts in excess of $4.0 \times 10^{12}$/liter stored for 3 days, pH never fell below 6.6. However, it should be noted that, although the pH fall was not as great, there continued to be a correlation between platelet count and pH in PE.

Importance of Agitation and Size of Container

Agitation of PC was absolutely essential if pH fall in large containers constructed of PE was to be minimized. If the PC were not agitated (Fig. 3), pH fell to levels at least as low as those which were observed in PVC. Furthermore (Fig. 3), when PC were stored with agitation in small ($5 \times 10$ cm) containers constructed of PE, pH fell to levels significantly lower than those observed with PVC. We have previously commented on a proteinaceous material which forms when PC are stored in PVC; it also forms in PC stored in PE. We have noted that this material does not form if PC are stored without agitation in PVC or PE or if platelet-poor plasma (PPP) is stored with agitation in PVC or PE.
Correlation Between pH and Lactate Concentration

Lactate concentration was measured after storage intervals of 1, 2, and 3 days in PC stored with and without agitation. In all instances (Fig. 4), there was an inverse relationship between pH and lactate concentration. For PC stored with agitation at platelet counts of 3.0–4.0 $\times$ 10$^{12}$/liter, the rate of lactate production was $3.5 \pm 0.3$ *$\mu$* mole per 10$^9$ platelets per day in PVC (six measurements) and $1.2 \pm 0.1$ *$\mu$* mole per 10$^9$ platelets per day in PE (six measurements), $p < 0.001$. However, at lower platelet counts, 1.0–2.0 $\times$ 10$^{12}$/liter, in PVC, the rate of lactate production was $1.4 \pm 0.2$ *$\mu$* mole per 10$^9$ platelets per day. This figure is significantly different from the value for PVC at the higher counts ($p < 0.001$). Therefore, lactate production rate depends on both the type of container and the platelet count within the container.

*All ± values refer to 1 SEM.
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Fig. 5. \( P_{O_2} \) and \( P_{CO_2} \) in \( PC \) stored for 24 hr. Gas tensions varied not only with the type of container but also with the platelet count of the PC. This was particularly evident in standard PVC containers. \( P_{CO_2} \) in freshly drawn blood commonly reaches 100 mm Hg due to the venous stasis and clenching-unclenching of the hand used to speed blood flow. Thus, the low \( P_{CO_2} \) observed in large PE containers represents a sizable escape of CO2.

Rate of \( O_2 \) Transport Across PVC and PE

\( O_2 \) transmission rate was 419 ± 22 ml/sq m/24 hr (four measurements) across Standard PVC and 966 ± 19 ml/sq m/24 hr (four measurements) across Fenwal PE (\( p < 0.001 \)).

\( P_{O_2} \) and \( P_{CO_2} \) in Stored PC

Figure 5 records measurements of PC \( P_{O_2} \) and \( P_{CO_2} \) after storage for 24 hr in three different types of containers. Over a wide range of platelet counts, PC \( P_{O_2} \) was high, 70-160 mm Hg, in large PE containers. In standard PVC, PC \( P_{O_2} \) was inversely proportional to the platelet count, while in small PE containers \( P_{O_2} \) was low even at low platelet counts. PC \( P_{CO_2} \) fell into three ranges: low for large PE, medium range for standard PVC, and high for small PE. It is important to note in this figure that, in standard PVC, \( P_{CO_2} \) was distinctly higher in those PC with higher platelet counts. Therefore, in those PC with high platelet counts stored in standard PVC, \( pH \) depression would be due not only to increased production of lactic acid but also to elevated \( P_{CO_2} \).

In a few instances, \( P_{O_2} \) and \( P_{CO_2} \) were measured at 48 and 72 hr of storage. In general, the gas tensions remained relatively constant except when \( pH \) of PC in PVC fell to 6.0 or below. In those instances, \( P_{O_2} \) rose and \( P_{CO_2} \) fell towards atmospheric levels. Apparently, the morphologic change (see below) which occurs at low \( pH \) is associated with a reduction in \( O_2 \) consumption and \( CO_2 \) production.

Morphologic Observations

Platelets in fresh PC showed typical discoid morphology. During storage in containers constructed of standard PVC or PE for 3 days, good discoid mor-
phology was almost always maintained if pH remained between 6.8 and 7.2. As pH fell to levels between 6.0 and 6.4, a transition to 40%-70% spheres was almost invariably seen. Disc-to-sphere transformation was seen between 6.4 and 6.8 on occasion, but just as frequently, retention of normal discoid morphology was observed. A drastic change occurred if pH fell to below 6.0. All the cells became spherical, and projections of rigid rods of cytoplasm developed. This abnormal morphology could not be returned toward normal by the addition of alkali to restore the pH to 7.0 or higher. We have previously shown that it is associated with a loss of in vivo viability.

