Platelet Metabolism During Storage of Platelet Concentrates at 22°C

By H. Kilkson, S. Holme, and S. Murphy

The development of methods for storing platelet concentrates (PCs) at 22°C for transfusion has been predominantly empiric, with minimal knowledge of metabolic events occurring during storage. It is known that a decrease in pH due to accelerated production of lactic acid in hypoxic conditions is a major cause for loss of platelet viability. In the current studies, we have measured metabolic parameters such as O₂ and glucose consumption rates and CO₂ and lactic acid production rates. We have also determined the O₂ and CO₂ transport capacities of various containers and the buffering capacity of plasma. The O₂ consumption rate was 1.10 ± 0.16 (SD) nmol/min/10⁹ platelets. In well-oxygenated systems, lactic acid formation was 1.74 ± 0.12 nmol/min/ml PC for PCs with a platelet count of 1 to 2 × 10⁹/1 ml, and 0.52 mol of glucose was consumed per mol lactic acid produced. In a completely oxygen-free system, lactic acid production increased 5-8 fold. These calculations suggest that 85% of energy generation is derived through oxidative metabolism and that glucose may not be the primary substrate for this metabolism. Bicarbonate concentration, initially 22.1 ± 1.6 mEq/L, decreased 1.41 ± 0.18 mEq/min/ml PC for PCs with counts 1 to 2 × 10⁹ platelets/ml. The loss of bicarbonate was caused by displacement by lactic acid and as a consequence of spontaneous CO₂ efflux from the container. CO₂ production, 2.3 ± 0.4 nmol/min/10⁹ platelets, was derived from oxygen consumption and the CO₂ liberated from bicarbonate as it was consumed. A rapid fall in pH to levels below 7.0 (22°C) took place when the bicarbonate concentration fell below 5 mEq/L as lactate concentrations reached 20 to 25 mmol/L. A further increase in lactate concentration from 25 mmol/L to 40 mmol/L correlated with a further fall in pH to 5.8. Thus, the ultimate storage life of a PC is determined by continuous lactate production and the fixed buffering capacity of plasma and by the glucose concentration of the PC. With knowledge of these parameters, methods for predicting pH as a function of time, platelet count, and O₂ and CO₂ transport capability of the container have been developed as guidelines for future work.

During the past ten years, many blood banks have adopted 20 to 24°C as the preferred temperature range for storage of PCs for transfusion. The polyvinylchloride (PVC) transfer packs that have been used are of a size (300 to 400 mL) that is optimal to hold plasma extracted from a unit of whole blood after centrifugation. They were designed long before PCs were stored at 20 to 24°C. It is only by chance that the plastic and the configuration are at all satisfactory for the storage of a 30- to 50-mL PC.

A major problem with the PVC containers has been that many PCs have a dramatic fall in pH from initial levels of 7.1 to below 6.0, even within 48 to 72 hours. As pH* falls from 6.8 to 6.0, platelets swell² and change shape from disc to sphere.³ These changes are reversible if the cells are returned to physiologic pH. At pH of 6.0 and below, the cells are irreversibly sphered and swollen, and they extrude long rigid rods of cytoplasm and progressively lose their capacity to consume oxygen.⁴ These changes are associated with removal of the cells from the circulation when they are infused in vivo.⁵ The pH fall has correlated strongly with a rise in the concentration of lactate. Furthermore, lactate production is stimulated by hypoxic conditions within the container, which are produced by the relative impermeability of PVC containers to gases. The pH fall can be retarded if containers with increased permeability to gases are employed,¹⁶ with resultant prolongation of the storage interval for PCs.

There has been an extraordinary amount of "trial-and-error" work in this area. To date, we have lacked the following fundamental pieces of information concerning PC storage: (1) the rate of O₂ consumption; (2) the rate of lactate production in PC with adequate O₂ supply; (3) the substrates used for oxidative and glycolytic metabolism; (4) the capacity and nature of the buffers in suspending plasma; and (5) the rate of CO₂ production and its effect on pH. In the current work, we have obtained this information. Because pH fall is the major limiting variable for prolonged PC storage, we have attempted to use this information to develop a model that would predict pH at any time during storage, given knowledge of PC volume and platelet count and the transport capability of the container for O₂ and CO₂.

