Platelet Storage for Transfusion in Synthetic Media: Further Optimization of Ingredients and Definition of Their Roles

By S. Murphy, T. Shimizu, and J. Miripol

Currently, most platelet concentrates (PC) are stored for transfusion at 20°C to 24°C in autologous plasma. There are potential advantages in replacing some of this plasma with a synthetic medium. In this study, our major goals were to define the optimal ingredients and their concentrations in such a medium and to gain insight into the mechanism by which each ingredient confers benefit. In addition, we wished to validate a new polyvinyl chloride container plasticized with di-n-decyl phthalate (DnDP) for PC storage. PC derived from donations of whole blood were stored for 7 days in autologous plasma or a basic synthetic medium (BSM) containing 15 mmol/L glucose, 21 mmol/L citrate, and physiologic concentrations of salts other than bicarbonate within either the DnDP container or a licensed polyolefin container, PL-732. Metabolic events were characterized and within either the DnDP container or a licensed polyolefin container, PL-732. Metabolic events were characterized and a panel of in vitro tests were used to monitor platelet quality as systematic changes in the BSM were made. Platelet quality was at least as good, if not better, after storage in DnDP in comparison to PL-732. pH consistently decreased to less than 6.0 because of inadequate buffering of lactic acid in BSM alone. However, pH and the in vitro tests were well maintained by either the serial addition of bicarbonate (BSM + B) or the addition of at least 15 mmol/L acetate and 10 mmol/L phosphate (BSM + AP). The benefits of BSM + AP were traced to a decrease in lactic acid production by 33% and 19% relative to plasma and BSM + B, respectively, and the vigorous oxidation of acetate (0.66 ± 0.09 mmol/d/10^12 platelets). The rates of lactate production and acetate consumption were similar and the pH during storage correlated with the difference between the two rates, suggesting that acetate oxidation has an alkalizing effect equivalent on a molar basis to the acidifying effect of production of lactate and a hydrogen ion. When pyruvate replaced acetate, it was also metabolized vigorously (0.52 ± 0.06 mmol/d/10^12 platelets). Its presence suppressed lactic acid production by 44% relative to BSM + B and allowed maintenance of pH and platelet quality similar to what is achieved with acetate. The results strongly suggest that the benefit from acetate (or pyruvate) is derived from its oxidation and the use of a hydrogen ion during that oxidation. For reasons that are not yet clear, the omission of phosphate resulted in pH decrease to less than 5.8 in 3 of 9 PC even with acetate present. The results allow for a more complete definition of the minimal essential ingredients of an optimal medium relative to the amount of plasma carried over into the PC. With less than 20% plasma carry-over, 15 mmol/L acetate, 14 mmol/L glucose, 10 mmol/L phosphate, and 5 mmol/L citrate produce excellent results. After 7 days of storage in such a medium, the results of in vitro tests reflecting platelet quality were at least as good as, if not better than, results after storage in plasma.

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the pH level in individual PC to develop a simple model that explains the beneficial effect of acetate. In a series of experiments, we replace acetate with pyruvate to show that this effect is not unique to acetate. The new understanding of the mechanism of acetate’s benefit allows definition of the optimal concentration of acetate required to achieve it.

The previous work suggested that phosphate was required for consistent success, but similar data were not provided for glucose or maltose. If glucose is required, how much is required? Will the glucose in residual plasma be sufficient? Similar questions can be raised about citrate. Providing answers to these questions allows us to establish principles that can be used in the further design and refinement of platelet storage medium.

Finally, there may be differences in the way different plastic containers interact with various media. Shimizu et al have reported on a container constructed from a new polyvinylchloride plasticized with di-n-decyl phthalate (DnDP). This container was used for the investigations reported and was compared with another licensed container currently in use.

