Characterization of Erythrocyte Quality During the Refrigerated Storage of Whole Blood Containing Di-(2-Ethylhexyl) Phthalate

By Timothy N. Estep, Robin A. Pedersen, Theresa J. Miller, and Kathleen R. Stupar

Di-(2-ethylhexyl) phthalate (DEHP) accumulates in blood brought into contact with materials utilizing this compound as a plasticizer. To determine whether this phthalate diester affects red blood cell integrity, we have compared cell morphology, plasma hemoglobin accumulation, microvesicle production, and the concentration of intracellular metabolites and electrolytes of erythrocytes from blood stored at 4 °C with and without DEHP. When sufficient emulsified DEHP was mixed with blood to give a final concentration of 300 μg/mL, plasma hemoglobin accumulation was reduced by an average of 70%, the percentage of cells exhibiting normal morphology was enhanced by at least 20-fold, and the volume of microvesicles released from red blood cells was reduced by 50% after 35 days of refrigerated storage compared to the values obtained from corresponding samples stored without added phthalate.

THE ACCUMULATION of phthalate esters in blood stored or circulated in contact with plastic was first reported over a decade ago, stimulating a widespread interest in the possible biologic effects of these compounds.1,2 Particular attention has been focused on the pharmacokinetics and toxicology of di-(2-ethylhexyl) phthalate (DEHP) because of its extensive use as a plasticizer in medical devices and blood storage systems.3,4 For example, Jaeger and Rubin found that DEHP is extracted into whole blood at a rate of 0.25 mg/100 mL blood/d during storage at 4 °C in polyvinylchloride containers formulated with this compound.5 Subsequent studies from a variety of laboratories have dealt with the distribution of DEHP among blood components, the effect of this phthalate diester on platelet function and survival, and the metabolism and toxicity of DEHP in vivo.5,6 On the other hand, there is a dearth of information concerning the biologic effects of DEHP on erythrocytes. To address this situation, we have assessed the effect of DEHP on a number of parameters that are commonly employed to evaluate the integrity of erythrocytes during the storage of whole blood.

MATERIALS AND METHODS

Whole blood was collected from normal human volunteers into citrate-phosphate-dextrose (CPD) anticoagulant after obtaining informed, written consent. In general, blood was collected and stored in containers that were free of DEHP. To assess the effect of plasticizer on red blood cells, aliquots of concentrated DEHP emulsions, concentrated emulsifier suspensions, or simple buffer solutions were aseptically mixed with blood during or prior to refrigerated storage. Addition volumes were less than or equal to 10% of the blood volume. Samples were stored at 4 ± 1 °C.

DEHP (Eastman, Rochester, NY; 99% purity) was emulsified by mixing with an equal weight of solubilizing agent, followed by mechanical suspension in isosmotic sodium phosphate buffer solution, pH 7.4. Unless otherwise noted, the solubilizing agent was a 3:1 (wt/wt) mixture of Tween 80 (Matheson, Coleman and Bell, Norwood, Ohio) and Pluradot HA 410 (BASF, Wyandotte, Mich). The crude DEHP emulsion was further dispersed by ultrasonic irradiation for a total of eight minutes using a probe sonifier and centrifuged at 6,500 g for ten minutes to pellet particulates. The same procedure was followed when egg phosphatidylcholine (Sigma, St Louis) or sodium deoxycholate (Eastman) were used as emulsifying agents. Solutions of emulsifier alone or emulsifier and mono-(2-ethylhexyl) phthalate (MEHP) and/or 2-ethylhexanol (EH) were prepared by mechanical suspension in isosmotic phosphate buffer solution.

