Effect of Oxygen and Temperature on the Potassium Efflux of Irradiated, Stored Red Blood Cells*

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ABSTRACT

Gamma irradiation of blood is performed to prevent transfusion-associated graft-versus-host disease and can result in an accelerated efflux of intracellular potassium ($K^+$). The oxygen content of the blood and the temperature at which the irradiation is carried out are two variables reported to affect this shift; they were investigated in two separate studies.

Study A—Four units of AS-5 red blood cells were each split equally into four transfer bags. One set of transfer bags was stored at 4°C for 42 days (Control), the second set (IRR) was irradiated and stored. The third and fourth groups were oxygenated; the third set (+O₂) was placed at 4°C. The fourth set (IRR + O₂) was irradiated, then stored. Supernatant $K^+$ and hemoglobin increased in the IRR + O₂ group over IRR and Control, + O₂ did not rise compared to Control.

Study B—Six units of CPDA-1 whole blood were each equally separated into four transfer bags. The first set of bags was placed at 4°C, the second set was irradiated and then stored (IRR). The third group of bags was irradiated and given an 8-hour 22°C incubation prior to storage at 4°C (IRR-22). The fourth set was irradiated, placed at 37°C for 2 hours, then stored. Supernatant $K^+$ was lower during the first 7 days of storage for the IRR-22 and IRR-37 groups compared to IRR, but these increased to IRR levels by day 35.

Irradiation of blood products results in an accelerated $K^+$ shift that can be exacerbated by the presence of oxygen. The injury can be partially and temporarily corrected by a post-irradiation incubation at 22°C or 37°C.

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Introduction

Gamma irradiation of blood and blood components before transfusion is done to prevent transfusion-associated graft-versus-host disease (TA-GVHD) in immunocompromised patients and those patients who opt to receive directed donations from family members. A survey of blood irradiation practice conducted in 1989 by Anderson et al found that approximately 10 percent of all blood transfused is irradiated. The logistics of providing irradiated red blood cells (RBC) for this group of patients has meant that in some instances there is a substantial storage period after irradiation. A well documented lesion occurs as a result of the irradiation of RBC containing components prepared for transfusion causing an accelerated shift of the intracellular potassium (K+) to the extracellular space ('potassium leak lesion'). The United States Food and Drug Administration has recommended that RBC containing blood products which have been irradiated be stored no longer than 28 days owing to reduced 24-hour post-transfusion recovery of these units after this time. These effects may be caused by the generation of free radicals and subsequent membrane damage and/or injury to important enzymes.

The ionizing effect of gamma rays on human tissue, including red blood cells, has been studied extensively to help predict the effects of radiation exposure by accident, war, or radiotherapy. The oxygen content of tissue and the temperature at which the irradiation is carried out are two variables reported to affect the efflux of K+. In two separate studies these variables were investigated in the irradiation process and how they may influence the efflux of K+ from conventionally handled RBC components.

**Study A: Potassium Leakage from Oxygenated, Irradiated RBC**

Availability of the Sterile Connecting Device* has allowed for the aseptic attachment of satellite bags to units of RBC. An occasional consequence of these manipulations is the mixing of the blood with the sterile air in the attached bags. This results in oxygenation of the unit of RBC as evidenced by its bright red color. Since radiation injury is directly related to oxygen content of the tissue, the K+ and hemoglobin leakage of oxygenated (O2), irradiated (IRR) RBC were determined.

**Study B: Effect of Temperature on the Post-Irradiation Repair of the “Potassium Leak” Lesion**

Irradiated RBC lose intracellular potassium at a rate twice that of non-irradiated RBC under normal blood bank conditions. Early studies on the effects of ionizing radiation on cell membranes demonstrated that the “potassium leak” lesion was minimized if the blood was at 37°C. The effects of irradiation were intensified when the RBC were incubated at 4°C, the temperature used in blood banks for RBC storage. The possibility for reduced intracellular K+ loss by irradiating RBC at temperatures above 4°C was investigated. A room temperature incubation was selected as one arm of the study since storage of whole blood is permissible for 8 hours after phlebotomy (to permit the harvesting of platelet-rich plasma).

