The Oxidation of Exogenously Added Organic Anions by Platelets Facilitates Maintenance of pH During Their Storage for Transfusion at 22°C

By Scott Murphy

Previous studies of platelet metabolism during the storage of platelet concentrates (PCs) at 22°C for transfusion have shown high rates of both oxygen consumption and aerobic glycolysis with little oxidation of pyruvate produced by glycolysis. During storage in plasma, free fatty acids are major oxidative fuels. Glutamine is also present as a potential fuel but relatively little is metabolized beyond glutamate. In synthetic media, acetate is oxidized and provides a poorly understood buffering function. In the current work, acetate and pyruvate (6 to 25 mmol/L, sometimes with their 14-C-labeled counterparts) were added to PCs stored in plasma. They were both vigorously metabolized, predominantly to CO₂ (0.51 ± 0.08 and 0.31 ± 0.06 mmol/d/10^12 plates for acetate and pyruvate, respectively). The metabolism of these exogenous substrates was associated with significantly increased oxygen consumption and decreased glucose consumption, fatty acid oxidation, and bicarbonate utilization for buffering. In a more limited number of studies, similar findings were observed with addition of beta-hydroxy-butyrate. Superior maintenance of pH in the presence of these additives could be attributed to the fact that the metabolism of an organic anion requires that a proton be brought with the anion into the metabolic pathway, thus providing an alkalinizing effect. Pyruvate (but not acetate) also stimulated the metabolism of glutamate. Studies with 14-C-labeled glucose suggested significant activity of the hexose-monophosphate shunt during PC storage and confirmed that little, if any, pyruvate derived from glucose was fully oxidized. Taken together, the results provide a relatively complete picture of the pathways of energy metabolism used by platelets during PC storage and suggest a strategy by which organic anions such as acetate can be used to improve the results of such storage.

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PLATELET TRANSFUSION continues to be a major component of the management of patients with thrombocytopenia. The ability to store platelets as platelet concentrate (PC) in approximately 50 mL of plasma at 22°C is critical in the logistics of supplying this transfusion product. Past experience has been that an understanding of metabolic patterns of platelets during such storage has led to improvements of methods used for storage. For example, in the mid 1970s, it was recognized that platelets have a high requirement for oxidative metabolism and that they increase their glycolytic rate if they are deprived of oxygen.1 The consequent increased production of lactic acid leads to fall in pH and loss of platelet viability in so-called “first-generation” plastic containers.2 This observation lead to the introduction of “second-generation” containers, which allow storage with a continuously adequate supply of oxygen.3

We have previously made observations concerning metabolic patterns when oxygen supply is adequate.4 In addition to a high rate of oxygen consumption (=1 mmol/d/10^12 platelets), there is also a high rate of aerobic glycolysis, with the number of moles of lactate produced equaling the number of moles of oxygen consumed.6 Because the utilization of one oxygen molecule in aerobic metabolism fuels the regeneration of six times as much adenosine triphosphate (ATP) as the production of one lactate molecule, we have estimated that the bulk of ATP regeneration (perhaps 85%) results from oxidative metabolism with only a small supplement coming from glycolysis.3

Even with adequate oxygenation, the ongoing production of lactic acid results in a continuing consumption of bicarbonate, the major buffer in plasma.5 When bicarbonate is completely depleted, pH falls quickly. Fortunately for current practice, the concentration of bicarbonate in plasma is adequate to cope with the production of lactic acid, but only for 5 to 7 days. Storage beyond 5 to 7 days in plasma or for even 2 to 3 days in a synthetic medium containing glucose, but lacking bicarbonate requires the development of a strategy for coping with the lactic acid being produced.