Plasma hemoglobin concentrations were determined in samples centrifuged twice at 1,000 g for five minutes by the measurement of pseudoperoxidase activity using tetramethylbenzidine, as described by Crosby and Furth7 as modified by Geissler and Stith,8 or by the modified cyanmethemoglobin procedure described by Moore et al9 using the turbidity correction procedure of Kandler et al.10

Erythrocyte shape was assessed by light microscopy by fixing 20 μL of well-mixed blood at room temperature for two hours with 0.5 mL of a solution containing 1.4% glutaraldehyde, 115 mM NaCl, 13 mM Na2HPO4, 13 mM glucose, 5 mM NaH2PO4, 5 mM KCl, 1 mM KH2PO4, 1 mM MgCl2, and 0.7 mM MgSO4, pH 7.4. At least 1,000 cells from each fixed specimen were classified as being of normal morphology (discocyte or echinocyte I in the Bessis nomenclature), crenated (echinocytes II or III and spherocinocytes I or II), or spherical (spherocytes).11 For examination by scanning electron microscopy,
Erythrocytes fixed as described above were sequentially washed by centrifugation at 400 g for one minute in buffer without fixative and in 50%, 75%, 95%, and 100% (2×) ethanol solutions. Approximately 10 μL of cell suspension in 100% ethanol was then air dried on a glass coverslip, which was attached to a planchet with a conductive adhesive, and gold coated. Scanning electron microscopy (SEM) examination was performed at 25 kV with a JEOL JXA-35 scanning electron microscope.

Microvesicle formation was assessed in plasma samples from which the formed elements were removed by centrifugation at 1,000 g for four minutes (2×). Microvesicles were pelleted by centrifugation at 50,000 g for two hours at 4 °C and resuspended in 0.5 or 1.0 mL of saline-phosphate buffer solution. The hemoglobin content of both supernatant and pellet suspensions was measured by the plasma hemoglobin assay methods described above.

Erythrocyte metabolites and electrolytes were measured on cells washed three times by centrifugation and resuspension in one to two volumes of 0.161 mol/L tetraethylammonium chloride. Preliminary experiments indicated that this procedure removed 95% of the flame photometry. The concentration of adenosine triphosphate (ATP) and washing three times by centrifugation and resuspension in one to two volumes of 0.161 mol/L tetraethylammonium chloride. Preliminary experiments indicated that this procedure removed 95% of the leukocytes originally present and essentially all of the remaining platelets. Aliquots of washed cell suspensions were assayed for total hemoglobin content by means of a commercial kit (Unopette, Becton Dickinson, Rutherford, NJ) based on the cyanmethemoglobin method, for hemocytocentrifugation at 13,000 g for four minutes in capillary tubes, and for sodium and potassium content by flame photometry. The concentration of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) were measured in trichloroacetic acid (TCA) extracts of cell suspensions by means of purchased kits (Sigma) based on the procedures of Bucher as modified by Adams (ATP) and Lowry et al (2,3-DPG). Phosphofructokinase (PFK) activity was measured in frozen-thawed hemolysates prepared and assayed as recommended by Beutler.

The DEHP content of whole blood was assessed in chloroform:methanol extracts by gas-liquid chromatography (GLC) as described by Miripol and Stern using a WCOT OV 101 glass capillary column, hydrogen carrier gas at a flow rate of 1 mL/min, and detector, injector, and column temperatures of 300, 290, and 180 °C, respectively.

Data were analyzed by applying the two-tailed Student’s t test to the average of differences between paired control and test values. This paired analysis was necessitated by the large donor-to-donor variability that we observed in a variety of parameters measured on blood samples that were drawn, manipulated, and stored in an identical manner. Such variability has previously been reported by others. P values less than 0.05 were considered statistically significant.

RESULTS

When DEHP solubilized with Tween 80:Pluradot HA410 was added to whole blood contained in non-plasticized polypropylene tubes, there was an inhibition of plasma hemoglobin accumulation and enhanced preservation of normal erythrocyte morphology during subsequent storage at 4 °C for 35 days (Figs 1 and 2). Both effects varied in a biphasic manner as a function of DEHP concentration and were maximal when 400 μg/mL phthalate diester was added to blood. Addition of this concentration of DEHP to blood resulted in an average reduction of plasma hemoglobin accumulation of 70% and more than a 20-fold improvement in the fraction of discocytes relative to buffer control samples after 35 days of refrigerated storage. In the absence of DEHP, the addition to blood of 400 μg/mL emulsifier resulted in only a moderate decrease in plasma hemoglobin accumulation (<25%) and a negligible inhibition of erythrocyte shape change (<twofold) relative to buffer control samples, although both parameters were altered significantly (P < .01) in the presence of higher concentrations (800 μg/mL) of solubilizing agent.

