Impairment of Red Cell Transit through the Canine Lungs following Injections of Hypertonic Fluids

By Richard M. Effros

ABSTRACT

Rapid injections of hypertonic sucrose, sodium chloride, and urea solutions were given in the jugular veins of anesthetized dogs, and samples of blood were collected from the aorta. Following each of these injections, hemoglobin and plasma protein concentrations in the collected blood declined because fluid was extracted from the lungs. Injections of sucrose and sodium chloride, but not of urea, caused the hemoglobin concentration to fall more than the plasma protein concentration and slowed the movement of labeled red cells relative to that of labeled albumin when these two substances were injected at the same time and site as were the hypertonic solutions. The net flux of plasma through the lungs exceeded that of red cells for several seconds, indicating that plasma might pass around the more slowly moving red cells and through the smaller vessels of the lung. Impairment of red cell movement through the lungs is attributed primarily to red cell dehydration and loss of deformability. Unlike sucrose and sodium chloride, urea rapidly equilibrates between red cells and plasma and can only transiently dehydrate red cells. No impairment of red cell movement was seen after injections of hypertonic solutions of urea.

KEY WORDS: erythrocyte deformability, pulmonary vascular resistance, indicator-dilution techniques, rheology, hypertoncity, microcirculation

Rapid injections or infusions of a variety of hypertonic solutions into the pulmonary vascular inflow transiently increase pulmonary vascular resistance (1-3). However, if these same solutions are administered more slowly and in lower concentrations, pulmonary vascular resistance decreases (4). Evidence that red cells are in some way responsible for the increase in vascular resistance which follows rapid hypertonic injections was provided by Read et al. (5, 6) and Semler et al. (7), who found that removal of red cells from the perfusing fluid abolished the effect. Read et al. (8) described significant red cell clumping in the arteriolar blood of the lungs, mesentery, and pia following rapid intraarterial injections of hypertonic sodium chloride or sucrose solutions. These findings were confirmed by Brown et al. (8), who studied the effects of hypertonic radiopaque media on the mesenteric circulation and emphasized the importance of the decline in the ability of red cells to deform as well as the presence of red cell clumping.

The present study shows that injections of hypertonic sodium chloride or sucrose solutions produce a transient dissociation between the flow of plasma and the flow of red cells through the pulmonary vasculature of the dog. Impairment of red cell movement relative to plasma protein flow through the lungs was demonstrated in two ways: (1) a decline in the ratio of hemoglobin concentration to plasma protein concentration in blood leaving...
the lungs ("base-line dilution") and (2) a slowing of the transit of labeled red cells relative to labeled plasma protein when these substances were injected at the same time and site as was the hypertonic solution ("multiple-indicator dilution").

Theory

Two approaches were used to study the effect of hypertonic injections on the movement of red cells and plasma protein through the pulmonary vasculature of the dog. The first approach, base-line dilution, was based on measurements of outflow concentrations of hemoglobin and T-1824 following the test injections. The second approach, multiple-indicator dilution, was based on the transit times of labeled red cells and plasma protein through the central volume after these substances were injected at the same time and site as was the osmotic bolus.

**BASE-LINE DILUTION**

A small volume of a hypertonic solution was rapidly injected into the superior vena cava of an anesthetized dog. The injected bolus was swept along by the flow of blood into the chambers of the right heart, the pulmonary vasculature, the chambers of the left heart, and through the aorta to the sampling site near the opening of the left carotid artery. Blood was pumped from the aorta into serial sample tubes mounted on a moving rack.

Because of the hypertonic nature of the injection solution, fluid was extracted from the tissues traversed by the bolus. Extraction of tissue fluid was reflected by a decline in red cell and plasma protein concentration. (Evidence has been obtained showing that most of this fluid was derived from the pulmonary vascular bed rather than from the walls of the cardiac chambers or the great vessels. No dilution of pulmonary artery blood by tissue fluids was found after injections of hypertonic solutions into the jugular vein.) After the hypertonic solution traversed the pulmonary capillary bed, fluid was returned to the tissues from the blood which followed, and the concentrations of red cells and plasma protein increased above base-line values.