MATERIALS AND METHODS

PCs were prepared from blood anticoagulated with citrate-phosphate-dextrose (CPD) or CPD-adenine, as previously described.¹ Informed consent was obtained from the donors, after approval of the local Human Investigation Committee, in accordance with an assurance filed with and approved by the HSS. PC
PLATELET METABOLISM DURING STORAGE

407

from PVC (P1-146 from Fenwal labs, Deerfield, Ill) or volume was adjusted so that the average volume during storage (considering sample volumes to be removed for study) would be 50 mL. PCs were stored in either commercially available transfer packs constructed from PVC (PL-146 from Fenwal Labs, Deerfield, Ill) or three experimental containers constructed from polyolefin films, hereafter referred to as packs A, B, and C. These packs have the same configuration as PVC bags, and the films were chosen so as to have increased rates of O2 transport. PCs were stored on a flat-bed, to-and-fro agitator at 70 cycles per minute (Eberbach Corp. Ann Arbor, MI) at 22 ± 2 °C. In most instances, storage was for one week, but in a few instances, storage was extended to 11 to 14 days.

PC PO2, PCO2, and pH at 37 °C were determined as previously described on PC using a pH/blood gas analyzer (Instrumentation Lab, Lexington, Mass). Using data from Wilhelm et al,8 P02, and measurements, pH at 22 °C was higher than pH at 37 °C by an average of 0.18.

PC platelet count and lactate concentration were determined as previously described.1 PC glucose concentration was measured enzymatically with hexokinase and glucose-6-phosphate dehydrogenase using a kit from Sigma Chemical Co, St Louis. PC bicarbonate concentration was measured by placing nitrogen bubbles of various sizes within the container along with 50 mL of cell-free plasma that had been exhaustively bubbled with nitrogen. The containers were then placed on the agitator, and measurements of PO2 were made at intervals. The determination of oxygen consumption rate is a constant that we determined experimentally by adding lactate acid to known concentrations of bicarbonate.

RESULTS

Determination of Oxygen Consumption Rate

In most studies, the oxygen consumption rate [C(O2) (nmol/min/109 platelets)] was determined by a steady-state technique.6 First, the capacity for O2 transport, KO2 (nmol/min/atm), of a container was determined by placing nitrogen bubbles of various sizes within the container along with 50 mL of cell-free plasma that had been exhaustively bubbled with nitrogen. The containers were then placed on the agitator, and measurements of PO2 were made at intervals. The calculation of KO2 from data of this type is described in Appendix I. Table 1 shows results for KO2 measurements for five different containers. To determine C(O2), PC of known volume and platelet count were placed in containers with known KO2 and allowed to equilibrate with agitation for at least 18 hours with ambient air at 22 °C. The PC PO2 (22 °C) was measured, and ΔPO2 (22 °C), the difference between ambient PO2 and PC PO2, was calculated. At steady state, the rate of oxygen consumption is equal to the rate of oxygen influx. Therefore:

\[
C(O_2) = \frac{\text{Platelet content} \times P(O_2) - P(CO_2) 
}{10^8} = KO_2 \times \Delta P(O_2) (22 \degree C)
\]

In the following results, C(O2) measurements include only those with values of pH (22 °C) > 6 and PO2 (22 °C) > 10 mm Hg. We have previously shown that oxygen consumption steadily declines if pH falls below 6.0.4 The Po2 values below 10 mm Hg were not included because, at low PO2, oxygen demand may be higher than the influx, which is limited by the capacity of the bag to transport oxygen into the PC. C(O2) was determined at 24 hours of storage on 50 occasions in four containers with different KO2 values: the 400-mL PL-146 bag from Fenwal and the three experimental polyolefin containers. The mean was 1.10 ± 0.16† nmol/min/109 platelets. The following means for the four containers were obtained: 1.07 ± 0.14 for PL-146; 1.07 ± 0.21 for bag A; 1.14 ± 0.16 for bag B, and 1.09 ± 0.16 for bag C. The means are not significantly different. Figure 1 shows the C(O2) determinations obtained at various PO2 levels (Fig 1A) and platelet contents (Fig 1B) of the PC. There was no significant correlation between C(O2) and PO2 for any of the four