We have also observed that the rate of production of lactate is approximately twice the rate of consumption of glucose, suggesting that most pyruvate, the immediate precursor of lactate in glycolysis, does not enter the tricarboxylic acid cycle for subsequent oxidation5 (Fig 1). This observation lead to a search for other possible substrates for oxidative metabolism. We showed that free fatty acids in plasma can be metabolized to CO₂ and performed calculations that suggested that they are the major oxidative substrate during storage in plasma.7 Some cells such as gastrointestinal epithelium8 use glutamine as a major oxidative substrate. We found that glutamine is present in PC plasma at approximately 0.4 mmol/L at the initiation of storage, and that it is deaminated to glutamate during storage.9 However, the bulk of the glutamate is not further metabolized, at least within 5 days. Calculations indicated that glutamine is only a minor oxidative substrate, but we were puzzled by the platelet's failure to metabolize glutamine carbon beyond glutamate.

Rock et al10 first reported the storage of platelets in a synthetic medium containing acetate. Guppy et al11 showed that acetate can be a major substrate for oxidative metabolism. We studied the storage of PC in synthetic media containing acetate and found that it is vigorously oxidized under the conditions of PC storage.12,13 Furthermore, the metabolism of acetate provides a buffering effect and slows or abolishes the depletion of bicarbonate expected due to the production of lactic acid.6,13 In the United States, storage of

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PC is limited to 5 days because of concern about bacterial overgrowth. However, in Sweden, acetate has been added to PC stored in plasma for clinical transfusion. Using a system to monitor bacterial growth, storage has been successfully extended to 11 days.

In the current work, we have continued studies of platelet metabolism during storage of PC in plasma. The goals have been (1) to study the metabolism of acetate and pyruvate when added to PC, (2) to further study the metabolism of glucose using glucose radiolabeled in its various carbons, (3) to study the effect of the metabolism of added acetate and pyruvate on the metabolism of glucose, free fatty acids, and glutamine, (4) to understand the mechanism by which anions benefit acid-base metabolism during the oxidation of acetate (and, as we will show, other organic acids), and (5) to make suggestions as to how the storage of PC might be improved based on these observations.

MATERIALS AND METHODS

PC were prepared as previously described from blood drawn into citrate-phosphate-dextrose from normal volunteers who gave written, informed consent to protocols approved by the Institutional Review Board of Thomas Jefferson University. Platelet-free plasma (PFP) was prepared as in Edenbrandt and Murphy. PC and PFP were stored as previously described in PL-732 transfer packs (Baxter) for as long as 12 days. At intervals during storage, the following measurements were made as previously described: platelet concentration, mean platelet volume, dispersion (geometric standard deviation) of the platelet size distribution, leukocyte concentration, pH at 22°C, rate of oxygen consumption, osmotic reversal reaction, percent discs by oil, phase microscopy, and concentrations of lactate, glucose, acetate, bicarbonate, glutamine, and glutamate.

The concentrations of pyruvate and D-3-β-hydroxybutyrate (β-OH-butyrate) were measured using kits from Sigma Chemical Co (St Louis, MO) and Boehringer Mannheim (Indianapolis, IN), respectively.

Sodium acetate (United States Pharmacopoeia [USP] for injection; American Reagent Labs, Shirley, NY), sodium pyruvate (Sigma) or sodium D, L-3-β-hydroxybutyrate (Boehringer Mannheim) was added to some PC at the initiation of storage. In addition, as indicated, radiolabeled metabolic substrates, all from New England Nuclear Corp (Boston, MA), were added to PC at the initiation of storage as previously described. They were acetic acid, sodium salt, [14-C-2], 2.7 mCi/mmol; acetic acid, sodium salt, [14-C-1], 59 mCi/mmol; acetic acid, sodium salt, [3-H], 87 mCi/mmol; pyruvic acid, sodium salt, [14-C-2], 10.4 mCi/mmol; pyruvic acid, sodium salt, [14-C-1], 11.9 mCi/mmol; hydroxybutyric acid, potassium salt, D(-)-B-[3-14C], 18.2 mCi/mmol; palmitic acid, [14-C-U], 800 mCi/mmol; glucose, D-[14-C-U], 10.6 mCi/mmol; glucose, D-[14-C-6], 51.8 mCi/mmol; glucose, D-[14-C-3], 53.6 mCi/mmol; glucose, D-[14-C-1], 53.4 mCi/mmol; glutamine, L-[14-C-U], 210 mCi/mmol; sodium bicarbonate, [14-C], 42.6 mCi/mmol. At intervals during storage, aliquots were removed from the PC for determining the amount of radioactivity in CO2. PC (0.5 mL) was placed directly into scintillation fluid. PFP was prepared from 1.2 mL PC by centrifugation for five minutes in a Beckman Microfuge E. PFP (0.5 mL) was placed directly into scintillation fluid. A second 0.5-mL aliquot of PFP was acidified to pH 5 with 2 mL of 85% lactic acid and shaken for 1 hour on a platform shaker to allow release of CO2 formed from bicarbonate. The PFP, so treated, was then added to scintillation fluid. Controls with [14-C]-bicarbonate were performed daily to be certain that all labeled bicarbonate was being released by acidification with lactic acid. Scintillation fluid and counting procedures were as previously described.