Measurement of plasma hemoglobin accumulation and erythrocyte shape as a function of time revealed that inhibition of the former by 300 μg/mL emulsified DEHP became evident after 14 days of storage, while differences in cell shape were apparent within a single day (Table 1). In both cases, the observed differences

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**Fig 1.** Accumulation of hemoglobin in the plasma of whole blood stored for 35 days at 4 °C in polypropylene tubes. Prior to storage, either sodium phosphate buffer solution (A), buffer solution with various amounts of Tween:Pluradot emulsifier (B), or buffer solution with emulsified DEHP (E) was added to blood. Each point represents the average ± SD of six samples. By paired comparison with buffer control samples, the lower average plasma hemoglobin concentration observed in blood containing added DEHP was statistically significant at all phthalate levels tested (P < .05 at 50 μg/mL DEHP; P < .01 at 100 to 1,600 μg/mL). By paired comparison with samples containing equivalent amounts of emulsifier, the lower average plasma hemoglobin concentration observed in samples containing added DEHP was statistically significant in blood containing 100 to 400 μg/mL phthalate (P < .02).

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**Fig 2.** Fraction of erythrocytes exhibiting normal morphology in whole blood stored for 35 days at 4 °C in polypropylene tubes. Prior to storage, either sodium phosphate buffer solution (A), buffer solution with various amounts of emulsifier (B), or buffer solution with emulsified DEHP (E) was added to blood. Each point represents the average ± SD of six samples. By paired comparison with both buffer control and emulsifier control samples, the preservation of normal erythrocyte morphology was significantly enhanced in blood containing 100 (P < .05), 150 (P < .02), 200 (P < .01), 400 (P < .0001), and 800 (P < .001) μg/mL DEHP.
Table 1. Plasma Hemoglobin Concentration and Erythrocyte Morphology in Whole Blood as a Function of Storage Duration at 4 °C With and Without 300 µg/mL Emulsified DEHP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blood Additive</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Plasma hemoglobin</td>
<td>Buffer solution</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>concentration (mg/dL)</td>
<td>Buffer solution + 300 µg/mL emulsifier</td>
<td>5 ± 1</td>
</tr>
<tr>
<td></td>
<td>Buffer solution + 300 µg/mL emulsifier + 300 µg/mL DEHP</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Percent cells of</td>
<td>Buffer solution</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>normal morphology</td>
<td>Buffer solution + 300 µg/mL emulsifier</td>
<td>38 ± 11</td>
</tr>
<tr>
<td></td>
<td>Buffer solution + 300 µg/mL emulsifier + 300 µg/mL DEHP</td>
<td>74 ± 2</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD of three samples stored in polypropylene tubes.

increased as storage progressed. The differences in cell morphology were particularly apparent upon sample examination by scanning electron microscopy, as shown by the micrographs illustrated in Fig 3.

Through most of the storage period the majority of abnormally shaped cells were of a spiculate form, either echinocytes or spheroechinocytes. After 35 days, however, the percentage of spherical cells averaged 11 ± 8 (n = 11) and 9 ± 9 (n = 11) in samples containing added buffer solution or emulsifier solution, respectively—values that were higher (P < .03) than the average of 2 ± 2 (n = 11) found in blood to which DEHP was added.

The inhibition of plasma hemoglobin accumulation

![Fig 3. Scanning electron micrographs of erythrocytes isolated from whole blood stored at 4 °C with isoosmotic sodium phosphate buffer solution (A,B); buffer solution with sufficient Tween:Pluradot emulsifier to give a final concentration of 300 µg/mL (C,D); or buffer solution with sufficient emulsified DEHP to give a final concentration of 300 µg/mL phthalate (E,F). The erythrocytes were from blood stored for one (A,C,E) or 21 (B,D,F) days. The scale of all micrographs is equal and is indicated by a 10-µm bar in A.](#)
EFFECT OF DEHP ON ERYTHROCYTES