MATERIALS AND METHODS

Blood (450 mL ± 10%) was drawn into citrate-phosphate-dextrose within a PL-146 container (Baxter Health Care, Deerfield, IL) from normal volunteers who gave written, informed consent to protocols approved by the Institutional Review Board of Thomas Jefferson University. In many studies, using a sterile connecting device (Terumo Medical Corp, Elkton, MD), the PL-732 transfer pack was replaced with a 300-mL experimental container constructed from polyvinylchloride plasticized with DnDP, which has been previously described. The capacity for oxygen transport for this container [K(O2); nanomoles per minute per atmosphere] was measured as previously described. PC were prepared from PRP in PL-732 or DnDP containers as previously described. In some studies, the platelets were suspended in autologous plasma; in others, as much as 60% DnDP containers as previously described. In some studies, additions were made to BSM. Additional phosphate was added to attain final concentrations of 5, 10, and 20 mmol/L and sodium acetate (USP-Abbott Laboratories; 2 meq/mL) was added to attain final concentrations of 15 and 20 mmol/L. Sodium pyruvate (Sigma Chemical Co, St Louis, MO) was added to attain a final concentration of 10 mmol/L. The osmolarity and pH (22°C) of all media were corrected to 300 mOsm and 7.0 by the addition of sterile water and 1N sodium hydroxide.

As will be described in the Results, the pH consistently decreased during storage in BSM without the addition of acetate and phosphate. Therefore, to study storage with BSM alone, the pH of the PC was measured daily and 8.4% sodium bicarbonate injection (USP-Abbott Laboratories) was added if necessary: 0.1 mL for pH 6.9 to 7.0, 0.2 mL for pH 6.8 to 6.9, and 0.3 mL for pH less than 6.8. This allowed storage for 7 days with pH at or just less than 7.0. Two additional media were used. Setosol was prepared as previously described. TRD B2 was prepared to contain 107 mmol/L sodium chloride, 4 mmol/L potassium chloride, 3 mmol/L magnesium chloride, 15 mmol/L sodium acetate, 5 mmol/L sodium citrate, 15 mmol/L sodium phosphate, and 14 mmol/L glucose (pH 7.02; 310 mOsm).

In most studies, the PC were sampled on days 1, 5, and 7 of storage. The following measurements were made as previously described: platelet concentration, mean platelet volume, dispersion (geometric standard deviation) of the platelet size distribution, pH at 22°C, pH at 37°C, rate of oxygen consumption [C(O2)], osmotic reversal reaction (Osm Rev), percentage of discs by oil phase microscopy, ATP concentration in platelets, lactate concentration, glucose concentration, acetate concentration, and bicarbonate concentration. Pyruvate concentration was determined with a kit from Sigma Chemical Co. The rates of consumption of glucose and acetate and the rate of production of lactate were determined from their concentrations on days 1 through 7 of storage by linear regression. In these studies, relative to day 1 of storage, the rate of oxygen consumption was slightly reduced to approximately 90% on day 5 and 80% on day 7. The rate for the entire storage interval was taken to be the mean of the three measurements.

Student’s paired and unpaired t-test was used to determine the level of significance of the difference between means.

RESULTS

Characterization of the DnDP Container

In four studies, the oxygen transport capacity [K(O2)] of the DnDP container was determined to be 1.028 ± 98 nmol/min/atm. This allowed the rate of oxygen consumption [C(O2); millimoles per day per 1012 platelets] to be calculated in the metabolic studies that follow. Figure 1 shows the relationship between pO2 and platelet count on day 1 of storage of PC. As described in studies of other containers, the linear relationship between the two measurements suggests that the C(O2) of a PC correlates directly with the platelet content of the PC. The fact that the pO2 did not reach 0 until the platelet concentration exceeded 2.5 × 1012/L (equivalent to PC platelet content >1.25 × 1011 platelets) indicates that the capacity for oxygen transport of the container is sufficient to meet the oxygen demands of PC encountered in routine practice.

Table 1 lists the results of a series of in vitro measurements that correlate with in vivo viability after infusion. The results on day 7 of storage for PC stored in plasma in the DnDP container were similar to those for storage in other second-generation containers for PC. Rates of oxygen consumption and lactate production are presented in Table 2.

