Storage of whole blood before separation: the effect of temperature on red cell 2,3 DPG and the accumulation of lactate

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BACKGROUND: Although whole blood intended for component preparation is commonly left to cool at ambient temperature, knowledge is insufficient as to the effects this may have on red cell quality, in particular after a prolonged hold.

STUDY DESIGN AND METHODS: Whole blood collected in ACD-A (7% wt/wt) and CPD (12% wt/wt) was incubated at 4, 10, 15, 20, 25, and 30°C for 24 hours. Blood gases, pH, bicarbonate, glucose, lactate, and red cell 2,3 DPG were investigated.

RESULTS: When the blood was stored at 30°C, the 2,3 DPG concentration decreased within 4 hours from 858 ± 106 to 316 ± 172 mmol per mol of hemoglobin (a 63% decrease); 99 percent was lost within 18 hours. At 25°C, 46 percent was lost within 4 hours and 94 percent within 18 hours; at 20°C, the decrease at 18 hours was 62 percent and that at 15°C was 24 percent. No loss of 2,3 DPG was observed at 4°C and 10°C storage. No difference was attributable to the anticoagulant used. After 24 hours, the lactate concentration at 15°C was 2.9 times the original, that at 20°C was 3.8 times the original, that at 25°C was 7.0 times, and that at 30°C was 9.2 times.

CONCLUSIONS: With current anticoagulants, storage of whole blood at temperatures of 25 to 30°C before separation causes a great and rapid loss of 2,3 DPG and an accumulation of acid metabolites. In a hold of blood for >4 hours, rapid cooling is desirable to avoid initial loss of 2,3 DPG.

ABBREVIATIONS: Hb = hemoglobin; RBC(s) = red cell(s).

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Under physiologic conditions, the concentration of 2,3 DPG is kept at about 950 mmol per mol of Hb. Patients with anemia commonly have an increased concentration of 2,3 DPG, which seems to compensate to some extent for their lesser capacity to transport oxygen. In contrast, during storage of whole blood in vitro, the RBCs become depleted of their 2,3 DPG within the first 2 weeks, mainly as a result of the increasing acidity when glucose is metabolized to lactate.

To obtain uniform centrifugation effects, it is important that the temperature of the blood units be the same. To achieve this, a hold of some duration after collection is common. Many blood banks allow the freshly collected whole-blood units to cool spontaneously by keeping them at room temperature for a few hours. As was shown by Pietersz et al., the temperature decrease is quite slow when a blood unit is left at ambient temperature; a span of about 6 hours is needed to reach 25°C. With melting 1,4-butanediol—a wax with melting temperature at 20°C—used as a heat absorber, the process was accelerated to 2 hours. In their study of ACD whole blood stored at various temperatures in vitro, Strauss et al. found that 2,3 DPG decreased by 50 percent during 5-day storage at 4°C, but that the same effect was achieved in 6 to 8 hours at 25°C. Prolonging the hold to 8 hours before separation has shown to improve the yield of platelets prepared from platelet-rich plasma.

A room-temperature hold of collected whole blood for up to 8 hours before separation into components is accepted by the regulatory agencies in the United States and used for practical and logistical reasons. An overnight hold, as used in some countries, reduces the need for staff members to work beyond usual office hours to produce components from blood collected late in the day, such as by mobile teams.

Under normal blood bank conditions, the blood will have been cooled by the surrounding air to about 30°C when collection is completed. A further decrease in temperature will depend on the circumstances; normally, it is a continuous process. In this study, we wanted to investigate the effects of clearly defined temperatures on the RBC metabolic rate and 2,3 DPG level, when blood was stored for 24 hours as whole blood in two commonly used anticoagulants. We decided to use CPD in the normal proportion to blood (12% wt/wt) and ACD-A in a smaller proportion (7% wt/wt) as is sometimes used in apheresis.