Table 1. KO2 Measurements

<table>
<thead>
<tr>
<th>Containers</th>
<th>No. of Studies</th>
<th>KO2 (nmol/min/atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenwal PL-146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300-mL</td>
<td>8</td>
<td>358 ± 14</td>
</tr>
<tr>
<td>400-mL</td>
<td>11</td>
<td>441 ± 43</td>
</tr>
<tr>
<td>Experimental pack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>14</td>
<td>789 ± 102</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>903 ± 37</td>
</tr>
<tr>
<td>C</td>
<td>18</td>
<td>1,082 ± 52</td>
</tr>
</tbody>
</table>

†Unless otherwise indicated, all ± refer to standard deviation.
bags when considered individually or for all bags considered together. $C(O_2)$ and platelet content showed significant correlation for bag A ($r = .90, P < .001$), but not for any of the other bags or for all bags considered together. Figure 2 shows determinations of $C(O_2)$ at intervals during storage for seven days in the various containers. There was no tendency for $C(O_2)$ to vary as time passed. In addition to time of storage, there was no significant correlation between $C(O_2)$ and PC pH.

$C(O_2)$ was also determined by a second method. Fifty milliliters PC with a platelet count of $1 \times 10^9/mL$, was placed in two PVC bags coated with a layer of Mylar polyester, 0.25 mm thick. This coating made the plastic essentially impermeable to gases. A 10- to 30-mL gas bubble (10% O$_2$/90% N$_2$) was then injected. The O$_2$ tension in the bubble was measured at intervals of 4, 8, 18, 24, and 45 hours. The bubble was replaced periodically to replenish O$_2$ and remove CO$_2$. Calculated $C(O_2)$ values were $1.36 \pm 0.22$ nmol/min/10$^9$ platelets, with no significant variation over a 45-hour period.

**Glucose Consumption and Lactate Production**

The concentrations of lactate and glucose were measured serially during storage in polyolefin containers for 11 days. The PO$_2$ was always $>10$ mm Hg. Representative results are graphed in Fig 3. Lactate production and glucose consumption were linear with time. Figure 4 shows the relationship between glucose consumption and lactate production. As expected, there was very strong correlation ($r = .923$). The slope of the regression lines was 0.52, suggesting 2 mol of lactate produced for each mole of glucose consumed.

In Fig 5, the lactate concentration on day 7 is graphed against the platelet count. We were surprised to find a nonlinear relationship between the two variables. In the range relevant for transfusion practice ($1 \to 2 \times 10^9$ platelets/mL), the average lactate concentration on day 7 was $17.5 \pm 1.2$ mmol/L. Therefore, the average lactate production rate, P(La), was $1.74 \pm 0.12$ nmol/min/mL PC with adequate oxygen supply. In six studies, PC with platelet counts $1.3 \to 1.7 \times 10^9/mL$ were stored for seven days in PL-146 containers. The PO$_2$ (37 ºC) at 24 hours was 0 mm Hg, indicating an oxygen demand greater than supply. The average day 7 lactate concentration was $38.2$ mmol/L. Finally, lactate production rate was measured under completely oxygen-free conditions. This was done by bubbling a humidified mixture of 10% CO$_2$ and 90% N$_2$ over PCs that were being stored in the usual fashion for six hours. The rate of lactate production in five studies was found to correlate linearly with the platelet count ($r = .99$). The mean per 10$^9$ platelets was $7.8 \pm 0.9$ nmol/min. For a PC with a count of $1.5 \times 10^9$ platelets/mL, this represents a

![Fig 2. Oxygen consumption, $C(O_2)$, during seven days of storage in four containers. $C(O_2)$ did not depend on the duration of storage.](image)

![Fig 3. Rise in lactate concentration and fall in glucose concentration during storage of four PC for 11 days in polyolefin containers. Platelet counts were $1.3 \to 1.7 \times 10^9/mL$. Oxygen supply was adequate. Under these circumstances, rates of lactate production and glucose consumption are remarkably constant as time passes.](image)

![Fig 4. Relationship between lactate production and glucose consumption during storage. The data points represent the difference between concentrations at 24 hours and intervals between days 3 and 11 of storage. The y intercept and slope are $-0.2$ and $0.52$, respectively ($r = .923$). For all data points, the mean of the ratios of glucose utilization to lactate production is $0.50 \pm 0.12$ (99% confidence interval, 0.44 to 0.56).](image)
PLATELET METABOLISM DURING STORAGE

Fig 5. Decrease in bicarbonate and increase in lactate concentration after seven days of storage related to platelet concentration. Open and closed circles represent studies carried out in PL-146 and experimental polyolefin containers, respectively. The artificially low platelet counts (open circles) were obtained by diluting PCs with autologous platelet-poor plasma. The regression lines calculated from data obtained from PCs with counts in the range of 1 to 2 × 10⁶ platelets/mL are given by: bicarbonate (mEq/L) = 4.2 · 10⁻⁸ · count (plt/mL) + 7.5, (r = .61); lactate (mmol/L) = 5.1 · 10⁻⁸ · count (plt/mL) + 10.7, (r = .88).