Fractional changes in the concentration of red cells in the collected blood were determined by measuring the hemoglobin concentration in whole blood. Alterations in hemoglobin concentration in whole blood must reflect proportional alterations in red cell numbers, since the amount of hemoglobin in each cell remains constant. Dilution of plasma protein by the fluid extracted from the lungs was similarly measured by following plasma protein concentration in whole blood. It must be emphasized that whole blood concentrations of hemoglobin and plasma protein are uninfluenced by shifts of fluid between red cells and plasma. Thus, alterations in these parameters primarily reflect fluid movement between the blood and the tissues rather than between the red cells and the plasma. Measurements of relative plasma protein concentrations in whole blood were facilitated by administering an intravenous loading dose of T-1824, a dye which is bound primarily to albumin (9), 30-60 minutes before the first injection of hypertonic or control solutions. Variations in T-1824 concentration in whole blood over the experimental interval were relatively slight following control injections, and it was assumed that the fractional changes in T-1824 concentration in whole blood reflected proportional changes in plasma protein concentration in whole blood produced by fluid movements between blood and tissues.

Base-line concentrations of hemoglobin and T-1824 in whole blood \((H_{gb_0} \text{ and } T-1824_0)\) were averaged, and all concentrations were factored by the base-line concentrations \((H_{gb_0}/H_{gb_0} \text{ and } T-1824_0/T-1824_0)\). These ratios \((c/c_0)\) provided a measure of proportional changes occurring in hemoglobin and T-1824 concentrations relative to base-line values. To compare the relative fall in hemoglobin concentration with the fall in T-1824 concentration, the areas \((D)\) below the base-line concentrations of these substances were calculated.

\[
D_{H_{gb}} = \sum_{a} \left[ 1 - \frac{H_{gb_1}}{H_{gb_0}} \right],
\]
\[ D_{T-1824} = \sum \left[ 1 - \left( \frac{T-1824}{T-1824_b} \right) \right], \] (2)

where \( a \) represents the first point at which a definite decline in hemoglobin was identified and \( b \) represents the point at which \( Hgb_b / Hgb \) again reached unity. The ratios of \( D_{T-1824} \) to \( D_{Hgb} \) were calculated and are presented for each indicator in Table 1.

The ratios of hemoglobin concentration to T-1824 concentration were also calculated for each sample of the base-line period and averaged \( \left[ \frac{Hgb}{T-1824} \right]_0 \). Subsequent values of \( \left[ \frac{Hgb}{T-1824} \right] \) were factored by the base-line value to obtain \( r \), where \( r = \frac{\left[ \frac{Hgb}{T-1824} \right]}{\left[ \frac{Hgb}{T-1824} \right]_0} \). Values of \( r \) provide a measure of proportional changes in hemoglobin concentration relative to protein concentration exiting from the organ compared with base-line levels.

**MULTIPLE-INDICATOR DILUTION**

A double-lumen catheter was introduced into the superior vena cava. As the hypertonic or control solution was injected through one lumen of the catheter, a small volume of a suspension of labeled red cells in an isotonic solution containing labeled albumin was injected through the other lumen. In this manner, the exposure of red cells to the hypertonic fluid occurred in vivo, and prolonged in vitro incubation of cells with very hypertonic solutions was avoided.

The activities of the injected indicators were measured in the collection samples and divided by the total activity of each in the injection volume yielding “fractional concentrations” (designated \( w \)) in units of ml\(^{-1}\).

Fractional concentrations of each indicator were plotted on a logarithmic ordinate against time on a linear abscissa, and an exponential decline was assumed to correct for recirculation. The areas (\( A \)) under each curve were calculated.

\[ A = \sum w \tau, \] (3)

where \( w \) is the fractional concentration and \( \tau \) is the time interval of each sample. The recovery (\( R \)) of each injected substance (\( X \)) relative to \(^{125}\)I-albumin in the aortic blood was also calculated.

\[ R_X = A_X / A_{125I}, \] (4)

where \( A_X \) designates the area under the indicator curve of \( X \) and \( A_{125I} \) represents the area under the indicator curve of \(^{125}\)I-albumin.