Accumulation of radioactivity in CO2 on any day during storage was the sum of the radioactivity which had left the bag through the walls of the container and the radioactivity in bicarbonate on that day. The former was determined by subtracting the radioactivity in CO2 in PC minus radioactivity in PFP not treated with lactic acid. Accumulation of radioactivity in CO2 was calculated using linear regression analysis. Unless otherwise indicated, means are expressed ± 1 SD. Statistical significance of differences was determined with Student’s paired and unpaired t-tests.

RESULTS

In these studies, the mean PC platelet concentration was 1.47 ± 0.46 × 10^11/L and the mean PC volume was 65.9 ± 7.0 mL. Mean PC leukocyte concentration was 2.2 ± 1.8 × 10^11/L.

Studies with addition of acetate to PC. In control PC, acetate was not detectable after 1 day of storage. Sodium acetate was added to PFP or PC resulting in concentrations ranging from 7 to 25 mmol/L. In 24 studies of addition to PFP, the concentration of acetate on day 7 of storage was 101% ± 4% of the concentration at the initiation of storage.
indicating that acetate was stable in the absence of cells. In 30 studies with PC, the concentration of acetate declined in a linear fashion ($r = -0.991 \pm 0.010$) over 7 days at a rate that correlated with the platelet concentration (rate, mmol/L/d = 0.05 + 0.55 × concentration, $r = 0.947$). The consumption of acetate was 0.51 ± 0.08 mmol/d/10^12 platelets. There was no correlation between the rate of consumption of acetate and the initial concentration of acetate.

14-C-1 acetate and 14-C-2 acetate were added along with unlabeled acetate in five and six studies, respectively. The accumulation of radioactivity in CO₂ occurred in a linear fashion ($r = 0.995 \pm 0.002$ for 14-C-1 acetate and $r = 0.996 \pm 0.002$ for 14-C-2 acetate). The fractions of total acetate consumed that could be accounted for in CO₂ were 0.87 ± 0.09 for 14-C-1 acetate and 0.88 ± 0.13 for 14-C-2 acetate. The 12% to 13% of acetate consumed that was not found in CO₂ could not be accounted for by incorporation into platelets because, for 14-C-1 acetate, only 0.1 ± 0.0% of the radioactivity on day 7 of storage was present in platelets when 39.1% ± 4.5% had been converted to CO₂ and, for 14-C-2 acetate, only 1.17% ± 2.92% of the radioactivity on day 7 of storage was present in platelets when 44.9% ± 10.1% had been converted to CO₂.

Controls were performed as in Cesar et al. 14-C-acetate was added to PFP and the percentage converted to CO₂ on day 7 was 3.96 ± 3.23 and 1.65 ± 2.31 for 14-C-1 acetate and 14-C-2 acetate, respectively. None of these figures are significantly different from zero. In another control, 3-H acetate was added to four PC. At the end of storage, the concentration of radioactivity in PC was 98.4% ± 1.5% compared with the start of storage.