Storage of PC in BSM With (BSM + B) and Without Bicarbonate

In eight studies, PC were stored in BSM without bicarbonate. pH decreased to the ranges of 5.4 to 6.3 and 5.4 to 5.8
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on days 5 and 7 of storage, respectively. In 25 studies, PC were stored identically except that sodium bicarbonate was added at intervals during storage to maintain pH above 6.8 as described in the Materials and Methods. The mean total bicarbonate added was 0.73 ± 0.15 mL so that pH was continuously maintained in the range of 6.7 to 7.4. As indicated by the in vitro results in Table 1, the quality on day 7 of platelets stored in this fashion was at least equivalent to those stored in plasma. In fact, they were significantly superior in regards to dispersion and ATP level. The metabolic rates in BSM + B are presented in Table 2. The rates of oxygen consumption and lactate production were significantly reduced relative to those with plasma.

Storage of PC in BSM With Added Acetate and/or Phosphate

In 19 studies, PC were stored in BSM to which sodium acetate and phosphate were added to bring their final concentrations to 20 mmol/L (BSM + 20A20P). These PC all had pH greater than 6.7 on days 5 and 7 of storage and showed satisfactory results in the measurements reflecting platelet quality (Table 1). To explore the minimal necessary concentrations of these two ingredients, the acetate and phosphate concentrations were reduced to 15 mmol/L and 10 mmol/L, respectively (BSM + 15A10P), in 19 additional studies. All of these PC also displayed adequate maintenance of pH for 7 days. Tables 1 and 2 indicate comparable in vitro and metabolic parameters for these two concentration levels except for ATP levels. In the following analyses, the results for these 38 PC have been combined and referred to as BSM + AP. Table 1 indicates that, for the percentage of discs, storage in BSM + AP was superior to storage in BSM + B, and that, for dispersion and ATP concentration, storage in BSM + AP was superior to storage in plasma. In addition, the addition of acetate and phosphate had an effect on metabolism. Relative to storage in BSM + B, C(O2) was significantly increased and lactate production and glucose consumption were significantly decreased (Table 2). Relative to storage in plasma, C(O2) and lactate production were significantly decreased (Table 2).

On the other hand, omission of either acetate or phosphate resulted in unacceptable decreases in pH. Omission of acetate resulted in a decrease in pH to less than 6.0 on day 7 of storage in four of four PC, whereas omission of phosphate resulted in a similar decrease in pH in three of nine PC. For the latter nine PC, there was no significant correlation between the extent of plasma carry-over and pH on either day 5 or 7 (data not shown). In five studies, acetate and phosphate additions were adjusted to 15 mmol/L and 5 mmol/L, respectively. The pH decreased to 6.3 on day 7 in one of these five PC.

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**Table 1. Results on Day 7—DnDP Container**

<table>
<thead>
<tr>
<th>Medium</th>
<th>n</th>
<th>% Discs</th>
<th>Dispersion*</th>
<th>Osm Rev (%)</th>
<th>ATP (μmol/10^11 platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>14</td>
<td>58.3 ± 20.3</td>
<td>1.77 ± 0.07</td>
<td>49.0 ± 10.9</td>
<td>2.98 ± 0.42</td>
</tr>
<tr>
<td>BSM + B</td>
<td>25</td>
<td>46.6 ± 15.3</td>
<td>1.72 ± 0.06†</td>
<td>50.7 ± 10.6</td>
<td>3.61 ± 0.61†</td>
</tr>
<tr>
<td>BSM + 20A20P</td>
<td>19</td>
<td>73.9 ± 13.9</td>
<td>1.73 ± 0.05</td>
<td>53.8 ± 9.3</td>
<td>4.69 ± 1.39</td>
</tr>
<tr>
<td>BSM + 15A10P</td>
<td>19</td>
<td>65.5 ± 22.8</td>
<td>1.71 ± 0.04</td>
<td>49.5 ± 8.6</td>
<td>3.58 ± 0.68</td>
</tr>
<tr>
<td>BSM + AP</td>
<td>38</td>
<td>69.7 ± 18.45</td>
<td>1.72 ± 0.05†</td>
<td>51.6 ± 9.2</td>
<td>4.12 ± 1.22†</td>
</tr>
<tr>
<td>TRDB2</td>
<td>7</td>
<td>52.1 ± 26.7</td>
<td>1.72 ± 0.06</td>
<td>49.9 ± 11.0</td>
<td>3.45 ± 1.19</td>
</tr>
</tbody>
</table>

