THE EFFICACY of di-2-ethylhexyl phthalate (DEHP) as a plasticizer in polyvinyl chloride (PVC) formulations has resulted in the widespread use of this compound in modern medical products. Since DEHP is not covalently bound within the PVC matrix, it may diffuse out of the plastic. When blood is stored in contact with DEHP-plasticized PVC, this lipophilic plasticizer accumulates in various components in a time- and temperature-dependent process. While most of the plasticizer is found in plasma, a proportion (approximately 5% to 10%) is associated with RBCs when whole blood is stored under typical blood bank conditions.

Recent in vitro studies have demonstrated that the storage of RBCs in the presence of DEHP results in a better retention of normal morphology, enhanced osmotic stability, and lower hemolysis than is observed during storage in the absence of this plasticizer. However, in none of the studies reported to date was the effect of DEHP on in vivo RBC survival investigated. The experiments reported here were performed to address this issue and to ascertain whether the previous in vitro observations were indicative of a clinically relevant improvement in RBC function.

MATERIALS AND METHODS

Research subjects entered the study with approval of the human use committees of the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH); the University of Rochester, and the New York State Department of Health; and after giving informed consent. All donors met the requirements of the American Association of Blood Banks and of the American Red Cross Blood Services with the exception that deferrals related to the transmission of infectious agents were waived.

This study was conducted in three phases (Table 1). In each phase the effect of storage containers on red cells was analyzed in a paired fashion, with each subject donating blood for storage in containers with and without DEHP. At each donation 450 ± 45 mL blood were collected into a collection vessel containing 63 mL of citrate-phosphate-dextrose-adenine-1 (CPDA-1) anticoagulant contained in polyvinylchloride (PVC) bags plasticized with DEHP or a trimellitate compound that is known to have low leachability. Aliquots of RBCs from each container were then labeled with chromium-51 and were reinfused into the original donors. For blood stored in DEHP-plasticized PVC bags, 24% more red cells survived in vivo 24 hours after reinfusion than was observed when the blood had been stored in trimellitate-plasticized bags (P < .001). Whole blood stored in glass bottles showed a similar improvement in in vivo survival when DEHP was added in weekly increments to mimic the accumulation of this plasticizer seen during storage in plastic containers. Survival of packed red cells stored in the presence of DEHP increased by 14% compared with storage in trimellitate-plasticized bags (P < .05). In agreement with previous studies, hemolysis and microvesicle formation were also reduced in the presence of DEHP. These results suggest that proposed new storage systems lacking DEHP should be carefully evaluated to determine whether adequate post-transfusion survival of RBCs may be achieved.

This is a US government work. There are no restrictions on its use.
EFFECT OF DEHP ON RED CELL SURVIVAL

Table 1. Experimental Protocol

<table>
<thead>
<tr>
<th>Phase</th>
<th>Component Stored</th>
<th>Group</th>
<th>Container*</th>
<th>Additions</th>
<th>Subjects Participating</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Whole blood</td>
<td>A†</td>
<td>PVC-TEHTM‡</td>
<td>None</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>Whole blood</td>
<td>B</td>
<td>PVC-DEHP§</td>
<td>None</td>
<td>5†</td>
</tr>
<tr>
<td>III</td>
<td>Packed red cells</td>
<td>C†</td>
<td>Glass</td>
<td>None</td>
<td>5†</td>
</tr>
<tr>
<td></td>
<td>(Hematocrit: 75%)</td>
<td>D</td>
<td>DEHP‡</td>
<td>None</td>
<td>8</td>
</tr>
</tbody>
</table>

*All blood collected into CPDA-1 anticoagulant and stored for 35 days. Blood collected directly into final storage container. Approximately 60 mL of blood removed on days 0 and 35 for analytic determinations.
†Blood drawn from donor using an aluminum-hubbed needle and polyethylene tubing. Standard blood-drawing sets used for other collections.
‡PVC bag plasticized with tri-2-ethylhexyltrimellitate, a plasticizer of low leachability and red cell effect (PL 1240; Fenwal, Deerfield, IL).24,21
§PVC bag plasticized with di-2-ethylhexyl phthalate (PL 146; Fenwal).
||Sterile, vented 1-L glass bottles (Travenol, Deerfield, IL). Bacterial cultures performed on day 28.
†These subjects also participated in phase I. Two system D units became bacterially contaminated and were not used.