Fig 6. Relationship among pH, bicarbonate, and lactate concentrations during storage in polyolefin bags. The data points represent measurements made throughout storage on multiple units of PC. There is a linear relationship between bicarbonate and lactate concentrations until lactate concentration reaches 30 mmol/L. Values below 30 mmol/L were used to calculate the indicated regression line which is given by: bicarbonate (mEq/L) = -0.79 lactate (mmol/L) + 21.9, (r = .96). With the exception of one experimental point, pH remains above 6.6 as lactate rises to 30 mmol/L. The four experimental points with lactate above 30 mmol/L were obtained by allowing storage of four PCs to proceed until 14 days. There is a rapid pH fall once the bicarbonate buffer is exhausted.

Bicarbonate Depletion

The decrease in plasma bicarbonate during storage, as measured in mEq/L, showed only moderate dependence on the platelet count in the range relevant to transfusion (Fig 5). For PCs with counts in the range 1 to 2 × 10⁶ platelets/mL, the mean decrease was 1.41 ± 0.18 nEq/min/mL PC. The figure demonstrates that the loss was not entirely caused by displacement of bicarbonate by lactic acid formation, but also occurred with storage of plasma alone. This indicates that there is a spontaneous loss of bicarbonate as CO₂ exits through the walls of the container.

Figure 6 shows the relationship among pH, bicarbonate, and lactate concentration during storage. A fall in pH to levels below 7.0 took place when the bicarbonate concentration reached 5 mEq/L and below. This occurred at a lactate concentration of approximately 20 mmol/L. A further increase in lactate concentration to levels approaching 40 mmol/L correlated closely with pH fall from 7.0 to below 6.0.

Capacity of Plasma for Lactate Buffering

Known amounts of lactic acid were added to samples from a pool of fresh platelet-poor plasma in a PVC transfer container. The PCO₂ was adjusted to various values covering a range of several orders of magnitude by addition of 100% CO₂ or room air, and the pH (22 °C) was measured. The results from six studies are shown in Fig 7. The figure demonstrates that at 25 and 35 mmol/L lactic acid there is a lesser influence of PCO₂, in the range relevant to storage of PC (20 to 60 mm Hg), on the pH of the plasma than at lower levels of lactic acid.

Rate of CO₂ Production, G(CO₂)

Using methods and calculations described in Appendix I, KCO₂, the capacity for CO₂ transport, was found to be 3,180 ± 145 nmol/min/atm in four experiments for 300-mL PL-146 bags. The rate of CO₂ generation, G(CO₂), could then be measured by the steady-state technique as described for C(O₂) determination. The equation is given in Appendix II, equation 7. G(CO₂) was determined at 72 hours of storage when a stable PCO₂ level was reached in 300-mL PL-146 bags from
Fig 7. Relationship between pH and Pco₂ at four lactate concentrations. In six studies, known amounts of lactic acid were added to fresh, cell-free plasma inside a 300-mL PVC container. The Pco₂ was varied by addition of 100% CO₂ or room air. The regression lines (solid lines) are calculated from data points of simultaneous pH and Pco₂ measurements. Dashed lines show ± SD of estimate.

Fenwal. The mean of 13 experiments was 2.3 ± 0.4 nmol/min/10⁹ platelets.