Studies with pyruvate. In control PC, the concentrations of pyruvate were 0.105 ± 0.018 mmol/L and 0.091 ± 0.003 mmol/L on days 1 and 5 of storage, respectively. Sodium pyruvate was added to PFP or PC resulting in concentrations ranging from 6 to 12 mmol/L. In 28 studies with PFP, the concentration fell gradually in a linear fashion ($r = 0.987 ± 0.173$) at a rate of 0.24 ± 0.03 mmol/L/d. In 29 studies with PC, the concentration also fell in a linear fashion ($r = 0.996 ± 0.008$), but at a greater rate than with PFP. This rate correlated with the platelet concentration ($r = 0.909$) and was 0.08 ± 0.06 mmol/d/10^12 platelets. Corrected for the spontaneous disappearance of pyruvate from PFP, this figure would be 0.31 ± 0.06 mmol/d/10^12 platelets.

The pyruvates 14-C-1 and 14-C-2 were added along with unlabeled pyruvate in each of five studies. The accumulation of radioactivity in CO₂ occurred in a linear fashion ($r = 0.968 ± 0.026$ for 14-C-1-pyruvate and $0.956 ± 0.053$ for 14-C-2 pyruvate). The fractions of total pyruvate consumed that could be accounted for in CO₂ were 0.88 ± 0.20 for 14-C-1 pyruvate and 0.64 ± 0.07 for 14-C-2 pyruvate. These differences were statistically different ($P = 0.036$). In a fashion similar to the studies with acetate, 14-C-pyruvate was also added to PFP controls. There was no significant accumulation of radioactivity in CO₂ in the absence of cells.

Effect of acetate and pyruvate on energy metabolism. As shown in Table 1, the presence of both acetate and pyruvate stimulated the rate of oxygen consumption on day 1 of storage. This stimulation persisted on day 5 and 7 (data not shown). Pyruvate significantly reduced the rate of lactate production to 68% of the control value. On the other hand, acetate did not have a statistically significant effect. Both compounds suppressed glucose consumption significantly, but pyruvate suppressed more than acetate.

In addition, pyruvate suppressed the accumulation of glutamate by 30%, whereas acetate had no effect. In six paired studies, 14-C-U glutamine was added to all 12 PCs and unlabeled sodium pyruvate was added to one member of each pair to achieve a final concentration of 10 mmol/L. As in previous studies, glutamine disappeared to be replaced by glutamate during the first few days of storage. The sum of the concentrations of glutamine and glutamate declined in a linear fashion ($r = -0.986 ± 0.008$ and $-0.961 ± 0.067$ with and without pyruvate, respectively) over 7 days. Consistent with the decreased accumulation of glutamate in the presence of pyruvate (Table 1), the rate of consumption of glutamine and glutamate was significantly greater ($P = 0.026$) with pyruvate than without pyruvate. 21.7 ± 4.2 and 15.6 ± 3.4 mmol/10^12 platelets/d, respectively. The accumulation of radioactivity from 14-C-U glutamine in CO₂ also occurred in a linear fashion ($r = -0.987 ± 0.014$ and $0.982 ± 0.012$ with and without pyruvate). The rate of conversion of glutamine to CO₂ were 12.7 ± 5.1 and 15.0 ± 4.3 mmol/10^12 platelets/d with and without pyruvate, respectively. The difference between these values is not statistically significant.

We have previously shown that radioactivity is incorporated into CO₂ during storage when 14-C-U palmitate is added to PC at the initiation of storage. In the current studies, there was rapid incorporation into CO₂ in control PCs with no additives during the first 24 hours, and substantial but lesser degrees of incorporation during the ensuing four days (Fig 2). However, the difference between days 5 and 7 was not statistically significant. In the controls, 20.1% ± 3.3% of the added radioactivity was incorporated into platelets during the first 24 hours with a slight further increase to 24.5% ± 3.8% on day 7. Figure 2 shows that the addition of acetate or pyruvate significantly suppressed the conversion of 14-C-U palmitate to CO₂. However, they did not significantly alter the incorporation of radioactivity into platelets (data not shown). These results can only be analyzed in a semiquantitative fashion because we have previously shown that levels of plasma-free fatty acids, including palmitate, increase gradually during PC storage, presumably because they are continuously released from plasma lipoproteins. Thus, the specific activity of palmitate