Mean transit times (\( t \)) of \(^{51}\)Cr-labeled red cells and \(^{125}\)I-albumin were calculated with the equation

\[ t = (\sum w \tau / \sum \tau) - \tau / 2 - t_{catb}, \] (5)

where \( t_{catb} \) designates the mean catheter delay time and \( n \) represents the number of the sample.

**Methods**

Mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv, and 50 mg as needed), intubated, and ventilated with a Harvard pump for the duration of the study. Intravenous injections of succinylcholine (20 mg and as needed), heparin (20,000-40,000 U.S.P. units) and T-1824 (300-500 mg in 0.154M NaCl, 30-60 minutes before the hypertonic injections were begun) were then administered. T-1824 was used as a convenient indicator of plasma protein concentration (primarily albumin) (9).

A volume of 3.5-6.0 ml of 2.02M NaCl, 2.25M sucrose, or 2.00-9.51M urea was injected within a 2-second interval through a catheter placed in the superior vena cava by way of the external jugular vein. Control injections consisted of equivalent volumes of 0.154M NaCl. U-14C-sucrose or 14C-urea (New England Nuclear) was incorporated with several of the hypertonic solutions to facilitate measurements of sucrose and urea concentrations in the collected samples (approximately 20 \( \mu \)l were injected in each experiment).

Blood was withdrawn at 4–6 ml/sec from the carotid artery with a peristaltic pump and collected in serial tubes which were changed at 1/3- or 1/2-second intervals by a moving rack collector.

**BASE-LINE DILUTION**

Whole blood was diluted 1:200 in Drabkin’s solution (10) or (as was found equivalent) in 15 mM LiCl solution containing detergent. Hemoglobin concentration was read at 540 nm in a Unicam 1800 spectrophotometer (Philips). In some studies, sodium concentration in whole blood was determined in the LiCl solution by flame photometry.

Additional samples of blood were diluted 1:26 in 0.154M NaCl and spun at 315 g. The supernatant fluid was then read at 620 nm, the absorption maximum of T-1824.
MULTIPLE-INDICATOR DILUTION STUDIES

In these studies, a bolus of 1.75 ml of a suspension containing labeled red cells and albumin was injected into the superior vena cava at the same time as the osmotic or the control solution by way of a smaller catheter (PE 160) threaded through the original catheter (PE 350). The catheters were initially filled with blood, and the injections through each catheter were completed within a 1-second interval.

The red cell suspension was made in the following manner. A 20-ml sample of blood was collected from the dog and incubated for 20 minutes with 100 µc of Na₂⁵¹CrO₄ (New England Nuclear). The red cells were centrifuged at 315 g for 10 minutes, and the plasma was discarded. The red cells were then washed twice with 0.154M NaCl and resuspended in a solution of 0.154M NaCl containing human serum albumin (2.5 g/100 ml) labeled with ¹²⁵I (tracer material, Abbott). Enough of the albumin solution was added to obtain the original hematocrit, and approximately 20 µc of ¹²⁵I and 10 µc of ⁵¹Cr were injected in each experiment.

⁵¹Cr-labeled red cell and ¹²⁵I-albumin activities of 0.2-ml samples of whole blood diluted with 2.0 ml of water were determined in an automatic two-channel well scintillation counter. In some studies, ¹⁴C-sucrose or ¹⁴C-urea activities were measured in protein-free supernatant fluids derived from these samples; 0.5 ml of 30% trichloroacetic acid was added to the blood hemolysates and the precipitated protein was centrifuged for 10 minutes at 315 g. A 0.2-ml sample of the supernatant fluid was diluted with 2.0 ml of 95% ethanol and 8 ml of a dioxane mixture (231 ml of dioxane, 40 g of naphthalene, 3 g of 2,5-diphenyloxazole, 75 mg of 1,4-bis-[5-phenyloxazolyl]-benzene), and ¹⁴C activity was determined in a liquid scintillation counter. ⁵¹Cr, ¹²⁵I, and ¹⁴C activities were divided by the total activity of each in the injection mixture yielding fractional concentrations in units of ml⁻¹.