The second test arm utilized a 37°C incubation phase after irradiation to provide a physiological temperature for potential recovery from the irradiation injury. It has been determined that the

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* SCD-312, Haemonetics, 400 Wood Road, Braintree, MA 02184.
introduction of $O_2$ into a RBC component enhanced extracellular K+ loss and that room temperature, and 37°C incubation temporarily reduced red cell K+ loss.

**Methods and Materials**

Blood was collected from subjects who gave informed consent and met the criteria for normal, volunteer blood donors. Gamma irradiation was performed using a 3300 cGy dose delivered by a Gamma-cell 1000. A dose of 3300 cGy was selected to insure that the entire volume of the canister containing the RBC in the irradiator received at least 2500 cGy. This dose (2500 cGy) was recommended recently as the most appropriate and is the minimum dose required by the most recent edition of the AABB Standards. Supernatant potassium (K+) measurements were performed with an ion specific electrode. Supernatant hemoglobin was measured spectrophotometrically on the last day of storage. Statistical analysis was performed using the paired t-test.

**STUDY A**

Four units of RBC, adenine-saline added, were each separated into four 300 mL transfer bags. A bag from each unit was stored at 4°C for 42 days (Control). The second set of bags was irradiated and stored at 4°C (IRR). The third and fourth sets were oxygenated by injection of 30 mL of $O_2$. Very gentle agitation was performed for ten minutes at 22°C; the gas was then withdrawn from the bags. The third set of bags were then placed at 4°C for storage (+$O_2$); the fourth was irradiated, then stored (IRR + $O_2$). Supernatant K+ of each unit was measured before subdividing and from each bag on days 1, 4, 8, 15, 22, 28, 35, and 42.

**STUDY B**

Six units of CPDA-1 whole blood were collected and each was separated into four 150 mL blood bags. One bag was placed at 4°C storage without irradiation (Control), the second was irradiated and then immediately placed at 4°C (IRR). The third bag was irradiated and given an 8-hour room temperature incubation before storage at 4°C (IRR-22). The fourth bag was irradiated and placed in a 37°C incubator for 2 hours before 4°C storage (IRR-37). Supernatant K+ of the unit was measured before splitting and from each bag on days 1, 2, 4, 7, 14, 21, 28 and 35.

**Results**

**STUDY A**

The mean K+ levels are shown in table I and figure 1; mean supernatant hemoglobin levels are shown in table II. Oxygenated, irradiated RBC (IRR + $O_2$) had 20 percent higher extracellular K+ levels than non-oxygenated irradiated (IRR) RBC ($p < 0.01$) throughout the storage period. The supernatant hemoglobin was increased between these two groups as well. Addition of $O_2$ to non-irradiated RBC (+$O_2$) did not result in a change of K+ efflux.

**STUDY B**

The mean K+ levels over the storage period are shown in table III and figure 2; mean supernatant hemoglobin levels

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†† Fenwal Laboratories, 1425 Lake Cook Road, Deerfield, IL 60015.
### TABLE I