### Table 1. Effects of Acetate and Pyruvate on Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetate</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose consumption</td>
<td>0.15 ± 0.31</td>
<td>0.96 ± 0.31</td>
<td>0.66 ± 0.31</td>
</tr>
<tr>
<td>Lactate consumption</td>
<td>2.10 ± 0.45</td>
<td>1.96 ± 0.45</td>
<td>1.43 ± 0.32</td>
</tr>
<tr>
<td>Lactate production</td>
<td>1.16 ± 0.36</td>
<td>0.95 ± 0.22</td>
<td>0.74 ± 0.28</td>
</tr>
<tr>
<td>Glutamate, day 7</td>
<td>0.345 ± 0.061</td>
<td>0.349 ± 0.088</td>
<td>0.240 ± 0.064</td>
</tr>
</tbody>
</table>

* P < .05 v control, † P < .05 v acetate.
decreases substantially as storage proceeds. This decrease could be a major reason for the slowing of the rate of incorporation of radioactivity into CO$_2$ during the latter half of the storage interval.

**Effect of acetate and pyruvate on acid-base balance and mean platelet volume.** Both acetate and pyruvate slowed the rate of bicarbonate consumption to 57% and 45% of the control, respectively (Table 1). Figure 3 shows the effect of preservation of bicarbonate on maintenance of pH. By day 12 of storage, mean pH for the control was 6.50 with six of ten PC having pH less than 6.6. On the other hand, with either acetate or pyruvate present, mean pH was greater than 7.1. The lowest pH observed on day 12 with either additive was 6.85. We have previously shown$^{19}$ that platelets swell progressively when exposed to pH below 6.8. Figure 3 also shows that control platelets swelled to a mean volume that is approximately one third greater than baseline by day 12 and that this swelling was prevented by the addition of acetate and pyruvate.

**Platelet quality control.** To examine the possibility that the metabolic changes noted upon addition of acetate or pyruvate might be caused by damage to the platelets or, conversely, by enhancement of their quality, four indices of platelet quality were determined on days 5 and 7 of storage: percent discs by oil, phase microscopy; the osmotic reversal reaction; platelet count as a percentage of the count on day 1; and the dispersion of the platelet size distribution determined by Coulter counter. There were no statistically significant differences for any of these four parameters among the three groups on either day. The results on day 7 are shown in Table 2.

**Studies with D-β-hydroxybutyrate (b-OH-butyrate).** In control PCs, b-OH-butyrate was not detectable during storage. b-OH-butyrate was added to PFP or PC resulting in concentrations in the range of 9 to 14 mmol/L. In seven studies of addition to PFP, the concentration of b-OH-butyrate on day 7 of storage was 100.6% ± 3.0% of the concentration at the initiation of storage indicating that b-OH-butyrate was stable in the absence of cells. In 13 studies with PC, the concentration of b-OH-butyrate declined in a linear fashion ($r = -.975 ± .036$) at a rate that correlated with the platelet concentration (rate, mmol/L/d = 0.05 ± 0.12 × platelet concentration, $r = .773$). The consumption of b-OH-butyrate was 0.16 ± 0.03 mmol/d/10$^{12}$ platelets. In eight

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**Table 2. Platelet Quality, Day 7**