Plasma osmolalities were directly determined in the plasma of the collected samples by freezing point depression in 14 experiments. In 15 experiments, plasma osmolalities were calculated from the concentrations of the osmotic substance, i.e., sucrose, urea, or sodium chloride (measured as sodium), in whole blood. The calculated osmolality values were based on the plasma osmolalities of equivalent dilutions of the test substances in whole blood. Since modest amounts of hypotonic fluid were extracted from the tissues, dilution of base-line concentrations of sodium chloride and other solutes had to be assumed, and the values calculated for overall serum osmolalities were consequently somewhat too high. In 10 experiments with hypertonic sodium chloride, osmolalities were calculated from the concentration of ¹²⁵I-albumin in whole blood. Justification for this calculation was based on the relatively close correspondence of fractional concentrations of ²⁴Na with those of ¹²⁵I-albumin when each of these substances was injected together with hypertonic saline (fractional concentrations of ²⁴Na remained within 15% of those of ¹²⁵I-albumin in 4 experiments).

Pulmonary arterial and capillary osmolalities were presumably higher than those found in the collected samples, and the latter were included.

![Effects of an intravenous injection of 5.1 ml of isotonic (0.154M) NaCl. Study 1. Top: Hemoglobin (Hb) or T-1824 concentrations factored by average base-line values (c/c₀) are plotted against time. Note that a small decline in these values occurred. Middle: The ratios of hemoglobin concentration to T-1824 concentration factored by the average base-line ratio (r) are plotted against time. No significant change in these ratios is observed. Bottom: Fractional concentrations (w) of ⁵¹Cr-labeled red cells (⁵¹Cr-RBC) and ¹²⁵I-labeled albumin (¹²⁵I-Alb) are plotted against time. The red cell curve precedes that of albumin.](http://circres.ahajournals.org/)

_Circulation Research, Vol. XXXI, October 1972_
primarily for comparison of concentrations obtained with each solute.

Chemical determinations of urea concentration were performed by a simple modification of the standard urease technique (11). A 0.2-ml sample of whole blood was diluted in 5 ml of saline; 0.1 ml of this suspension was diluted in 1.0 ml of a urease reagent (50 mg of Sigma type III urease, 1 g of disodium ethylenediaminotetraacetae, 100 ml of water, and 1 ml of phosphate buffer, adjusted to pH 7.0) and incubated at 50°C for 10 minutes. Then, 1.5 ml of a phenol color reagent was added (50 g of phenol, 0.25 g of sodium nitroprusside, and 1 liter of water) followed by 1.5 ml of a hypochlorite solution (25 g of NaOH, 50 ml of 5% NaOCl, and 1 liter of water). This mixture was incubated for 10 minutes at 50°C, and the samples were read at 620 nm.

Sucrose concentrations were determined by a modified direct resorcinol method (12). Whole blood, 0.01 ml, was diluted in 1.0 ml of water. Then, 0.5 ml of 30% trichloroacetic acid was added, and the sample was centrifuged at 315 g.

![Figure 2](image1.png)

**FIGURE 2**

Effects of intravenous injection of 5.1 ml of a 2.25M sucrose solution. Study 3. Top: The decline in hemoglobin concentration is proportionately greater and earlier than that in T-1824. Middle: A relative fall in the ratio of hemoglobin concentration to T-1824 concentration is followed by a rise to levels above base-line values. Bottom: Fractional concentrations of 14C-sucrose are plotted with those of 51Cr-labeled red cells and 151I-labeled albumin against time. Portions of the 151I-labeled albumin curve preceded that of 14C-labeled red cells. Note that the relative concentration of hemoglobin rises above that of T-1824 before a significant decline in the red cell and albumin indicators has occurred (vertical line). See Figure 1 for abbreviations.

![Figure 3](image2.png)

**FIGURE 3**

Effects of intravenous injection of 5.1 ml of a 2.25M sucrose solution. Study 3. Top: The decline in hemoglobin concentration is proportionately greater and earlier than that in T-1824. Middle: A relative fall in the ratio of hemoglobin concentration to T-1824 concentration is followed by a rise to levels above base-line values. Bottom: Fractional concentrations of 14C-sucrose are plotted with those of 51Cr-labeled red cells and 151I-labeled albumin against time. Portions of the 151I-labeled albumin curve preceded that of 14C-labeled red cells. Note that the relative concentration of hemoglobin rises above that of T-1824 before a significant decline in labeled red cell, albumin, or sucrose concentrations has occurred (vertical line). See Figure 1 for abbreviations.
PULMONARY RED CELL TRANSIT

for 10 minutes. A 0.5-ml sample of the supernatant fluid was removed and to this was added 5 ml of a resorcinol reagent (2 parts of a solution containing 200 mg of resorcinol in 200 ml of 95% ethanol plus 5 parts of a solution containing 1,000 ml of concentrated HCl and 224 ml of water). The samples were heated at 80°C for 25 minutes and were then read at 490 nm.