50% concentration was at least the utilization of a particular emulsifying agent. After and red blood cell shape change was not dependent on the utilization of a particular emulsifying agent. After 35 days of 4 °C storage, the plasma hemoglobin concentration was at least 50% lower and the percentage of normally shaped erythrocytes at least sevenfold higher in blood containing 300 μg/mL DEHP solubilized with either Tween:Pluradot, sodium deoxycholate, or egg phosphatidylcholine (egg PC) as compared to control samples containing added buffer solution or 300 μg/mL emulsifier. The erythrocyte stability observed in the presence of DEHP was also not attributable to the hydrolysis products mono-(2-ethylhexyl) phthalate (MEHP) or 2-ethylhexanol (EH) (Table 2). When sufficient MEHP and/or EH were added to blood to simulate the degree of hydrolysis typically observed during 35 days of storage (~20% of a 300 μg/mL dose of DEHP as assessed by GLC), there was a 17% to 22% reduction in plasma hemoglobin accumulation relative to the emulsifier control samples, compared to the corresponding 66% decrement observed when DEHP was added. Moreover, there was no improvement in the preservation of normal erythrocyte morphology in the presence of MEHP and/or EH in contrast to the marked differences observed in samples containing the intact phthalate diester.

Table 2. Plasma Hemoglobin Accumulation and RBC Morphology in Whole Blood Stored for 35 Days at 4 °C With Emulsified DEHP or DEHP Hydrolysis Products

<table>
<thead>
<tr>
<th>Blood Additive</th>
<th>Plasma Hemoglobin Content (mg/dL)</th>
<th>Percent Cells of Normal Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer solution</td>
<td>142 ± 35</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier</td>
<td>99 ± 37</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier + 60 μg/mL MEHP</td>
<td>82 ± 27</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier + 30 μg/mL EH</td>
<td>77 ± 6</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier + 60 μg/mL MEHP + 30 μg/mL EH</td>
<td>78 ± 33</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier + 300 μg/mL DEHP</td>
<td>34 ± 13</td>
<td>43 ± 17</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD of three samples stored in polypropylene tubes.

Besides data obtained from samples stored in non-plasticized polypropylene tubes, we also performed experiments utilizing PVC packs. The addition of 300 μg/mL emulsified DEHP to samples stored in tri-(2-ethylhexyl) trimellitate (TEHTM) plasticized PVC bags was found to effect an inhibition of plasma hemoglobin accumulation and erythrocyte shape change similar to that detected during blood storage in polypropylene tubes (Table 3). Furthermore, when matched blood samples were stored for 35 days at 4 °C in transfer packs formulated from PVC plasticized with TEHTM or DEHP, the average plasma hemoglobin concentration was reduced from 69 ± 22 mg/dL to 32 ± 14 mg/dL (P < .01, n = 6) and the fraction of normally shaped erythrocytes remaining after storage improved from 2% ± 1% to 11% ± 2% (P < .05, n = 3) in the latter as compared to the former.

Correspondence between results obtained during storage in PVC packs and polypropylene tubes was also demonstrated during experiments designed to test whether or not the addition of DEHP to blood could reverse erythrocyte shape changes that had already occurred (Fig 4). In these studies, matched blood samples were stored with and without 300 μg/mL emulsified DEHP in TEHTM plasticized PVC transfer packs and, after two weeks at 4 °C, aliquots of the control samples were transferred to polypropylene tubes. Immediately after transfer, half of the samples placed in polypropylene tubes were admixed with buffer solution, the other half being combined with sufficient emulsified DEHP to yield a final concentration of 300 μg/mL phthalate. While there was no difference in the fraction of normally shaped cells present in control samples transferred to polypropylene tubes and admixed with additional buffer as compared to their counterparts retained in PVC packs, the addition of emulsified DEHP to transferred control samples resulted in a reversion of approximately half of the echinocytes present to the discocyte morphology. The extent of this reversion was such that the resulting overall population shape distribution was similar to that found in blood stored in PVC packs with emul-

Table 3. Plasma Hemoglobin Content and Erythrocyte Morphology After the Refrigerated Storage of Whole Blood for 35 Days in TEHTM Plasticized Packs With and Without 300 μg/mL Emulsified DEHP