DISCUSSION

Now that we have a value for C(O₂), equation 6 in Appendix II allows us to predict Pₒ₂ during storage when KO₂ is known for the container and platelet content is known for the PC. We can now see how critical the KO₂ for the PL-146 bag is. If PC volume is 50 mL in a 300-mL PL-146 bag and KO₂ = 358 (Table 1), Pₒ₂ (22 °C) will be 0 when the platelet count is 1.3 × 10⁹/mL, a routine level seen in practice. At progressively higher platelet counts, the PC will be progressively more hypoxic. In a container with KO₂ twice this value, Pₒ₂ (22 °C) would be 0 when the platelet count is 2.6 × 10⁹/mL, a count rarely seen in practice. When one considers that transfer packs constructed of polyvinylchlorides such as PL-146 were designed long before PCs were stored at 22 °C, it is remarkable that their gas transport characteristics are exactly at this critical level. The KO₂ required to maintain Pₒ₂ (22 °C) = 10 mm Hg for a given number of platelets in a container is shown in Fig 8. We chose Pₒ₂ = 10 mm Hg for this graph because of the data in Fig 1. We found no evidence for a decline in oxygen consumption for Pₒ₂ between 10 mm Hg and 120 mm Hg, suggesting that mitochondria could function even at this low O₂ tension. In fact, recent studies indicate that cells do not demonstrate the metabolic changes of hypoxia until intracellular Pₒ₂ is less than 1 mm Hg. Thus, critical PC Pₒ₂ may be far less than 10 mm Hg, but our current techniques for measuring Pₒ₂ are not accurate enough to make such fine distinctions. It is of interest to note that the experimentally determined oxygen consumption rate translates to approximately 0.12 mL/h of O₂ for a bag with 50 mL PC at a platelet count of 1.5 × 10⁹/mL. Hence, an air bubble of 5 mL (1 mL of oxygen), as is frequently found in bags, provides approximately eight hours of O₂ supply for the platelets.

The constancy of both C(O₂) and lactate formation over seven days of storage was somewhat surprising, given that platelets, even under the best of circumstances, exhibit a substantial amount of morphological decay by days 3 through 7, as judged by phase-contrast microscopy and other in vitro measurements. However, Tegas and Beutler et al. have shown that platelet glycolytic enzymes and intermediates remain intact during storage.

Several other observations in this study surprised us. In Fig 5, it is apparent that lactate concentration after seven days of storage is not linearly correlated with the platelet count over the range 0 to 2 × 10⁹/mL. In the range relevant to transfusion practice, 1 to 2 × 10⁹/mL, the rate of lactate production per 10⁹ platelets is higher in PCs with lower counts. This is in accordance with studies by Rock et al., who observed a decreased rate of lactate production per platelet with increasing platelet concentration. Thus, in practice, using adequately permeable containers, the rate of lactate production is only moderately affected by the platelet count. Figure 5 indicates that the rate of lactate production at 2 × 10⁹/mL is only 32% higher than at 1 × 10⁹/mL.

PCs stored in less oxygen-permeable containers, such as PL-146 (300 mL), will be progressively hypoxic when the platelet count is increased above 1.3 × 10⁹/mL (Fig 8). In order to meet energy requirements when oxygen supply is limited, glycolysis is accelerated. This effect (the Pasteur effect) has been demonstrated for platelets. This results in a progressive increase in lactate production per platelet as the platelet count is raised, accounting for the correlation between count and rate of lactate production in PVC containers observed previously. In the present

Fig 8. Oxygen transport capability of container, KO₂, needed to maintain Pₒ₂ (22 °C) at 10 mm Hg for a given platelet content. The relationship is derived from equation 6 in Appendix II. The arrows indicate KO₂ values for Fenwal’s PL-146 bag and the three experimental polyolefin containers used in this study.

From www.bloodjournal.org by guest on October 22, 2017. For personal use only.
study, we showed that lactate production was increased twofold for hypoxic PCs with counts of 1.3 to 1.7 x 10^9 platelets/mL stored in PVC containers, and that under completely oxygen-free conditions, lactate production was increased 6.7-fold. Some lactate production may be from contaminating lymphocytes, which would obscure the relationship between lactic acid production and platelet count. There is controversy, but lymphocytes appear to produce little lactate when oxygen supply is adequate, and they have a marked increase in lactate production (up to 30-fold increase) if there is an oxygen deficit. Hence, they may make a significant contribution to lactate production in these hypoxic situations. They also may contribute to the variability in pH fall that has been observed in the critical platelet count range (1.4 to 2.0 x 10^9/mL) in PVC containers.