<table>
<thead>
<tr>
<th>Day 7 of Storage</th>
<th>Control</th>
<th>Acetate</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Discs</td>
<td>51.7 ± 23.6</td>
<td>51.1 ± 19.2</td>
<td>49.9 ± 20.2</td>
</tr>
<tr>
<td>Osmotic reversal reaction, % recovery</td>
<td>45.4 ± 8.5</td>
<td>44.8 ± 6.4</td>
<td>45.8 ± 8.8</td>
</tr>
<tr>
<td>Platelet count, % of day 1 count</td>
<td>93.5 ± 6.0</td>
<td>93.1 ± 5.7</td>
<td>92.2 ± 6.4</td>
</tr>
<tr>
<td>Dispersion</td>
<td>1.802 ± 0.100</td>
<td>1.825 ± 0.069</td>
<td>1.841 ± 0.149</td>
</tr>
</tbody>
</table>
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studies, 14-C-b-OH-butyrate was added along with unla-
beled b-OH-butyrate. The accumulation of radioactivity in
CO₂ occurred in a linear fashion (r = .971 ± .023). The
fraction of b-OH-butyrate consumed that could be accounted
for in CO₂ was 0.96 ± 0.15. On day 7 of storage, no significant
radioactivity was incorporated into platelets. In a fash-
ion similar to the studies with acetate, 14-C-b-OH-butyrate
was also added to PFP controls and there was no significant
accumulation of radioactivity in CO₂. Relative to controls
(Table 1), the addition of b-OH-butyrate reduced lactate pro-
duction and glucose and bicarbonate consumption to 1.67 ±
0.22 mEq/L/d, 0.75 ± 0.17 mmol/L/d, and 1.08 ± 0.14 mEq/
L/d, respectively, resulting in maintenance of pH in the range
of 6.9 to 7.5, through 12 days of storage, mean = 7.25 ±
0.30 on day 12.

Glucose metabolism to CO₂. In 34 studies, glucose, ra-
diabeled either uniformly or in C-1; C-3,4; or C-6, was
added to PC. The rates of conversion of the various 14-C
glucoses to CO₂ were measured and expressed as a percent-
age of the total utilization of glucose. For 14-C-U glucose,
12.2% ± 3.1% of glucose carbon converted was metabolized
to CO₂. Significantly (P < .05) more CO₂ was formed from
14-C-1 glucose (21.9% ± 5.2%) and from 14-C-3,4 glucose
(12.2% ± 3.3%) than from 14-C-6 glucose (4.5 ± 3.3%). The
amount of 14-C-6 glucose converted to CO₂ was not signifi-
cantly different from zero statistically. When the four
14-C glucoses were added to PFP controls, there was no
significant accumulation of radioactivity in CO₂ (data not
shown).

DISCUSSION

The current work documents the following new observa-
tions concerning the storage of PC in plasma at 22°C: (1)
Exogenously added acetate is metabolized at =0.5 mmol/d/
10¹² platelets and almost 90% of the acetate metabolized is
oxidized to CO₂. (2) exogenously added pyruvate is metabo-
lized at =0.3 mmoles/day/10¹² platelets. Nearly 90% of py-
ruvate's C-1 carbon is oxidized to CO₂, whereas approxi-
ately two thirds of the C-2 carbon is fully oxidized. (3)
Exogenously added b-OH-butyrate is metabolized at =0.15
mmol/d/10¹² platelets with 96% converted to CO₂. (4) Glu-
cose is metabolized at = 1.2 mmol/d/L of PC (equivalent to
0.8 mmol/d/10¹² platelets under the conditions of our stud-
ies). Approximately 12% of glucose carbon is oxidized to
CO₂ with more coming from the C-1, -3, and -4 carbons
than from the C-6 carbon. (5) Both acetate and pyruvate
stimulate oxygen consumption and suppress glucose con-
sumption and fatty acid oxidation. (6) With no additive,
glutamine is converted to glutamate, which is then slowly
oxidized to CO₂. Pyruvate (but not acetate) stimulates the
utilization of glutamate formed from glutamine, but not
through its oxidation to CO₂. (7) The addition of acetate and
pyruvate both slow the rate of bicarbonate consumption and
inhibit fall in pH. Their ability to preserve bicarbonate is
greater than can be attributed merely to suppression of pro-
duction of lactic acid.