<table>
<thead>
<tr>
<th>Blood Additive</th>
<th>Plasma Hemoglobin Content (mg/dL, Mean ± SD)</th>
<th>Percent Cells of Normal Morphology (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer solution</td>
<td>71 ± 32 (n = 3)</td>
<td>4 ± 1 (n = 3)</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier</td>
<td>51 ± 28 (n = 3)</td>
<td>12 ± 4 (n = 3)</td>
</tr>
<tr>
<td>Buffer solution + 300 μg/mL emulsifier + 300 μg/mL DEHP</td>
<td>32 ± 24 (n = 6)*</td>
<td>45 ± 10 (n = 6)*</td>
</tr>
</tbody>
</table>

*P < .05 by paired comparison with either buffer or emulsifier control samples.
sivated DEHP added at the outset of the experiment. After maximum reversion was achieved, the subsequent rate of decrease in the percentage of discocytes was comparable in all samples, regardless of the time at which DEHP was added or the storage container utilized.

To further explore the effect of DEHP on erythrocytes, we measured several additional parameters commonly used to assess cell integrity (Table 4). These experiments were performed on samples stored in TEHTM plasticized packs. No difference was detected in the concentration of intracellular ATP, 2,3-DPG, sodium, or potassium in matched samples stored with or without 300 μg/mL emulsified DEHP, although all of these parameters varied as a function of storage duration, as reported by others who have employed CPD as anticoagulant.21-24 There was, however, a significant decrease (P < .02) in the extent of microvesicle formation in blood containing DEHP. The latter result was confirmed in a separate experiment in which a reduction of microvesicle encapsulated hemoglobin from 12 ± 4 mg/dL to 3 ± 1 mg/dL (P < .05, n = 3) was observed upon the addition of 300 μg/mL emulsified DEHP to blood that was subsequently stored in polypropylene containers.

**DISCUSSION**

In light of the low solubility of DEHP in aqueous media, a necessary prerequisite to our experiments was consideration of methods by which various amounts of this compound could be efficiently and reproducibly introduced into blood. While DEHP may be solubilized directly into plasma by incubation with plasticized materials or coated celite, such extraction has been shown to vary as a function of temperature, incubation time, and lipoprotein content.25-27 Plasma incubation may also result in the alteration of constituent lipids and proteins which can, in turn, affect the integrity of erythrocytes.28-30 Furthermore, DEHP is subject to hydrolysis by plasma enzymes.31-34 To mitigate these difficulties, we chose to introduce DEHP into blood as part of a preformed emulsion. In this manner, a wide range of DEHP concentrations in blood was rapidly and conveniently achieved. It should be noted that when plasma is used to solubilize DEHP, one is in fact using an analogous approach, since the phthalate is primarily incorporated into lipoprotein structures that are essentially emulsions stabilized by endogenous phospholipid and protein amphiphats.25-33

**Table 4.** Effect of 300 μg/mL DEHP on Red Blood Cell Metabolites, Electrolytes, and Microvesicle Formation During the Refrigerated Storage of Whole Blood

<table>
<thead>
<tr>
<th>Parameter Value</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Additive</td>
<td>Day 1</td>
</tr>
<tr>
<td>ATP concentration</td>
<td>300 μg/mL emulsifier</td>
</tr>
<tr>
<td>(μmol/g Hb)</td>
<td>300 μg/mL emulsifier + 300 μg/mL DEHP</td>
</tr>
<tr>
<td>2,3-DPG concentration</td>
<td>300 μg/mL emulsifier</td>
</tr>
<tr>
<td>(μmol/g Hb)</td>
<td>300 μg/mL emulsifier + 300 μg/mL DEHP</td>
</tr>
<tr>
<td>Na⁺ concentration</td>
<td>300 μg/mL emulsifier</td>
</tr>
<tr>
<td>(μmol/L cells)</td>
<td>300 μg/mL emulsifier + 300 μg/mL DEHP</td>
</tr>
<tr>
<td>K⁺ concentration</td>
<td>300 μg/mL emulsifier</td>
</tr>
<tr>
<td>(μmol/L cells)</td>
<td>300 μg/mL emulsifier + 300 μg/mL DEHP</td>
</tr>
<tr>
<td>Plasma Hb in microvesicles</td>
<td>300 μg/mL emulsifier</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD of four samples. Blood was stored in transfer packs formulated from TEHTM plasticized PVC.