Studies in the literature, as reported in recent reviews, show a range of 2 to 9 nmol/min/10^9 platelets for oxygen consumption and a range of 3 to 23 nmol/min/10^9 platelets for glucose utilization. These values are significantly higher than those found in this study and are probably due to the fact that the studies in the literature were carried out at 37 °C. The coefficient of variation of our C(O_2) measurement was 14.5%, large but not unusually so for a biologic measurement. There are several potential causes for the variability. First, the coefficient of variation for mean platelet volume in the normal population is 9.4%. A recent report suggested that metabolic rates during PC storage are more closely related to platelet mass in the PC than to platelet number. Secondly, as suggested above, variability in the number of contaminating leukocytes might make some contribution. We did not include measurements of platelet volume or leukocyte count in our experimental design, because our previous work had shown a strong correlation between platelet number and the rate of oxygen consumption by PCs. However, to be precise, we must point out that the metabolic data that we are reporting are those for the cell mix that is present in PCs as they are currently prepared. From the practical point of view, these data are the most important because this is the cell mix with which we must deal in designing methods for PC storage. A future study dissecting the relative contributions of platelets of various sizes and of leukocytes to rate of oxygen consumption will be of great interest. However, the results of such a study may not be clearcut. Since we began our work, two publications have suggested a relationship between white cell contamination and metabolic rates, while two have shown no such relationship.

Because platelets are consuming oxygen during PC storage, we were surprised to find the ratio of glucose consumption to lactate production to be so close to 0.5 (Fig 4). If glucose were the sole substrate for oxidation, our results suggest that the following reactions would be occurring each minute in a milliliter of PC with a platelet count of 1.5 x 10^9/mL and adequate oxygen supply:

\[
0.28 \text{ nmol glucose} + 1.65 \text{ nmol O}_2 \\
\rightarrow 1.65 \text{ nmol CO}_2 + 1.65 \text{ nmol H}_2\text{O} \\
0.87 \text{ nmol glucose} \rightarrow 1.74 \text{ nmol lactate}
\]

The ratio of glucose consumption to lactate production should be 0.66 rather than 0.52. The calculated ratio depends on our measurement, C(O_2). Its variability makes the lower limit of the 99% confidence interval for the calculated ratio 0.64. The upper limit of the 99% confidence interval for the measured ratio was 0.56, so that the data suggest that oxidation has substrates other than glucose. Recent work suggests that citrate and arachidonic acid are unlikely to be oxidized during storage. Platelet glycogen should be considered, but quantitative considerations make it an unlikely candidate. As indicated in Fig 4, glucose consumption and lactate production values were determined as the differences between concentrations at 24 hours and at intervals between days 3 and 11 of storage. In previous work, we found that platelet glycogen fell rapidly during the first 24 hours of storage to a mean of 0.05 μmol/10^9 platelets, the equivalent of 0.075 nmol/L glucose equivalents for a PC with a platelet count of 1.5 x 10^9/mL. Because lactate concentration rises at 2.5 mmol/L daily, platelet glycogen could make only a trivial contribution over a storage period of 1 week or more. There is ample evidence that platelets can oxidize fatty acids. Furthermore, plasma free fatty acids actually increase during storage, so adequate levels are present for metabolism. It has also been reported recently that ammonia accumulates during PC storage, suggesting that deamination of amino acids is occurring. Therefore, fatty acids and amino acids are potential substrates for oxidative metabolism. In future studies, it will be of interest to examine these possibilities.

The rate of adenosine triphosphate (ATP) production can be calculated from the rates of lactate production and oxygen consumption. The net yield of ATP production by glycolysis of glucose is 1 ATP equivalent per molecule lactate produced, and the number of ATP molecules produced per O atom consumed in oxidative metabolism is approximately 3. In a 50-mL PC with a count of 1.5 x 10^9 platelets/mL, we have:

- Glycolytic ATP (Glyc ATP) production: 1.7 nmol/min/mL PC
- Oxidative ATP (Ox ATP) production: 9.9 nmol/min/mL PC
The ratio of Ox ATP/Glyc ATP, 5.7, is close to the value 5.9, which has been reported by Holmsen et al for platelet-rich plasma (PRP). Furthermore, the model predicts that lactate production would have to increase 6.7-fold in the complete absence of oxygen in order to maintain an undiminished ATP production. This value is the same as the one we determined experimentally.