* Favorable in vivo results correlate with low dispersion.
† P < .05 versus plasma.
‡ P < .05 versus BSM + 15A10P.
§ P < .01 versus BSM + B.
Metabolic Patterns in BSM + AP

Figure 1 shows the relationship between \( P_O_2 \) and platelet concentration on day 1 of storage in BSM + AP. There is almost complete overlap with the data for storage in plasma, supporting the same conclusion that the rate of oxygen consumption correlates with the platelet concentration. Figure 2A, B, and C shows the relationship between the rate of acetate consumption and the platelet concentration on day 1, the rate of oxygen consumption, and the rate of lactate production. The rate of acetate consumption and the platelet concentration were correlated (\( r = .765 \)). Acetate consumption rate was \( 0.66 \pm 0.09 \) mmol/d/10\(^{12} \) platelets. Because the rate of oxygen consumption was also highly correlated with the platelet concentration (Fig 1), it is not surprising that the rates of acetate and oxygen consumption were highly correlated (\( r = .824 \), Fig 2B). For these studies, the ratio of acetate consumption/oxygen consumption was 0.51 \( \pm 0.07 \). This implies that approximately two molecules of oxygen were consumed for each molecule of acetate consumed.

There was also a positive correlation between the rates of acetate consumption and lactate production (Fig 2C), but the correlation was less strong (\( r = .435 \)). The reason for this will be discussed below. However, the ratio of the rate of acetate consumption/rate of lactate production was 0.88 \( \pm 0.23 \), suggesting that approximately one molecule of acetate was metabolized for each molecule of lactate that was produced.

Figure 3A and B shows the relationship between the rate of lactate production and platelet concentration on day 1 and the rate of glucose consumption. As has been observed in previous studies of storage in plasma, the rate of lactate production was correlated with the platelet count but not strongly (\( r = .528 \)), particularly in the range of 1 to 2 \( \times 10^{12} \)/L. On the other hand, the rates of lactate production and glucose consumption were highly correlated (\( r = .911 \)). The ratio of the rate of lactate production/rate of glucose consumption was 2.06 \( \pm 0.34 \), implying that two molecules of lactate were produced for each molecule of glucose used.

Metabolic Patterns and pH Regulation During Storage of PC in BSM + AP

In previous studies of storage of PC in plasma, PC pH at any time point correlated strongly and inversely with the degree of increase in the concentration of lactate. Figure 4A shows that this correlation was present but less strong during storage in BSM containing acetate (\( r = -.620 \)). However, if the pH was compared with the difference between the increase in lactate concentration and the decrease in acetate concentration (Fig 4B), the correlation was much stronger (\( r = -.890 \)). These results are compatible with the concept