Study A and Study B

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>IRR</th>
<th>+O₂</th>
<th>O₂ + IRR</th>
<th>Day</th>
<th>Control</th>
<th>IRR</th>
<th>IRR-22</th>
<th>IRR-37</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>6.5 ± 2.6</td>
<td>5.6 ± 2.0</td>
<td>5.7 ± 1.4</td>
<td>7.5 ± 2.4</td>
<td>0</td>
<td>3.2 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>3.2 ± 0.2</td>
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<tr>
<td>1</td>
<td>8.6 ± 2.4</td>
<td>17.7 ± 2.1</td>
<td>7.6 ± 1.0</td>
<td>22.3 ± 2.6</td>
<td>1</td>
<td>5.0 ± 0.4</td>
<td>8.2 ± 0.7</td>
<td>5.6 ± 0.4</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>12.8 ± 2.0</td>
<td>31.0 ± 3.0</td>
<td>12.1 ± 0.6</td>
<td>37.3 ± 3.9</td>
<td>2</td>
<td>7.3 ± 0.3</td>
<td>13.3 ± 0.7</td>
<td>9.9 ± 0.8</td>
<td>10.2 ± 0.6</td>
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<td>8</td>
<td>17.4 ± 1.6</td>
<td>37.0 ± 3.1</td>
<td>15.9 ± 0.4</td>
<td>44.8 ± 4.5</td>
<td>4</td>
<td>11.1 ± 1.7</td>
<td>20.6 ± 2.3</td>
<td>15.3 ± 2.1</td>
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<td>15</td>
<td>23.2 ± 1.8</td>
<td>43.3 ± 3.3</td>
<td>22.4 ± 0.8</td>
<td>51.1 ± 4.6</td>
<td>7</td>
<td>13.7 ± 2.1</td>
<td>25.8 ± 3.8</td>
<td>19.2 ± 2.8</td>
<td>20.2 ± 3.4</td>
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<tr>
<td>22</td>
<td>30.9 ± 2.4</td>
<td>49.6 ± 3.5</td>
<td>29.0 ± 1.1</td>
<td>60.4 ± 6.3</td>
<td>14</td>
<td>18.0 ± 2.1</td>
<td>31.1 ± 2.2</td>
<td>25.7 ± 2.1</td>
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<td>28</td>
<td>38.0 ± 2.3</td>
<td>53.0 ± 3.3</td>
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<td>64.0 ± 3.2</td>
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<td>21.7 ± 2.1</td>
<td>35.4 ± 2.3</td>
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<td>32.3 ± 2.4</td>
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<tr>
<td>35</td>
<td>41.0 ± 2.0</td>
<td>54.0 ± 3.6</td>
<td>39.0 ± 1.2</td>
<td>65.0 ± 4.2</td>
<td>28</td>
<td>24.9 ± 1.9</td>
<td>37.7 ± 2.5</td>
<td>34.6 ± 2.2</td>
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<td>42</td>
<td>44.0 ± 1.9</td>
<td>55.0 ± 3.1</td>
<td>42.0 ± 1.0</td>
<td>66.0 ± 3.5</td>
<td>35</td>
<td>27.9 ± 2.2</td>
<td>39.9 ± 2.7</td>
<td>37.8 ± 3.0</td>
<td>38.7 ± 2.4</td>
</tr>
</tbody>
</table>

**Study A** — Supernatant potassium (mmol/L) in irradiated red blood cells with and without prior oxygenation compared to non-irradiated controls.

**Study B** — Supernatant potassium levels (mmol/L) of whole blood given post-irradiation incubations at 22° C and 37° C prior to 4° C storage compared to non-irradiated and irradiated units placed immediately into 4° C storage.

Results expressed as Mean ± S.D.

IRR = irradiated.

+O₂ = oxygenated.

O₂ + IRR = irradiated and oxygenated.

IRR–22 = irradiated, then incubated at 22° C.

IRR–37 = irradiated, then incubated at 37° C.
Fig 1. The effect of prestorage irradiation (IRR), addition of O\textsubscript{2} (NONIRR + O\textsubscript{2}), or addition of O\textsubscript{2} with irradiation (IRR + O\textsubscript{2}) on supernatant potassium compared to Control throughout 42 days of storage at 4°C.

![Graph](image)

are shown in table IV. The post-irradiation incubations at both 22°C and 37°C significantly reduced the efflux of intracellular K\textsuperscript{+} from the RBC during the first 7 days of storage when compared to those irradiated and placed at 4°C (p < 0.0001). Despite the short term improvement, by day 35, IRR-22 and IRR-37 had attained the same mean extracellular K\textsuperscript{+} levels as the IRR units. Supernatant hemoglobin levels were significantly higher at 35 days in the IRR-22 group (p = 0.035) and the IRR-37 group (p = 0.002) when compared to the IRR group.