On the other hand, equally impressive morphologic changes occur if pH rises above 7.2. Prior to storage, PC pH is 7.0–7.2. Elevations above this level occur if escape of CO₂ (Fig. 5) through the plastic container allows a greater loss of hydrogen ion than that generated by glycolysis. As pH rises, an increasing percentage of cells becomes spherical. In addition, small aggregates, degranulated forms, and extracellular granules occur with increasing frequency. If pH reaches 7.6, all the cells become spherical, and large numbers of degranulated forms are observed.

Phase-microscopic platelet counts were performed in 24 PC before and after storage for 3 days in large PE. The mean platelet count after storage was 76% ± 5% of that obtained prior to storage. Only PC with final pH less than 7.3 were included in this calculation since above this level morphologic distortion made counting difficult.

*In vivo Viability*

Figure 6 shows the recovery in the circulation during the first 3 hr after infusion of platelets which have been concentrated, stored for 3 days in large PE containers, labeled with ⁵¹Cr, and then reinfused into their original donors. There was an inverse correlation between PC pH after storage and in vivo recovery; the higher the pH, the lower the recovery. The recoveries for those PC with final pH < 7.2 are at least as good as those obtained in previous studies. The mean survival \( t_\frac{1}{2} \) was 3.6 ± 0.2 days* for the 14 studies in which the initial recovery was greater than 25%. Half-time for the six studies with recovery less than 20% could not be measured accurately because of the low level of circulating radioactivity.

Because of the observation that disc-to-sphere transition with pH fall began at levels as high as 6.8, four PC were obtained and stored for 48 hr in bags constructed of standard PVC so that final pH was 6.3–6.5. By phase-contrast microscopy, approximately 70% of these cells were spheres and 30% were discs. After storage, the platelets were labeled with ⁵¹Cr and reinfused into the original donors. In vivo recovery was 44.8% ± 8.0% with a subsequent \( t_\frac{1}{2} \) of 3.6 ± 0.3 days.* These studies indicate that disc-to-sphere transition in itself does not impair in vivo viability more than would be expected after 2 days of storage but that the more drastic changes seen below pH 6.0 are required to render the cell nonviable.

*In previous studies, t_\frac{1}{2} was 4.1 ± 0.1 days for fresh platelets and 3.8 ± 0.1 and 2.2 ± 0.3 days for PC stored in standard PVC for 2 and 3 days, respectively.
Studies With Thin PVC

The studies to this point indicated that pH falls in PC stored in standard PVC due to production of lactic acid and CO₂, that this pH fall is lethal if pH falls to 6.0, and that this can be prevented if PC are stored in containers constructed of material, such as PE, which is more permeable to O₂ and CO₂. O₂ transport is approximately twice as rapid across PE as it is across standard PVC. However, standard PVC is 0.015 inches thick, whereas PE is 0.003–0.005 inches thick. Since rate of gas transport across plastic is inversely proportional to the thickness of the film, PVC is intrinsically more permeable per unit thickness, and it seemed likely that thinner PVC would produce the same effect as PE.

Figure 7 graphs PC pH versus initial platelet count when PC were stored in thin PVC. pH maintenance is much better with the thinner bags, essentially identical to that seen with PE. In addition, six of these PC were labeled after storage for 3 days and rein infused into their original donor. Figure 6 shows that the in vivo recovery for these units was similar to that which had been observed with PE. The survival ± for these six studies was 3.4 ± 0.4, not significantly different from what had been observed with PE. Furthermore, in thin PVC, when the initial platelet count was relatively low and the final pH high, the same morphologic deterioration was observed that had been seen with PE. We assume that the viability of such PC would be similarly compromised, but this point was not directly tested by in vivo studies.
The proteinaceous material to which we have previously referred forms to the same degree in PC agitated in thin PVC as it does in PE and standard PVC.

*Storage in 10% CO₂ Atmosphere*

The studies to this point have indicated that PC stored in either large PE or thin PVC containers developed abnormal morphology if pH rose above 7.2. Furthermore, we have demonstrated that in vivo viability was compromised as this morphology developed. Based on the fact that platelet aggregation is enhanced at higher pH levels, we hypothesized that the morphologic changes resulted from platelet aggregation and release, and that storage in a 10% CO₂ atmosphere would lower pH and improve results. In fact, this proved to be the case. When 30 ml PPP in large PE containers was stored in room air and a 10% CO₂ atmosphere, P的日, equilibrated at approximately 10 mm Hg and 70 mm Hg, respectively. PC pH never rose above 7.05 in 10% CO₂ even at low platelet counts. Under these conditions, good disc morphology was maintained. During 3 days of storage in 10% CO₂, PC pH did not fall below 6.0 unless the platelet count was greater than 4.0 × 10¹²/liter.

To test the hypothesis that the improved morphology observed with a 10% CO₂ atmosphere would result in an improved in vivo recovery, PC were prepared, diluted if necessary with autologous PPP to obtain a platelet count of 1.3–1.7 × 10¹²/liter in the final 30-ml volume, and stored for 72 hr in large containers constructed of PE. This count was chosen because, in room air, pH rose and morphology deteriorated at this low platelet concentration. In vivo recovery was 36.1% ± 2.5% (eight studies) in a 10% CO₂ atmosphere and 14.6 ± 4.8% (five studies) in room air (p < 0.005). After storage in 10% CO₂, t½ was 3.9 ± 0.2 days. By phase-contrast-microscopic counting, the mean platelet count after storage for 72 hr in a 10% CO₂ atmosphere was 79% ± 2% of that obtained prior to storage (30 studies).