### Table 2. Metabolic Rates per Day

<table>
<thead>
<tr>
<th>Container</th>
<th>Medium</th>
<th>( n )</th>
<th>Oxygen Consumption (mmol/10(^{12} ) platelets)</th>
<th>Acetate Consumption (mmol/10(^{12} ) platelets)</th>
<th>Glucose Consumption (mmol/L)</th>
<th>Lactate Production (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DnDP</td>
<td>Plasma</td>
<td>14</td>
<td>1.47 ( \pm 0.22 )</td>
<td>ND</td>
<td>iphertexts.</td>
<td>2.17 ( \pm 0.28 )</td>
</tr>
<tr>
<td>DnDP</td>
<td>BSM + B</td>
<td>25</td>
<td>0.86 ( \pm 0.31^* )</td>
<td>1.10 ( \pm 0.35 )</td>
<td>1.79 ( \pm 0.54^* )</td>
<td>1.38 ( \pm 0.26 )</td>
</tr>
<tr>
<td>DnDP</td>
<td>BSM + 20A20P</td>
<td>18</td>
<td>1.22 ( \pm 0.19 )</td>
<td>0.67 ( \pm 0.06 )</td>
<td>0.71 ( \pm 0.16 )</td>
<td>1.53 ( \pm 0.40 )</td>
</tr>
<tr>
<td>DnDP</td>
<td>BSM + 15A10P</td>
<td>18</td>
<td>1.32 ( \pm 0.20 )</td>
<td>0.65 ( \pm 0.10 )</td>
<td>0.75 ( \pm 0.25 )</td>
<td>1.45 ( \pm 0.34^* )</td>
</tr>
<tr>
<td>DnDP</td>
<td>BSM + AP</td>
<td>36</td>
<td>1.27 ( \pm 0.20^+ )</td>
<td>0.66 ( \pm 0.09 )</td>
<td>0.73 ( \pm 0.21 )</td>
<td>1.47 ( \pm 0.17 )</td>
</tr>
<tr>
<td>DnDP</td>
<td>TRDB2</td>
<td>7</td>
<td>1.06 ( \pm 0.24^$ )</td>
<td>0.55 ( \pm 0.17^$ )</td>
<td>0.65 ( \pm 0.12 )</td>
<td>0.22 ( \pm 0.01 )</td>
</tr>
<tr>
<td>PL732</td>
<td>BSM + AP</td>
<td>16</td>
<td>0.91 ( \pm 0.15^$ )</td>
<td>0.57 ( \pm 0.06^$ )</td>
<td>0.63 ( \pm 0.20 )</td>
<td>0.33 ( \pm 0.28 )</td>
</tr>
<tr>
<td>PL732</td>
<td>BSM + PP</td>
<td>8</td>
<td>0.82 ( \pm 0.91 )</td>
<td>0.65 ( \pm 0.17 )</td>
<td>1.01 ( \pm 0.31^$ )</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* \( P < .05 \) versus plasma.
† \( P < .05 \) versus BSM + B.
‡ \( P < .05 \) versus DnDP, BSM + AP.
§ \( P < .05 \) versus PL732, BSM + AP.
that lactate production has its expected acidifying effect while acetate consumption has an offsetting alkalinizing effect.

Similarly, in previous studies of storage of PC in plasma, there has been an inverse correlation between pH after storage and the platelet concentration of the PC. As indicated in Fig 5, the reverse was true for PC stored in BSM + AP. This is expected based on the concept just proposed and the data provided in Figs 2A and 3A that indicate that the rate of lactate production was rather constant at all platelet counts, whereas the rate of acetate consumption was directly and tightly correlated with the platelet concentration. Thus, at high platelet concentrations, the ratio of acetate consumption to lactate production is higher than it is at low concentrations.

Bicarbonate concentrations were 3.6 ± 0.7 and 3.4 ± 1.1 on days 1 and 7 of storage, respectively. These means were not different statistically.

Modifications of BSM + AP

Omission of glucose (BSM + AP - G). It would be advantageous to omit glucose from the storage medium because glucose caramelizes during steam sterilization of solutions with neutral pH. We refer to this medium as BSM + AP - G. With no glucose in the medium, there will still be glucose present at the start of storage depending on the amount of plasma carry-over. Assuming a 25 mmol/L glucose concentration for CPD plasma, this will be 2.5 mmol/L at 10% plasma carry-over and 5.0 mmol/L at 20% plasma carry-over. As indicated in Fig 3B, the average daily glucose consumption will be 0.8 mmol/L/d, with a range of 0.4 to 1.2 mmol/L/d. Therefore, with 10% plasma carry-over, the average PC should be depleted of glucose on day 3 and lactate production will cease at that time. Figure 6 indicates that that was indeed the case for DnDP, which has mean plasma carry-over of approximately 10% (see Materials and Methods). Omission of glucose did not affect the rate of acetate consumption (0.70 ± 0.07 mmol/d/10^11 platelets). Therefore, continuing acetate consumption in the absence of lactate production resulted in an increase of pH between day 5 and day 7. However, with 17% plasma carry-over, as is the case for PL-732, lactate production in the average PC will not cease until after day 5. Figure 6 indicates that lactate production did continue beyond day 5 in PL-732. As would be expected from the results in Fig 4B, pH on day 7 was less in PL-732 than in DnDP (Fig 6). However, in both containers, pH was in an acceptable range throughout storage. As indicated in Table 3, PC stored in BSM + AP lacking glucose were somewhat inferior to those stored with glucose in dispersion (DnDP) and ATP level (DnDP and PL-732).