Results

BASE-LINE DILUTION

In the control studies in which isotonic saline was injected, changes in hemoglobin and T-1824 concentrations were relatively minor. Changes in these concentrations relative to base-line values are indicated in Figure 1 (top). No consistent variation of relative concentrations of hemoglobin and T-1824 were observed (Fig. 1, middle).

Injections of the same volumes of hypertonic sodium chloride, sucrose, or urea solutions resulted in an initial fall in both hemoglobin and T-1824 concentrations, presumably produced by the movement of fluid from the pulmonary tissues into the capillary blood. However, the decline in hemoglobin concentration preceded and exceeded that in T-1824 concentration following hypertonic sodium chloride injections in each of 10 studies (Fig. 2, top) and following hypertonic sucrose injections in each of 14 studies (Fig. 3, top). This phenomenon is reflected by the initial fall in the ratio of hemoglobin to T-1824 below base-line values followed by a subsequent rise above these levels (Figs. 2 and 3, middle). The initial decline in T-1824 concentration averaged only 0.42 ± 0.11 (so) of the concomitant decline in hemoglobin concentration after injections of hypertonic sodium chloride and 0.64 ± 0.11 of the concomitant decline in hemoglobin concentration after injections of hypertonic sucrose (as calculated from the expression D T-1824/DHgb, Eqs. 1, 2 and Table 1). It must be concluded from these data that proportionately more hemoglobin than T-1824 was retained within the lungs for a brief interval after the injection of hypertonic sucrose or sodium chloride solutions.

Red cell retention was apparently greater after injections of sodium chloride than it was after injections of sucrose, perhaps because of the greater osmolalities found following some of the sodium chloride injections. (The maximum increases in osmolality attained with sodium chloride tended to be greater than those attained with sucrose. The ranges of these increases are shown in Table 1.) However no correlation was found between DT-1824/DHgb and peak osmotic concentration values following injections of sodium chloride or sucrose. It is also possible that the greater viscosity of the sucrose solutions slowed the movement of the plasma phase through the lungs to a greater extent than did the less viscous sodium chloride solutions.

Injections of hypertonic urea in each of eight studies were followed by equivalent

### Table 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>No. studies</th>
<th>Solute injected (mmoles)</th>
<th>Δ cmalone (mM/kg)</th>
<th>DT-1824/DHgb</th>
<th>Δ c/Δ cmalone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>14</td>
<td>8.1-15.8</td>
<td>32-108</td>
<td>0.64 ± 0.11 (P &lt; 0.001)*</td>
<td>0.94 ± 0.09</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
<td>10.3-13.2</td>
<td>75-181</td>
<td>0.42 ± 0.11 (P &lt; 0.001)*</td>
<td>0.98 ± 0.10</td>
</tr>
<tr>
<td>Urea</td>
<td>8</td>
<td>12.5-66.9</td>
<td>55-386</td>
<td>1.01 ± 0.10 (P &gt; 0.80)*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Δcmalone = maximum increase in plasma osmolality above base-line levels following injection of the hypertonic solution; Δc = increase in plasma osmolality above base-line levels when r returns to 1.0. Since an isotonic saline solution was used for the control injections, no change in osmolality occurred. Decreases in the base-line values of hemoglobin and T-1824 following control injections were too small and indistinct to provide values for DT-1824/DHgb. Also, following injections of urea, r remained close to 1.0 and Δc/Δcmalone was not calculated.