**DISCUSSION**

When incubated in vitro at 37°C in a physiologic buffer, platelets convert more than 95% of metabolized glucose to lactic acid, with only 1%–2% proceeding through the Krebs cycle to CO₂. Nevertheless, oxidative metabolism appears to be critical for the cell since it will augment its production of lactic acid fivefold if it is deprived of oxygen. This response, common to many cells, is known as the Pasteur effect. In vivo, the amount of hydrogen ion produced is trivial, and the lactate can be converted to glucose by the liver. However, as our data indicate, when PC are stored for transfusion, lactate accumulates and pH falls. This is lethal for the cell if pH falls to 6.0 or below.

Our data indicate that PC stored in standard PVC bags are not maximally oxygenated, particularly those with high platelet counts. In PVC, the rate of pH fall is roughly proportional to the platelet count. At any given platelet count, pH fall is less rapid in PE, and this correlates with a reduced rate of lactate production. PE is more permeable to oxygen than standard PVC, and the measured P₀₂ levels of PC stored in PE are higher. We assume that greater access to oxygen allows a reduced rate of glycolysis without impairment of viability. This hypothesis receives very strong support from the fact that we
obtained the same results with containers constructed of thin PVC as were observed with PE. PC in thin PVC have a reduced rate of pH fall and excellent maintenance of viability.

In current transfusion practice, using standard PVC containers, three factors combine to cause a lowering of pH in PC with high platelet counts stored at 22°C. First, the mere increase in platelet number results in an increase in lactic acid production. Second, $P_{O_2}$ tension is low (Fig. 5), further accelerating the lactic acid production per platelet by the Pasteur effect (see results). Third, $P_{CO_2}$ is higher than at lower counts. We assume that the increased volume of CO$_2$ produced by the increased number of platelets exceeds the capacity for diffusion across the plastic container so that $P_{CO_2}$ rises until CO$_2$ can diffuse at a more rapid rate.

Unfortunately, the application of these results at the practical level will be complex. Figure 3 emphasizes that containers must be agitated and must be of adequate size to take advantage of increased permeability to gases. pH falls very rapidly in PE containers if they are small or if they are not agitated. We assume that this results from inadequate exposure of PC to the plastic surface for gas exchange. We do not yet know the optimal rate and type of agitation. In any event, these studies emphasize important variables to consider in future studies.

Furthermore, we now find that we have difficulty with PC storage if pH is too high as well as too low during storage. During the course of these studies, we have placed great emphasis on platelet morphology as judged under phase-contrast microscopy (100 x). There are few, if any, exceptions to the generalization that platelet viability is well maintained if normal discoid morphology is retained. We have found that good morphology is retained for 3 days if pH remains between 6.8 and 7.2. As pH falls to 6.4–6.8, disc-to-sphere transformation begins, and the majority of cells are spherical between 6.0 and 6.4. As indicated in Results, these spherical cells retain quite acceptable viability. As we have reported previously, dramatic and lethal changes occur below 6.0; long, rigid protrusions of cytoplasm extend from the cell, and there is prominent clumping. We rarely see these changes in PC stored in PE or thin PVC because pH rarely falls to this low level.

On the other hand, when PC with low platelet counts are stored in PE or thin PVC, lactic acid production is low, CO$_2$ escape is enhanced, and pH rises to levels of 7.4–7.6. Platelets in these PC demonstrate a different type of morphologic deterioration. One sees clumping, disc-to-sphere transformation, many degranulated forms, and what appear to be platelet granules in the extracellular space. These changes are also associated with a loss of viability (Fig. 6). Although we have no proof, we suspect that these platelets have aggregated and undergone the release reaction. This interpretation is consistent with the fact that platelet aggregation and release are highly sensitive to pH; aggregation is progressively inhibited as one lowers the pH of the suspending medium. Whatever the explanation for these morphologic changes may be, it is certain that they can be prevented when pH is maintained below 7.05 by storing in a 10% CO$_2$ atmosphere. Maintenance of good morphology is associated with maintenance of viability (see Results). We were encouraged to find that a
10% CO₂ atmosphere could be used without an unacceptable decline in pH in PC with high platelet counts.

These studies emphasize the variables which determine the success or failure of PC storage at 22°C. At this point, we would prefer not to outline a definite proposal for practical application. The basic problem is that, for all storage conditions, the PC platelet count markedly affects the storage characteristics of the unit, and, unfortunately, routine blood banking produces PC with widely varying platelet counts. We believe that the results of storage of PC at 22°C will be markedly improved by maintenance of optimal oxygenation and prevention of deleterious rises in pH. Utilizing the concepts derived from the data in this report, our current studies are directed towards the development of a practical technique applicable to the extremes of PC platelet counts found in clinical practice.

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