Replacement of acetate with pyruvate (BSM + PP). In previous studies of PC storage in plasma, we found that
acetate could be replaced by other anions that could be oxidized by platelets. Therefore, in eight studies, sodium pyruvate was added to BSM with 10 mmol/L phosphate and no acetate. The final pyruvate concentration in the PC to be stored in PL-732 containers was $9.01 \pm 0.49$ mmol/L. Pyruvate was consumed at a rate of $0.52 \pm 0.06$ mmol/10$^{12}$ platelets/d. The mean $	ext{pH}$ was well maintained for 7 days ($6.95 \pm 0.11$ on day 7). The measurements reflecting platelet quality were not statistically different from those for BSM + AP except for a slight reduction in the osmotic reversal reaction (Table 3). Pyruvate suppressed lactate production to an even greater degree than did acetate (Table 2).

**Inclusion of maltose.** We have previously$^{14}$ worked with a storage medium (Setosol) that includes 28.8 mmol/L maltose and higher concentrations of acetate (23 mmol/L) and phosphate (25 mmol/L) than are present in BSM + AP. A direct paired study between the two was performed (Table 3). BSM + AP was statistically superior for dispersion, osmotic reversal reaction, and ATP.

**TRDBZ.** This solution is described in Materials and Methods. It contains no calcium or sulfate, less citrate, and more magnesium than did BSM + AP. The results of in vitro tests reflecting in vivo viability were not statistically different from those observed during storage in BSM + AP (Table 1). Similarly, there were no significant differences in metabolic patterns comparing this medium with BSM + AP, except for a slight decrease in the rate of oxygen and acetate consumption (Table 2).

**DISCUSSION**

In this study, we have stored PC derived from whole blood donations for 7 days at 22°C in either an experimental polyvinylchloride container (DnDP) or a licensed polyolefin container (PL-732). In addition to validating the DnDP container, we wished to expand on previous work$^{14}$ to define the optimal ingredients and their concentrations for a synthetic storage medium to replace plasma and to determine how the various components of the synthetic medium contributed to its success. In this way, ground rules for the necessary components of a medium could be established. We used four in vitro measurements as reflections of platelet quality: the percentage of discs by oil phase microscopy; dispersion (geometric standard deviation) of the platelet size distribution by Coulter counter; the osmotic reversal reaction; and the platelet ATP level. The rationale for the use of these tests and their past correlation with results after infusion in vivo have been recently reviewed.$^{20}$ To simplify presentation, the measurements reflecting platelet quality are presented for day 7 (Tables 1 and 3) recognizing that, at least in the United States, storage is limited to 5 days. This was done because success at 7 days allows additional confidence that 5-day storage will be successful.

These studies confirm the work of Shimizu et al$^{15}$ with platelets obtained by apheresis, suggesting that the DnDP container has the qualities required for PC storage. Figure 1 shows that its permeability to oxygen is more than adequate for platelets stored in either plasma or synthetic medium and Table 1 shows that the in vitro measurements reflecting platelet quality after 7 days of storage are excellent as well.

The greatest problem in devising a synthetic medium for PC storage is control of pH. In the BSM, which contains glucose and essentially the same concentrations of major electrolytes as are found in citrated plasma except for bicarbonate, pH decreased to less than 6.0 in 5 to 7 days because of the production of lactic acid and the absence of a buffer for that lactic acid. When we examined the effect of the serial addition of bicarbonate to BSM (BSM + B), there was maintenance of pH, and the in vitro results reflecting platelet quality were at least as good as, if not better than, those with storage in plasma (Table 1). Furthermore, the rate of production of lactic acid was reduced by 18% (Table 2). Thus, there was a modest advantage derived from simply replacing plasma with a medium. Because bicarbonate is difficult to deal with in manufacturing, we decided to expand...
However, neither acetate nor phosphate was sufficient by itself. With only phosphate present, lactate accumulates at a rate of approximately 1.8 mmol/L per day and the buffering effect of phosphate alone is not sufficient to deal with this acid load. There are two explanations for the required, additional beneficial effect of acetate. First, as shown in Table 2, the rate of production of lactate in BSM + AP was reduced to 81% of that in BSM + B and 67% of that in plasma. Thus, the presence of acetate resulted in a further suppression of lactate production beyond what was achieved simply by replacing plasma with a medium. The mechanism of suppression of lactate production probably relates to acetate’s oxidative metabolism. Acetate was consumed at a rate that correlated with the platelet concentration and the rate of oxygen consumption (Fig 2) with two molecules of oxygen consumed for each molecule of acetate consumed. As indicated in Fig 7, acetate is initially converted to acetyl CoA in the mitochondrion. This initial step consumes two high energy phosphate bonds, but the acetyl CoA then enters the tricarboxylic acid cycle and its full oxidation regenerates 12 ATP molecules. Thus, the metabolism of acetate probably raises the intracellular concentration of citrate and ATP. Both of these inhibit phosphofructokinase, a rate-limiting enzyme in glycolysis. Thus, lactate production is inhibited.