*P < .02 by paired comparison with control samples.
Employing emulsions of DEHP with Tween 80 and Pluradot HA410, we found that the addition of 100 to 400 µg/mL of this phthalate diester to blood prior to refrigerated storage resulted in lower levels of plasma hemoglobin accumulation, an inhibition of erythrocyte shape change, and a decrease in microvesicle formation compared to control samples that were identical save for the presence of DEHP. Similar reductions were observed in plasma hemoglobin accumulation and erythrocyte shape change when blood was stored either in polypropylene tubes or in TEHTM plasticized transfer packs. The latter result implies that TEHTM is exerting little or no protective effect on erythrocytes in our experiments. This is consistent with the fact that emulsified TEHTM is somewhat less potent on an equal weight basis than DEHP in inhibiting erythrocyte deterioration (T.N. Estep and K.R. Stupar, unpublished observations, June 1981) and that TEHTM leaches into blood at approximately 1/100 the rate of DEHP.36

In dose–response studies, we observed significant inhibition of plasma hemoglobin accumulation and erythrocyte shape change with DEHP concentrations as low as 100 µg/mL. These concentration levels are comparable to those frequently achieved during the routine storage of blood for transfusion purposes,5,6,32 suggesting that the protective effects we have detected in blood containing emulsified DEHP should be observable under normal blood bank conditions. This hypothesis was confirmed by experiments with matched blood samples stored in transfer packs plasticized with TEHTM or DEHP in which there was a statistically significant reduction in plasma hemoglobin content and erythrocyte crenation in the latter as compared to former. In addition, the differences observed in the study performed with transfer packs were comparable to those found when 100 µg/mL of emulsified DEHP was added to blood prior to storage in polypropylene tubes, indicating there is a quantitative correspondence between the effects of DEHP added to blood in the form of an emulsion or after extraction from the container material. Inhibition of plasma hemoglobin accumulation and improved osmotic stability of erythrocytes during blood storage in a variety of containers have also been reported by Stern and Carmen37 and Rock et al.38

DEHP was also found to inhibit both plasma hemoglobin accumulation and erythrocyte shape change when solubilized with sodium deoxycholate or egg PC. The significance of the results obtained with sodium deoxycholate stems from the fact that this compound actually induces echinocyte formation when added alone to washed erythrocytes,39,40 while the studies performed with egg PC are of note because this phospholipid closely resembles one of the major lipid components of the red blood cell plasma membrane, as well as the predominant plasma phospholipid.35 Egg PC is very dissimilar structurally from either Tween 80, pluradot HA410, or sodium deoxycholate. These results suggest that the protective effects of DEHP on erythrocytes are not dependent on the method by which the phthalate diester is introduced into blood.

In studies directed to the mechanism by which DEHP enhances erythrocyte storage stability, we found that the protective effects observed in the presence of intact phthalate diester were not duplicated by the addition of the hydrolysis products MEHP and EH in quantities comparable to those generated under our typical experimental conditions. This result could be attributed to a lower inherent efficacy of MEHP and EH toward the inhibition of erythrocyte deterioration and/or the greater water solubility of these substances. The latter would reduce the tendency of these compounds to associate with cells. We were also unable to detect any influence of DEHP on the intracellular concentration of ATP, 2,3-DPG, sodium, or potassium, suggesting that this phthalate derivative does not alter the intermediary metabolism or ion transport properties of erythrocytes. On the other hand, the results we have obtained are consistent with the hypothesis that the cell membrane is the major locus of DEHP interaction, since erythrocyte crenation, plasma hemoglobin accumulation, and microvesicle formation are all believed to be manifestations of a deterioration of membrane structure.11,41,42 Experiments are currently in progress to determine which membrane component may be involved in this process.

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