These observations will be discussed in order. Figure 1
outlines the various pathways involved. Much previous work
indicates that the metabolic patterns described are not caused
by contaminating leukocytes. For example, the patterns of
glucose consumption, lactate production, acetate oxidation,
and glutamine metabolism described have all been observed
previously in PC that have been subjected to leukodeple-
tion.⁷,⁸,¹⁷

Acetate is present in normal human plasma at a concentra-
tion of only ≈0.05 to 0.25 mmol/L, and it contributes only
a small fraction to daily CO₂ output.²² However, in the past,
it was used as the anion against which patients with renal
failure were dialyzed. It was shown that acetate is transported
into the circulation of the patient to achieve concentrations
of ≈5 mmol/L and that it can provide 55% to 73% of the
body's oxidized fuel under these circumstances.²¹ In other
species as diverse as the termite²² and the cow,²³ acetate is
a major, if not the major, oxidative substrate. Thus, there is
ample precedence for its vigorous oxidation when added
exogenously to PC. Similarly, we have shown that b-OH-
butyrate can be converted to acetyl CoA for oxidation.

It is surprising that exogenously added pyruvate is also
vigorously oxidized because only a small fraction of py-
ruvate coming from glucose through glycolysis is oxidized.
However, the enzyme complex, pyruvate dehydrogenase
(PDH), which catalyzes the conversion of pyruvate to CO₂
and acetyl CoA is tightly regulated in a complex fashion.²⁴,²⁵
It is converted to an inactive form by phosphorylation
through the action of PDH kinase, and it can be reconver-
ted to its active form by dephosphorylation through PDH
phosphatase. It is known that pyruvate binds to PDH kinase
and inhibits its activity,²⁶ so that high concentrations of pyruvate
added exogenously favor the activity of PDH. Furthermore,
the generation of pyruvate during glycolysis requires the
conversion of nicotinamide adenine dinucleotide (NAD) to
reduced nicotinamide adenine dinucleotide (NADH) during
the conversion of glyceraldehyde-3-phosphate to 1,3-diphos-
phoglycerate. Because the activity of lactate dehydrogenase
in platelet cytoplasm is high, any accumulation of NADH
will favor the conversion of pyruvate to lactate, thus recon-
verting NADH to NAD.²⁷ This keeps the concentration of
pyruvate low favoring the activity of PDH kinase. For a
substantial amount of pyruvate to be decarboxylated, the
reducing equivalents of the NADH generated in the cyto-
plasm during glycolysis must be transferred for oxidation
into the mitochondria by the malate/aspartate and glycerol
phosphate shuttles.²⁸ It is a reasonable hypothesis for study
that these shuttles are relatively inactive in platelets, thus
favoring formation of lactate from pyruvate.

Quantitative considerations suggest that most of the oxy-
gen consumption by platelets is devoted to acetate or py-
ruvate when either is present as an additive. The rate of
oxygen consumption is ≈1 mmol/d/10¹² platelets (Table 1). The
rates of acetate and pyruvate consumption are ≈0.5 and
0.3 mmol/d/10¹² platelets, respectively. Full oxidation of 1
molecule of acetate or pyruvate requires 2 and 2.5 molecules
of oxygen, respectively.

The relatively small fraction (≈12%) of the glucose car-
bon used which is eventually oxidized to CO₂ is not evenly
distributed among the six glucose carbons. C-1, C-3, and C-
4 are favored relative to C-6. The C-1-carbon is removed
during the conversion of glucose-6-phosphate to ribulose
5-phosphate in the hexose monophosphate (HMP) shunt. Therefore, our results suggest that the shunt is active during PC storage. The C-3 and C-4 carbons of glucose become the C-1 carbons of the two pyruvate molecules formed from glucose during glycolysis, whereas the C-6 carbon becomes the C-2 carbon of pyruvate. It is the C-1 carbon of pyruvate that is decarboxylated by PDH. Our observations of greater rates of metabolism of C-3,4 glucose relative to C-6 glucose and of C-1 pyruvate relative to C-2 pyruvate both suggest that some acetyl-CoA formed by decarboxylation of pyruvate is not oxidized further to CO₂. The fate of this acetyl-CoA is not currently known.