Secondly, in previous work,14 we proposed that the metabolism of one molecule of acetate would result in the production of one molecule of bicarbonate, thus explaining its buffering effect. The current work suggests a more straightforward explanation. The rates of acetate consumption and lactate production were similar quantitatively (Fig 2), and, as shown in Fig 4, the pH during storage in media containing acetate correlated best with the difference between the increase in lactate concentration and the decrease in acetate concentration rather than simply with the concentration of lactate as is the case during PC storage in plasma.15 For acetate to enter the mitochondrion for oxidation, it must carry a hydrogen ion with it (Fig 7). Thus, it is expected that equal rates of acetate consumption and lactate production would result in no net production or consumption of hydrogen ion or bicarbonate and stable pH, as we have observed experimentally.

Nonetheless, the correlation \( r = .435 \) between the rates of acetate consumption and lactate production is not as close as the correlation between the rates of acetate consumption and oxygen consumption \( r = .824 \); Fig 2). This can be
traced to the fact that the rate of acetate consumption is closely correlated with the platelet concentration ($r = .765$; Fig 2), whereas the rate of lactate production is not ($r = .528$; Fig 3). The relative independence of rate of lactate production from platelet concentration has also been observed during storage in plasma.16 Also, as in plasma,16 the rate of lactate production is tightly correlated with the rate of glucose consumption ($r = .911$; Fig 3), with approximately two molecules of lactate produced for every molecule of glucose consumed. Thus, the rate of glucose consumption is also relatively independent from the platelet concentration. It is for this reason that we express lactate production and glucose consumption as millimoles per liter per day, whereas we express oxygen and acetate consumption as millimoles per $10^{12}$ platelets per day.

These results explain the surprising data shown in Fig 5. In past studies of PC storage in plasma, pH at the end of a storage interval correlated inversely with the platelet concentration.15 The reverse occurs in BSM + AP (Fig 5). This is expected because the rate of lactate production is relatively constant regardless of the platelet concentration (Fig 3), whereas the rate of acetate consumption is greater at high platelet concentrations than it is at low platelet concentrations (Fig 2). The alkalinizing effect of acetate metabolism (Fig 7) predominates at high platelet concentrations, resulting in a higher pH.

We have previously shown in studies of PC storage in plasma that organic anions other than acetate, such as pyruvate, can be oxidized by platelets. Because their oxidation also withdraws hydrogen ions from the medium, their presence achieves the same alkalinizing effect as acetate. Tables 2 and 3 indicate that this is also true for pyruvate in the synthetic medium (BSM + PP), in which pyruvate replaces acetate. pH control was similar to that achieved with acetate, and, in fact, pyruvate had a significantly greater suppressive effect on lactate production than acetate. We do not mean to suggest that pyruvate is in any way superior to acetate for practical purposes, but the results with it provide support for the generality of the principle proposed in Fig 7.

Acetate alone was not sufficient to allow pH maintenance in all PC. In line with previous experience,14 the addition of at least 10 mmol/L phosphate assured success in 38 consecutive PC, whereas accelerated lactate production and pH level decrease were seen in some PC without it. The mechanism of phosphate’s benefit is not definitely known. It may provide a buffering effect early in storage and/or prevent depletion of adenine nucleotides through its inhibition of AMP deaminase.14 On the other hand, others3,4,10,11 have reported suc-
cessful PC storage in synthetic media with the only apparent necessary additive being acetate. An explanation for the discrepancies between the results of these studies and those that we report here will require further study.