Table 2. Effect of DEHP on Post-Transfusion Survival of Red Cells

<table>
<thead>
<tr>
<th>Storage System</th>
<th>Container Type</th>
<th>Subjects Studied</th>
<th>24-h Survival (%)</th>
<th>T₉₀ (0-10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>PVC-TEHTM (PL 1240)</td>
<td>10</td>
<td>69.9 ± 2.99</td>
<td>32.4 ± 5.16</td>
</tr>
<tr>
<td>B</td>
<td>PVC-DEHP (PL 146)</td>
<td>84.1 ± 2.13</td>
<td>76.8 ± 12.4</td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Glass, No DEHP</td>
<td>3</td>
<td>73.3 ± 2.69</td>
<td>20.5 ± 2.50</td>
</tr>
<tr>
<td>D</td>
<td>Glass + DEHP</td>
<td>81.7 ± 2.23</td>
<td>22.7 ± 4.56</td>
<td></td>
</tr>
<tr>
<td>Phase III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>PVC-TEHTM (PL 1240)</td>
<td>8</td>
<td>66.5 ± 3.29</td>
<td>42.7 ± 8.32</td>
</tr>
<tr>
<td>F</td>
<td>PVC-DEHP (PL 146)</td>
<td>75.8 ± 1.87</td>
<td>61.1 ± 16.1</td>
<td></td>
</tr>
</tbody>
</table>

T₉₀ (0-10 min) is the time taken for survival to fall to 50% of original value according to regression line for survival curve during first ten minutes after reinfusion.
*Pairs statistically different by paired t test, P < .001.
†Pairs statistically different by paired t test, P < .05.

RESULTS

RBCs stored in systems containing DEHP exhibited better survival after transfusion than did red cells stored in similar containers that lacked DEHP (Table 2). After storage for 35 days as whole blood, red cells stored in PVC bags with DEHP (system B) exhibited a 24-hour post-transfusion survival of 84.1% ± 2.13%, 17% higher than that of red cells stored as whole blood in PVC bags lacking DEHP (system A: 69.9% ± 2.99%, P < .001). Only three pairs of data from phase II could be analyzed because of inadvertent bacterial contamination of two system D units. Despite this small sample, storage of whole blood in glass bottles to which DEHP was added (system D) resulted in a 24-hour red cell survival, 81.7% ± 2.23%, that was statistically significantly higher than storage in system C, which lacked DEHP.

...
The rate of red cell disappearance from circulation from ten
donors after storage in each container type. Note
that difference in survival at 24 hours
is quantitatively apparent within the first ten minutes after reinfusion. Vertical bars repres-
ent SEM; asterisks indicate statistical significance, P < .05.

Red cells from phase III systems were tested for the
presence of C3 on their surface by a direct antiglobulin test
using anti-C3 after 35 days of storage. All samples from
systems E and F were negative.

The paired in vitro study of red cells stored in PVC with or
without DEHP and then subjected to radiolabeling and in
vitro elution estimation demonstrated similar uptake and
elution of the 51Cr label regardless of the plastic formulation
used to store the red cells (Table 3).

Also in agreement with previous studies, the presence of
DEHP did not alter the disappearance of ATP from red cells
during the storage period. It did mitigate the normal increase
of plasma hemoglobin accumulation, however (Table 4). The
relative increase in plasma hemoglobin concentration in
blood stored without DEHP was accompanied by an increase
in microvesicle content, but the latter could not quantita-
tively account for the total increment of plasma hemoglobin
concentration.