MATERIALS AND METHODS

Design of the study

From each of five normal, healthy donors, 2 half-units were collected in poly(vinyl)chloride containers (PL 146, Baxter, La Chatre, France): 236 g (225 mL) in 32 mL of CPD solution and 236 g in 18 mL of ACD-A solution. After careful mixing, blood was distributed into sterile, stoppered glass test tubes (Vacutainer 7-mL 367609, Becton Dickinson Vacutainer Systems Europe, Meylan, France), which were put in water baths at 15, 20, 25, and 30°C, ensuring rapid and steady cooling to the test temperatures. One test tube was used per sampling occasion. Samples for testing in a blood gas analyzer were withdrawn with a syringe without allowing contact with air and tested as quickly as possible. Perchloric acid extracts of RBCs were prepared under continuous mixing, centrifuged, neutralized with N,N-dioctyl methylamine, and stored frozen at −30°C until they were analyzed.

The extracellular pH, pO2, pCO2, and bicarbonate concentration were analyzed in a blood analyzer (Omni-9, AVL LIST GmbH Medizintechnik, Graz, Austria). Lactate and 2,3 DPG concentrations were analyzed in neutralized perchloric acid extracts of RBCs by using kits (Boehringer Mannheim Diagnostica, Mannheim, Germany) adapted for use with an automated spectrophotometer (Cobas Mira, Roche, Basel, Switzerland). Glucose analysis was performed with a kit (Roche) adapted for the Cobas Mira.

Statistics

The results are presented as the mean ±SD. Significance was determined by Wilcoxon’s signed-rank test using software (StatView, Abacus Concepts, Berkeley, CA); p < 0.05 was considered statistically significant.

RESULTS

Storage effects on pH, plasma bicarbonate, blood glucose and lactate

As is shown in Table 1, the initial pH values in the two blood mixtures were rather similar. The fact that the pH was not lower but instead slightly higher after collection in ACD-A than in CPD, although the former is more acid, is explained by the lower volume used. When stored at 15 and 20°C, the blood showed a minor decrease in pH with time; the choice of anticoagulant did not have an effect on the pH. When the blood is kept at 25 and 30°C for 24 hours, the pH decreased to 6.77 to 6.79 and 6.73, respectively.

The consumption of bicarbonate during 24 hours, as indicated by the decrease in bicarbonate concentration, was about 1.5 times higher at 30°C than at 15°C (Table 1). A substantial part of the buffering capacity of the plasma bicarbonate can be used up during a prolonged hold; time seems more important than temperature in this respect. However, the figures do not exactly reflect what is happening in a normal whole-blood unit, as blood bags have a partly gas-permeable container wall that can allow carbon dioxide to diffuse out, whereas this experiment was performed in stoppered glass test tubes.

The consumption of glucose corresponded to the formation of lactate, with 1 mole of glucose consumed per 2 moles of lactate formed (data not shown). As expected, this process was both temperature- and time-dependent (Fig. 1).
No significant differences were attributable to the anticoagulants used. After 24 hours, the concentrations of lactate at 15, 20, 25, and 30°C were 2.9, 3.8, 7.0, and 9.2 times those at the initial level, respectively.

2,3 DPG

In a preliminary study using a design similar to that of the main experiment, it was observed that, during storage of aliquots of whole blood from two donors at 4°C and 10°C for 24 hours, the concentration of 2,3 DPG increased by 13 percent, whereas, with storage at >15°C, a decrease was found. The results of the main experiment are summarized in Fig. 2. The 2,3 DPG concentration in the freshly collected blood was 858 ± 106 mmol per mole of Hb. This is about 100 mmol per mole of Hb lower than expected from previous studies of the levels in normal persons. It cannot be excluded that some loss occurred during blood collection and distribution into test tubes.