Our measured G(CO₂) of 2.3 nmol/min/10⁹ platelets is well in excess of our measured C(O₂), 1.1 nmol/min/10⁹ platelets. This is expected since CO₂ is produced both as an end-product of oxidative metabolism and as a result of disappearance of the bicarbonate buffer. Figure 6 indicates that there is a close inverse relationship between bicarbonate and lactate concentrations, with 0.8 mol of bicarbonate being transformed to CO₂ for each mole of lactate produced. The mode of bicarbonate disappearance is complex. Certainly, some is consumed during the displacement of bicarbonate by lactic acid, but there is a component of spontaneous loss, as indicated by the disappearance of bicarbonate during the storage of cell-free plasma (Fig 5). Nonetheless, we know the rate of CO₂ production due to bicarbonate depletion per milliliter of PC with a platelet count of 1.5 × 10⁹/mL to be 1.4 nmol/min (Fig 5). Assuming that the respiratory quotient is 1.0, a PC with platelet count of 1.5 × 10⁹/mL will produce 1.65 nmol/min/mL CO₂ due to oxidative metabolism. Therefore, the expected rate of CO₂ production (1.4 + 1.65 = 3.1 nmol/min/mL) is quite close to the measured value (2.3 × 1.5 = 3.5 nmol/min/mL).

The results in Fig 7 demonstrate the limited role of PCO₂ in establishing PC pH, particularly at lactic acid levels above 25 mmol/L. Even with PC with high platelet counts in containers constructed of relatively impermeable material, PCO₂ does not rise above 100 mm Hg. Thus, PCO₂ levels will modulate the pH established by the lactic acid concentration over a range of 0.6 pH units, but CO₂ elevations cannot be responsible for lethal pH falls to 6.0 or below, as some investigators have assumed.

We have used our results to attempt to construct a model that would predict PCO₂ and pH at any time during storage if it is established that KO₂ is adequate (Fig 8) and if KCO₂ and the PC platelet concentration are known. The mathematics and reasoning behind these calculations are given in Appendix II. The model is consistent with recently published experience in which PCs were stored for seven days in containers constructed from Fenwal’s plastic PL-732, which is known to have increased oxygen permeability. The pH at seven days was 7.17 ± 0.18. Assuming a lactate concentration of 17.5 mmol/L (Fig 5) and PCO₂ (37 °C) of 20 mm Hg, this is the pH range that Fig 7 would predict. The PCO₂ estimate is based on data in reference 1. In addition, Fig 9 shows results of experiments in which PCs with a volume of 50 to 70 mL and platelet counts of 0.8 to 1.2 × 10⁹/mL were stored in PL-146 containers. At that count, the PC were not hypoxic. The shaded area represents the predicted pH during storage based on calculations described in Appendix II. In general, the model predicts pH behavior, although at five to seven days of storage, the pHs were somewhat higher than predicted. This may have resulted from sampling earlier in storage, thereby reducing the number of platelets in the container generating acidic metabolites. Alternatively, other factors, as yet unidentified, may play a modulating role.

The production of lactic acid occurs during PC storage even in the presence of adequate oxygen. A first absolute barrier to truly prolonged storage is the finite buffering capacity of the PC. Bicarbonate buffers the initial 25-mmol/L lactate rise. An obvious approach is to add buffering capacity, as Kotelba-Witkowska et al did in a recent study. The next known barrier will be glucose concentration, as glucose will be exhausted as lactate reaches 40 to 50 mmol/L. This problem will be eliminated with the use of newer primary anticoagulants, such as CPD-A₃, which provides twice as much glucose as CPD. With understanding of these known barriers, truly prolonged storage can be explored with the expectation that other problems, as yet unrecognized, will be found.

APPENDIX

I. Determination of O₂ Transport Capacity of a Container, KO₂

Let a container containing V₉ mL of cell-free plasma with a gas bubble of volume V₈ be exposed to ambient air. The total volume of
O₂ in the bag at any time is

\[ V_{O_2} = (V_b + \kappa_2 \cdot V_c) \frac{P_{O_2}}{760} \]

(1)

where \( P_{O_2} \) - O₂ tension in mm Hg in the bag and \( K_1 \) - solubility constant of O₂ in plasma [0.0286 (22 °C)].

Let \( K_{O_2} \) - O₂ transport capability of the bag (mL/min/atm) at 760 mm Hg and 22 °C.