DISCUSSION

The post-transfusion survival of RBCs stored under refriger-
ation for 35 days in CPDA-1 anticoagulant in DEHP-
plasticized PVC containers was an average 17% to 24% higher than the survival of cells stored in containers plasticiz-
ed with TEHTM. Enhanced survival was also observed
when DEHP was added to whole blood stored in glass bottles,
offering further evidence that the improved post-transfusion
survival was indeed attributable to the presence of plasticizer.
This improvement was not due to an effect of DEHP on
51Cr uptake or elution, since there was no difference in the
efficiency of uptake or rate of elution of the radiolabel after
storage with or without DEHP.

The smaller effect of DEHP on red cells stored in glass
bottles compared with PVC containers is probably related to
the relatively lower concentration of DEHP achieved in the
glass containers. However, a confounding, detrimental effect
of glass per se on RBCs during storage cannot be excluded in
light of the historic evidence for such a phenomenon. The
lower DEHP concentration achieved on addition to glass

Table 3. Effect of DEHP on Red Cell Uptake and Elution of 51Cr in Vitro

<table>
<thead>
<tr>
<th>Storage Container</th>
<th>Proportion of Radioactivity Eluting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>PVC-TEHTM (PL1240)</td>
<td>96.9% ± 0.36%</td>
</tr>
<tr>
<td>PVC-DEHP (PL146)</td>
<td>96.4% ± 0.51%</td>
</tr>
</tbody>
</table>

Whole blood from 11 single donations was split between PVC bags lacking (PL1240) or containing (PL146) DEHP and stored for 35 days in CPDA-1. After 51Cr labeling, red cells were held in AB plasma at 37°C, and plasma radioactivity was determined as a percentage of the radioactivity originally taken up by the red cells. There were no statistically significant differences in the uptake or loss of the radiolabel between red cells stored in the two bag types.
bottles may have been due to hydrolysis of the phthalate ester during the incubation of plasma at 37°C necessary to effect rapid plasticizer dissolution. This method of plasticizer dissolution was selected over incubation of plasma in PVC-DEHP bags to avoid dissolution of other components of the plastic bag that might leach into plasma.)

As has been demonstrated in other in vitro studies, the presence of DEHP in clinically relevant quantities does not directly affect the internal metabolism of red cells. We documented no effect of DEHP on ATP levels; previous studies have also shown that 2,3-DPG levels and intracellular electrolyte concentrations are not affected by the presence of DEHP.

While other mechanisms cannot be completely excluded, the enhanced post-transfusion survival of red cells stored in the presence of DEHP probably reflects a protective effect of this compound with regard to the deterioration of the cell membrane. Previous in vitro studies have demonstrated that RBCs stored in the presence of DEHP exhibit more normal morphology, less hemolysis, lower osmotic fragility, and better filterability than do comparable cells stored in the absence of this plasticizer. Each of these parameters is believed to reflect the integrity of the cell membrane. Cell morphology, in particular, has been correlated closely with in vivo cell survival. The results reported in this communication demonstrate that the improvement observed during RBC storage in the presence of DEHP as assessed by in vitro measurements is indicative of an improvement in vivo cell survival.

It is interesting to note that improved RBC survival due to the presence of DEHP is manifested in blood storage systems that are currently in widespread use. This suggests that the removal of DEHP from such systems could result in decreased red cell post-transfusion survival if no compensatory additives were used to replace the protective effects of DEHP. This in turn suggests that cell viability should be carefully evaluated in any new storage system in which DEHP is eliminated or reduced.

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REFERENCES

1. Graham PR: Phthalate ester plasticizers—Why and how they are used. Environ Health Perspect 3:3, 1973
7. Sasakawa S, Mitomi Y: Di-2-ethylhexylphthalate (DEHP) content of blood or blood components stored in plastic bags. Vox Sang 34:81, 1979
The effect of the plasticizer di-2-ethylhexyl phthalate on the survival of stored RBCs

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