*Probability that value does not differ from 1.0 and Δc/Δcmalone was not calculated.
decreases in hemoglobin and T-1824 (Fig. 4, top and middle and Table 1). This finding held at osmotic concentrations in the aortic blood which were equivalent to or well above those obtained with sodium chloride or sucrose. $D_{T-1824}/D_{Hb}$ averaged $1.00 \pm 0.10$ after injections of urea.

MULTIPLE-INDICATOR DILUTION

A relative decline in the efflux of hemoglobin from the lungs could represent the retention of either red cells already in the lung and not in contact with the injection solution or red cells directly exposed to a hypertonic environment. That red cells which were directly exposed to the hypertonic solution traversed the pulmonary vasculature more slowly than did plasma protein was demonstrated by injecting labeled red cells and albumin concomitantly with the hypertonic solutions.

Following control injections of water or isotonic saline, the emergence of $^{51}$Cr-labeled red cells from the pulmonary vasculature preceded that of $^{125}$I-albumin (Fig. 1, bottom). After injections of hypertonic sodium chloride, the $^{51}$Cr-labeled red cell indicator curve followed that of $^{125}$I-albumin in each of five studies (Fig. 2, bottom). A similar, though somewhat smaller effect, was observed in five studies with hypertonic sucrose (Fig. 3, bottom). Slowing of red cell transit through the lungs relative to plasma protein transit is reflected by an increase in the mean transit time ratio, $t_{51Cr}/t_{125I}$. Significant increases in this ratio above control values followed injections of hypertonic sodium chloride and sucrose solutions (Table 2). In contrast $t_{51Cr}/t_{125I}$ was not altered significantly from control values following injections of hypertonic urea at osmotic concentrations which were equivalent to or greater than those following the sucrose and sodium chloride injections.

The initial drop in the ratios of hemoglobin to T-1824 following sucrose and sodium chloride appeared to coincide with the arrival of the labeled red cells and albumin as well as with the osmotic substance in the outflow blood (Figs. 2, 3, and 5). Retention of red cells directly exposed to the hypertonic solution would appear to be responsible at least in part for the decline in the ratio of hemoglobin concentration to T-1824 concentration in the collected blood.

A reversal in the relative concentrations of hemoglobin and T-1824 was noted when concentrations of the injected indicators and osmotic material were still high. This phenomenon is illustrated in Figure 2, in which it is shown that, following an injection of hypertonic sodium chloride, the ratio of hemoglobin concentration to T-1824 concentration increased to the base-line value before
any significant decline in labeled red cells or plasma was observed. This event occurred before sodium concentrations were significantly diminished (Fig. 5 and Table 1). Similarly, the ratio of hemoglobin concentration to T-1824 concentration following sucrose injections increased to the baseline level when high concentrations of labeled sucrose and the vascular indicators were still present in the collected blood (Fig. 3 and Table 1).

For the entire series of studies, the area under the labeled albumin curve averaged $0.99 \pm 0.05$ ($n = 20$) of the area under the $^{51}$Cr-labeled red cell curve. No significant differences between areas were observed following injection of control or test substances. (Table 1). This finding indicates that the recoveries of the substances were equivalent and presumably complete. Furthermore, recoveries of $^{24}$Na, $^{14}$C-sucrose, and $^{14}$C-urea relative to labeled albumin following hypertonic injections also appeared to be complete, averaging $0.99 \pm 0.07$ ($n = 4$), $1.00 \pm 0.01$ ($n = 4$), and $0.99 \pm 0.04$ ($n = 3$), respectively.

### Discussion

Following rapid intravenous injections of hypertonic urea, sucrose, or sodium chloride there was a decline in the concentrations of both hemoglobin and T-1824-labeled plasma protein in the aortic blood. The fall in the concentrations of these blood constituents reflected extraction by the osmotic bolus of fluid derived primarily from the lungs. However, the initial decline in hemoglobin concentration following injections of hypertonic sodium chloride or sucrose solutions was proportionately greater and earlier than that in T-1824, indicating that, for a short period of time, the blood leaving the lungs contained relatively fewer red cells compared to plasma protein than did the blood which was entering the lungs. There must, consequently, have been an increase in the relative quantity of red cells compared with plasma protein within the lungs. The phenomenon was quite transitory.