If one begins an experiment with a nitrogen bubble of volume, \( V_c \), and plasma that has been bubbled exhaustively with \( N_2 \), then the change in \( V_{O_2} \) with time \( t \) is given by:

\[ \frac{dV_{O_2}}{dt} = K_{O_2} \cdot \frac{P_{O_2} - P_{O_2}}{760} - K_{O_2} \cdot \Delta P_{O_2} \]

(2)

where \( P_{O_2} \) represents the ambient \( P_{O_2} \) in air at 22 °C. Combining equations 1 and 2 we have:

\[ \frac{dP_{O_2}}{dt} = \frac{P_{O_2}}{760} - K_{O_2} \cdot \Delta P_{O_2} \]

(3)

This integrates into:

\[ \ln \frac{P_{O_2}(t - 0)}{P_{O_2}(t - t)} = K_{O_2} \left[ \frac{V_b + \kappa_2 \cdot V_c}{P_{O_2}} \right] \cdot t \]

(4)

where \( \kappa_2 \) = solubility constant of CO₂ in plasma [0.73 (22 °C)].

In practice, \( \Delta P_{O_2} \) was measured at several time points after one hour. Each result was used to calculate a value for \( K_{O_2} \), and these values were averaged.

The CO₂ transport capability, \( K_{CO_2} \), can be measured in similar manner. The bag would be filled with a mixture of CO₂ and \( N_2 \) and the decrease in CO₂ concentration followed. The pertinent equation now becomes:

\[ \ln \frac{P_{CO_2}(t - 0)}{P_{CO_2}(t - t)} = K_{CO_2} \left[ \frac{V_b + \kappa_2 \cdot V_c}{P_{CO_2}} \right] \cdot t \]

(5)

where \( \kappa_2 \) = solubility constant of CO₂ in plasma [0.73 (22 °C)].

In practice, this measurement was made using only a gas bubble (5% CO₂, 95% \( N_2 \)) and no liquid. Percent CO₂ was measured by gas chromatography at intervals of time over at least six hours. A finite time interval is required for the walls of the container to establish a steady CO₂ flux value.

II. Prediction of \( P_{O_2}, P_{CO_2}, pH \)

At any time, \( P_{O_2}, P_{CO_2}, \) and pH in a PC can be predicted from the following. At steady state:

\[ C(O_2) (pc) (V_1) = K_{O_2} \frac{\Delta P_{O_2} (22°C)}{760} \]

or

\[ P_{O_2} (22°C) = P_{O_2} \frac{C(O_2) (pc) (V_1)}{760} \]

(6)

where \( pc \) = platelet count.

\( P_{CO_2} \) would be described by a similar equation:

\[ P_{CO_2} (22°C) = \frac{C(CO_2) (pc) (V_1)}{K_{CO_2}} \]

(7)

Values for \( C(O_2) \) and \( G(CO_2) \) have been determined as described in the Results section.

For example, for a 300-ml PL-146 bag containing a PC with a volume of 50 mL and a platelet count of 1.0 × 10⁹/mL, we have:

\[ K_{O_2} = 356 \text{ nmol/min/atm} \]

\[ K_{CO_2} = 3,180 \text{ nmol/min/atm} \]

\[ V_b = 50 \text{ mL} \]

\[ V_c = 148 \text{ mm Hg} \]

\[ \kappa_2 = 1.0 \times 10^9 \text{ platelets/mL} \]

\[ P_{O_2} = 148 \text{ mm Hg} \]

These results correlate well with reports in the literature. This \( P_{CO_2} \) would not be established immediately. The \( P_{CO_2} \) (22 °C) in freshly anticoagulated PC starts near 70 mm Hg (22 °C) due to acidification of bicarbonate by anticoagulant. A considerable amount of CO₂ must be expelled before the steady state is reached.

From the \( P_{CO_2} \), one can predict the pH, using Fig 7. The pH is, of course, dependent on the amount of lactate present or, under storage conditions, on the time of storage. One would predict the following pH behavior:

<table>
<thead>
<tr>
<th>Storage Days</th>
<th>Lactate (mmol/L)</th>
<th>( P_{CO_2} ) (22 °C)</th>
<th>pH (22 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>99</td>
<td>7.1</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>39</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>39</td>
<td>7.3</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
<td>39</td>
<td>7.1</td>
</tr>
<tr>
<td>7</td>
<td>17.5</td>
<td>39</td>
<td>7.0</td>
</tr>
<tr>
<td>10</td>
<td>25.0</td>
<td>39</td>
<td>6.7</td>
</tr>
</tbody>
</table>

REFERENCES


7. Murphy S, Kahn RA, Holme S, Phillips GL, Sherwood W,
Platelet metabolism during storage of platelet concentrates at 22 degrees C

H Kilkson, S Holme and S Murphy