The concentration of 2,3 DPG decreased according to temperature and incubation time; the values with the two anticoagulants were very similar at corresponding temperatures and time points. Combined data show that the loss of 2,3 DPG at 15°C was 9 percent during the first 4 hours and 24 percent and 29 percent after 18 and 24 hours, respectively. At 20°C, the level decreased by 25 percent during the first 4 hours. After 18 hours and 24 hours, the 2,3 DPG concentrations had decreased to 38 percent and 23 percent of initial levels, respectively.

At 25°C, the concentration after 4 hours was 461 ± 148 mmol per mole of Hb, a loss of 46 percent; 94-percent depletion occurred after 18 hours and 99 percent after 24 hours. At 30°C, the 2,3 DPG had decreased to 316 ± 172 mmol per mole of Hb in 4 hours, a loss of 63 percent; within 18 hours, 99 percent was lost.

**TABLE 1. Storage of whole blood collected in CPD solution (12% wt/wt) and ACD-A solution (7% wt/wt) at different temperatures for up to 24 hours: effects on pH and bicarbonate concentrations**

<table>
<thead>
<tr>
<th>Time (hours)/ temperature (°C)</th>
<th>pH</th>
<th>Bicarbonate (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPD</td>
<td>ACD-A</td>
</tr>
<tr>
<td>0</td>
<td>7.02 ± 0.09</td>
<td>7.07 ± 0.04</td>
</tr>
<tr>
<td>2/15</td>
<td>7.01 ± 0.09</td>
<td>7.05 ± 0.02</td>
</tr>
<tr>
<td>4/15</td>
<td>6.99 ± 0.09*</td>
<td>7.03 ± 0.03*</td>
</tr>
<tr>
<td>8/15</td>
<td>6.96 ± 0.08*</td>
<td>6.99 ± 0.03*</td>
</tr>
<tr>
<td>12/15</td>
<td>6.93 ± 0.08*</td>
<td>6.97 ± 0.04*</td>
</tr>
<tr>
<td>2/20</td>
<td>7.01 ± 0.09</td>
<td>7.05 ± 0.03</td>
</tr>
<tr>
<td>4/20</td>
<td>6.97 ± 0.09*</td>
<td>7.01 ± 0.03*</td>
</tr>
<tr>
<td>8/20</td>
<td>6.91 ± 0.08*</td>
<td>6.94 ± 0.03*</td>
</tr>
<tr>
<td>12/20</td>
<td>6.87 ± 0.07*</td>
<td>6.90 ± 0.03*</td>
</tr>
<tr>
<td>2/25</td>
<td>7.00 ± 0.09</td>
<td>7.03 ± 0.03*</td>
</tr>
<tr>
<td>4/25</td>
<td>6.95 ± 0.07*</td>
<td>6.98 ± 0.04*</td>
</tr>
<tr>
<td>8/25</td>
<td>6.83 ± 0.06*</td>
<td>6.84 ± 0.05*</td>
</tr>
<tr>
<td>12/25</td>
<td>6.77 ± 0.05*</td>
<td>6.79 ± 0.06*</td>
</tr>
<tr>
<td>2/30</td>
<td>6.98 ± 0.08*</td>
<td>7.02 ± 0.04*</td>
</tr>
<tr>
<td>4/30</td>
<td>6.92 ± 0.08*</td>
<td>6.94 ± 0.04*</td>
</tr>
<tr>
<td>8/30</td>
<td>6.77 ± 0.05*</td>
<td>6.79 ± 0.05*</td>
</tr>
<tr>
<td>12/30</td>
<td>6.73 ± 0.05*</td>
<td>6.73 ± 0.06*</td>
</tr>
</tbody>
</table>

* Significant difference from time 0.