Our studies allow us to suggest the optimal concentrations for ingredients in a synthetic medium in addition to 10 mmol/L phosphate. Reference to Fig 2A indicates that the maximal rate of acetate consumption would be 2 mmol/L/d. Based on its mechanism of action, one would want to have a starting concentration of at least 10 or 14 mmol/L for 5- or 7-day storage intervals, respectively, to be assured that acetate would be available for metabolism throughout storage. The lack of benefit for Setosol relative to BSM + AP suggests that the maltose in Setosol is not crucial. The requirement for glucose in a synthetic medium remains controversial. In this study (Table 3), ATP levels were consistently and significantly lower if glucose was omitted from BSM + AP. In addition, our PC were actually not stored in the absence of glucose for the entire storage period. Assuming a 25 mmol/L concentration of glucose in plasma, the concentration of glucose at the initiation of storage would be 2.75 mmol/L in DnDP (11% plasma carry-over) and 4.25 mmol/L in PL-732 (17% plasma carry-over). Plasma carry-over is significantly higher in PL-732 because of the rigid pockets at the top of the container. As indicated in Fig 3B and Table 2, the maximum and mean rates of glucose consumption in BSM + AP are 1.2 and 0.68 mmol/L/d, respectively. Thus, on average, there is enough glucose to last for 3 to 4 days in DnDP and for 6 days in PL-732 even if glucose is not present in the medium. This explains the results shown in Fig 6 that show that lactate production continues between days 1 and 5 in DnDP and between days 5 and 7 in PL-732. Certainly, when one works with 30% plasma carry-over, there is more than enough glucose in the plasma to support prolonged storage.

Similarly, the concentration of citrate required for PC storage requires definition. Gulliksson et al. have suggested that at least 8 mmol/L is required to inhibit coagulation. We had good success with the TRDB2 solution (Table 1) that contains 5 mmol/L citrate. With an 11% plasma carry-over, the final concentration of citrate would be 7.3 mmol/L, assuming the plasma citrate concentration to be 21 mmol/L. The TRDB2 solution has manufacturing advantages in that calcium is absent eliminating the precipitation of calcium salts that can occur during sterilization at close to neutral pH. With the good results shown in Table 1, we believe that the combination of the new container (DnDP) and storage medium (TRDB2) provide an ideal combination for in vivo, clinica study.

In conclusion, these in vitro studies provide ground rules that can be used in the application of platelet storage media to new methods of preparing PC for clinical use. We have studied here whole-blood-derived PC, but the generalizations should apply to PC prepared by apheresis as well. With 40% to 50% plasma carry-over, saline with no other ingredients appears to be sufficient at least for platelets derived from pooled buffy coats. Below 40% plasma carry-over, the addition of some form of buffering is required. Assuming that bicarbonate is too difficult to work with from the manufacturing point of view, an organic anion such as acetate whose metabolism suppresses lactate production and provides the buffering effect described in Fig 7 needs to be added. Our data suggest that a 15 mmol/L concentration of acetate should suffice for a 7-day storage interval. A 30% plasma carry-over provides enough glucose (7.5 mmol/L) for 7 days for all but the most metabolically active PC. Below this level of plasma carry-over, the provision of supplemental glucose should be considered although, as discussed above, this point is controversial. This could be accomplished by addition to the primary anticoagulant or to the medium itself. Although steam sterilization of solutions containing glucose at neutral pH is technically demanding because of the tendency of glucose to caramelize, recent work has suggested that this can be accomplished by performing sterilization under nitrogen. The addition of citrate to achieve a final concentration of 8 mmol/L should also be considered. For storage of PC with less than 15% plasma carry-over, we have found that the requirements are even more rigorous. There appears to be benefit from adding phosphate at a concentration of at least 10 mmol/L.

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Platelet storage for transfusion in synthetic media: further optimization of ingredients and definition of their roles

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