During PC storage, both acetate and pyruvate stimulate oxygen consumption and suppress the metabolism of fatty acids and glucose. Similar effects have been described in other cell types. Both substrates can enter the cell and its mitochondria for metabolism to acetyl CoA that may stimulate oxygen consumption. Fatty acids must be transferred into the mitochondria by a complex series of reactions using carnitine as a carrier and then be broken down into acetyl CoA by the equally complex process of β-oxidation. Acetate and pyruvate do not require such complex pathways to be metabolized. It is likely that acetyl CoA is formed relatively easily from them and it is known that acetyl CoA is the major inhibitor of β-oxidation. Furthermore, acetyl CoA condenses with oxaloacetate to form citrate, which then proceeds through the tricarboxylic acid cycle where oxidative phosphorylation results in the synthesis of ATP. Both citrate and ATP inhibit phosphofructokinase, the rate-limiting enzymatic step in glycolysis, thus reducing glucose oxidation.

Pyruvate (but not acetate) increased the rate at which glutamate derived from glutamine was further metabolized. Glutamate may be converted to α-ketoglutarate by directamination by glutamate dehydrogenase or by transamination using pyruvate or oxaloacetate as a transamination partner. The most straightforward explanation for the effect of pyruvate on glutamate utilization is that it does act as a transamination partner. We expected that increased glutamine carbon would be found in CO₂ due to pyruvate’s effect because it would be expected that the α-ketoglutarate formed would be oxidized through the tricarboxylic acid cycle, but this was not the case. The fate of the increased glutamate metabolized in the presence of pyruvate is unknown. In fact, the role of the high level of glutaminase activity which we have previously demonstrated in platelets is not known since past calculations have indicated that glutamine and glutamate are not major substrates for oxidative metabolism.

With regard to the practical problems of PC storage, the most important finding in this study is that the addition of organic anions such as acetate, pyruvate, or β-OH-butyrate to PC spared the consumption of bicarbonate and allowed prolonged stabilization of pH (Fig 3). This sparing was beyond what would be expected simply because of suppression of production of lactic acid. In fact, the extent to which the rate of bicarbonate consumption was reduced beyond that expected from the reduction in lactic acid production was similar to the rate at which the organic anion was being metabolized. This concept is presented graphically for acetate and pyruvate in Fig 4. The reason for this is that the metabolism of an organic anion requires that a proton be brought with the anion into the metabolic pathway, i.e., the anion is metabolized in its acid form. Thus, during hemodilution as acetate, the alkalinizing effect is achieved as acetate anions and an equal number of protons are metabolized to CO₂ by the tissues. The optimal concentration of organic anion to be added to PC would be that required to provide substrate for the maximal number of platelets anticipated for the longest storage duration planned. For acetate, that concentration can be easily calculated knowing the rate of acetate metabolism per platelet provided in the current work.

In this study, we used in vitro tests to monitor platelet quality. Of course, in vivo studies of increments in thrombocytopenic patients are the ultimate test of new approaches to storage of PC. There are already such in vivo studies in the literature showing that acetate can be a beneficial additive for PC storage in both a synthetic storage solution and in plasma. Our work indicates that the addition of any metabolizable organic anion can have a beneficial effect on pH for PC that are stored beyond 5 days in plasma. Acetate is particularly attractive because it is easy to prepare and work with, and there is long experience with its use clinically. Pyruvate has been explored as a potential additive for red blood cell storage, but it is less attractive because of its instability as witnessed by our observation of its slow decline in concentration during storage in cell-free plasma. The current storage interval, 5 days, is based entirely on appropriate concern about the risks of bacterial overgrowth. However, future research may lead to even more practical methods for detecting significant bacterial contamination than those used by Jonsson and Ahlgren. In addition, methods to decontaminate PC or prevent bacterial growth in them
may be developed. Were this to occur, the storage interval for PC could be extended routinely. Furthermore, the metabolic principles outlined in this work can be used as new methods of PC preparation and storage are investigated.

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The oxidation of exogenously added organic anions by platelets facilitates maintenance of pH during their storage for transfusion at 22 degrees C

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