**DISCUSSION**

In many blood banks, there is a lack of standardization concerning postcollection cooling of the units. A whole-blood unit left on the bench to cool at ambient temperature may, by chance, maintain a temperature of 25 to 30°C for several hours. This study shows that the initial hold of citrated whole blood may reduce the 2,3 DPG concentration profoundly, depending on the temperature of the blood mixture. The decrease was already significant after 2 hours at storage temperatures between 15 and 30°C. After 4 hours, the losses at 25 and 30°C were 46 and 63 percent, respectively, which indicated that, within an 8-hour hold, several RBC units may be depleted of most of their 2,3 DPG, even before the storage of the RBCs has begun. Because of the association between the 2,3 DPG concentration of the RBCs and their oxygen affinity, RBCs with impaired oxygen-releasing function may well appear, in blood units intended for transfusion, much earlier than the 10- to 12-day minimum commonly accepted for normality in this respect.

The effect of temperature on blood pH is illustrated in Fig. 3. It can be seen that blood, which has a pH of 7.05 at 37°C, will reach pH values of 7.3 at 20°C and 7.55 at 4°C. This is an effect caused by the differences in dissociation of hydrogen ion (H+) and binding to Hb at the different temperatures. In addition, pH will be influenced by the metabolic activity due to the temperature-dependent production of lactate and protons and by a change in the buffering capacity of plasma bicarbonate. After 24 hours’ storage, the concentrations of lactate were 4 and 13 mmol per L at 15°C and 30°C, respectively, and those of bicarbonate were reduced by 19 and 31 percent at these temperatures.

An important question is of course whether the loss of 2,3 DPG in stored RBCs has any clinical consequences. It is known that the loss is reversible in the circulation of the trans-
fusion recipient. The recovery in vivo has been studied in healthy persons and anemic patients. The following procedure has been used: transfusion of stored blood group O RBCs to a recipient, who is commonly of blood group A. In posttransfusion samples, the donor cells have been tested after the recipient's cells have been agglutinated with anti-A and removed.

In five studies, the levels of 2,3 DPG were back to 40 to 50 percent of normal in 6 hours and 50 to 75 percent in 24 hours. In three of these studies, the levels were 75 to 90 percent of normal after 48 hours. In another study, full recovery was obtained in 3 hours. The reason for this deviation is not known. The authors suggested that 2,3 DPG synthesis may be more rapid in anemic patients than in healthy persons, but a preparation artefact may not be excluded.

The clinical importance of the 2,3DPG depletion in stored RBCs is still not clear. Experimental work and some clinical observations indicate that, in massive transfusions to critically ill individuals, the poor oxygen-transporting function of the transfused RBCs may be clinically important.

A further question is whether storage at elevated temperatures causes impairment of RBC viability. In early studies, Jandl and Tomlinson showed that 30 percent of RBCs that had been incubated for 24 hours at 37°C were removed from the circulation in 20 minutes, but without clinical symptoms or hemoglobinemia; virtually all RBC viability was lost after 48 hours at 37°C. The RBCs were sequestered in the liver, and a rise in indirect bilirubin was observed. Mollison found that a 2-hour incubation at 37°C, immediately after the blood was taken from the donor, followed by storage for 28 days,
reduced the 24-hour in vivo recovery from 73.0 percent to 65.3 percent. Evidence has been obtained that elevation of the temperature to 22 to 25°C for 24 hours, after initial cold storage, reduces the time of further acceptable storage. However, incubation of 42-day-stored saline-adrenaline-glucose-mannitol-suspended RBCs at 37°C for 1 hour before injection improved morphology and fluidity but caused a decrease in ATP; the in vivo recovery did not change. It seems likely that the time of storage with strongly reduced metabolic activity, such as that due to acidity, has a negative effect on RBC viability. It would be undesirable, then, to increase, more than necessary, the metabolic acidity in the blood unit at the beginning of storage. But the significance of an initial hold at elevated temperatures on RBC viability after cold storage needs further study.

Rapid cooling of collected blood is necessary if preseparation loss of 2,3 DPG is to be avoided. Our study indicates that cooling to 15°C or slightly below should be preferred in an overnight hold to preserve 2,3 DPG, but this may have negative effects on platelet viability.

Fig. 3. Extracellular pH in blood collected in CPD at various temperatures.

REFERENCES