**Discussion**

Irradiation of cellular blood components is the only method known to avert TA-GVHD.\textsuperscript{1,16} The number of RBC undergoing irradiation has continued to increase since a survey conducted in 1989 determined that more than 10 percent of blood components were being irradiated prior to transfusion.\textsuperscript{1} After the survey was conducted, a Standard published by the AABB suggested that all cellular blood components from first-

**TABLE II**

<table>
<thead>
<tr>
<th>Study A</th>
<th>Study B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>198 ± 21</td>
</tr>
<tr>
<td>IRR</td>
<td>390 ± 37</td>
</tr>
<tr>
<td>+ O\textsubscript{2}</td>
<td>212 ± 26</td>
</tr>
<tr>
<td>O\textsubscript{2} + IRR</td>
<td>454 ± 42</td>
</tr>
</tbody>
</table>

**Study A** — Supernatant hemoglobin (mg/dL) after 42 days storage in irradiated red blood cells with and without prior oxygenation compared to non-irradiated controls.

**Study B** — Supernatant hemoglobin (mg/dL) after 42 days storage from whole blood given post-irradiation incubations at 22° C and 37° C prior to 4° C storage compared to non-irradiated and irradiated units placed immediately into 4° C storage.

Results expressed as Mean ± S.D.

IRR = irradiated.

+ O\textsubscript{2} = oxygenated.

O\textsubscript{2} + IRR = oxygenated, then irradiated.

IRR-22 = irradiated, then incubated at 22° C.

IRR-37 = irradiated, then incubated at 37° C.
degree relatives should be irradiated prior to transfusion. Although the transfusion of irradiated blood is thought to be safe, numerous studies have affirmed that irradiated RBC have an increased concentration of extracellular K+. The results of this study confirm these observations (table I and table III).

Only 12 percent of hospital blood banks and transfusion services have the capability for irradiating blood. Because of changes in clinical circumstances or postponement of surgery, irradiated RBC may undergo considerable storage prior to transfusion. The increased accumulation of supernatant K+ and hemoglobin in these units does not appear to be clinically important except when given in large volumes to small children.

While ubiquitous and of primary importance in cell function in its normal state, oxygen can also exist as a free radical generated from the interaction of a gamma ray with a molecule of oxygen or water. The RBC components contain relatively low concentrations of O₂ since they are collected by venous phlebotomy, but numerous components have been seen in our laboratory that obviously contain oxyhemoglobin. These products usually have been manipulated by the sterile addition of satellite blood bags so the RBC may be subdivided for use by neonates and children. Our findings of increased K+ efflux and release of hemoglobin from irradiated RBC in the presence of increased oxygen confirm earlier observations.

The intracellular concentration of K+ in fresh blood is 102 mmol/L RBC. In Study B, the whole blood had a mean hematocrit of 35 percent (data not shown); the calculated level at which intracellular and extracellular K+ concentration would have been in equilibrium is 38 mmol/L. The three sets which had been irradiated ultimately reached this equilibrium despite less leakage in the IRR-22 and IRR-37 groups during early storage.

The difference seen during the first week may reflect membrane repair or the continued activity by the Na-K pump which maintained intracellular K+ during the initial room temperature or 37°C.
incubation period. The pump is inhibited at 4°C so passive loss of K+ would no longer be compensated once the blood was refrigerated. If the Na-K pump compensated the K+ efflux during the initial incubations, it would be expected that the slope would match the irradiated control once the units were placed at 4°C. The slope of the rise in K+ concentration during the period day 0 to day 4 for the IRR-22 and IRR-37 units, the irradiated control, and non-irradiated control are 3.1, 3.2, 4.2, and 2.1, respectively (data not shown). This difference in the rate of K+ leakage indicates that some repair of the membrane is probably the reason for the early reduced extracellular K+ of the test arms of the study compared to the irradiated control. A similar conclusion was also reached by Brugnara and Churchill when maximal activities of the Na-K pump, the Na-K-Cl cotransport system, and K-Cl cotransport system were measured in irradiated RBC.4

While the effect these modifications have on the lymphocyte’s ability to proliferate after irradiation was not within the scope of these studies and adequate evidence of efficacy would need to be documented prior to any change in current practice, these investigations suggest that the physical conditions associated with blood irradiation are important considerations in developing and modifying procedures for clinical practice.

References