### Table 2

<table>
<thead>
<tr>
<th>Substance</th>
<th>No. studies</th>
<th>Solute injected (mmoles)</th>
<th>$\Delta C_{max}$ (mM/moles/kg)</th>
<th>$t_{1/2}$</th>
<th>$I_{max}/I_{0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>11.6-15.8</td>
<td>52-106</td>
<td>0.971 ± 0.007 ($P &gt; 0.8$)*</td>
<td>0.980 ± 0.008 ($P &lt; 0.01$)*</td>
</tr>
<tr>
<td>NaCl</td>
<td>6</td>
<td>10.4-13.2</td>
<td>108-280</td>
<td>1.013 ± 0.043 ($P &gt; 0.3$)*</td>
<td>1.037 ± 0.028 ($P &lt; 0.001$)*</td>
</tr>
<tr>
<td>Urea</td>
<td>6</td>
<td>7.7-66.9</td>
<td>57-360</td>
<td>1.013 ± 0.071 ($P &gt; 0.5$)*</td>
<td>0.954 ± 0.023 ($P &gt; 0.4$)*</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.982 ± 0.065</td>
<td>0.949 ± 0.016</td>
</tr>
</tbody>
</table>

Values are means ± sd.

*Probability that value is the same as control.

Figures 5 and 6 illustrate these findings. As shown in Figure 5, the ratio of hemoglobin concentration to T-1824 concentration ($r$) returns to baseline values before plasma sodium concentrations have declined significantly.

Circulation Research, Vol. XXXI, October 1972
Within a few seconds, there was a rise in the ratio of hemoglobin concentration to protein concentration in blood exiting from the lungs to values above base-line levels, and the ratio of the mass of hemoglobin to that of protein within the lungs was restored towards base-line levels.

Two alternative explanations may be proposed for this decline in the relative recovery of hemoglobin compared with T-1824 in blood leaving the lungs: (1) slowing of red cells relative to plasma protein or (2) mobilization of plasma protein stores from some intravascular or extravascular site. The latter possibility is suggested by the observation that the lungs, like most other organs, contain greater amounts of plasma protein compared with red cells than does the blood in the large vessels (13, 14). However, the distribution of these additional quantities of plasma protein between the vascular and the interstitial compartments remains in doubt. Furthermore, it is not at all clear how the osmotic bolus would mobilize protein from either of these compartments. Although fluid is extracted from the pulmonary tissue by hypertonic injections, it seems unlikely that plasma protein would be carried from the interstitium into the bloodstream in this fashion. If the pulmonary capillaries are sufficiently permeable to permit solvent drag of plasma protein molecules from the interstitium, then it is very improbable that small lipophobic solute molecules such as urea or sucrose would remain confined to the vascular space. Presumably, they would diffuse out into the interstitium, and the effective osmotic gradient between the vascular and the extravascular spaces would be relatively small. Stated in irreversible thermodynamic terms, it is improbable that the reflection coefficient of the capillary wall to a very large molecule would be low (as would be estimated from solvent drag) while that to a small lipophobic molecule is high (as would be determined from the relative flows of fluid induced by equivalent gradients of solute concentration and hydrostatic pressure).

Documentation that red cell movement through the pulmonary vessels is impaired by hypertonic sodium chloride and sucrose injections was obtained by multiple-indicator dilution. Labeled red cells and albumin were injected at the same time and site as was the hypertonic solution, ensuring in vivo exposure of both the red cells and the albumin to a hypertonic environment. Under control circumstances, red cells traversed the pulmonary vasculature more rapidly than did albumin. Exposure to the hypertonic solution reversed this effect, and labeled red cells emerged from the lungs more slowly than did labeled albumin. The decline in hemoglobin concentrations relative to T-1824 concentrations in the aortic blood appeared to coincide with the arrival of the hypertonic solution. There was no evidence that red cells entering the lungs before the osmotic bolus and never directly exposed to hypertonic fluid were slowed or that protein stores were flushed out of the lungs in advance of the osmotic bolus.

A variety of studies indicate that the impairment of red cell transit through the lungs observed in these experiments following sodium chloride and sucrose injections may be due to the loss of the ability of red cells to deform. Red cells placed in hypertonic solutions not only shrink and become crenated but also are more viscous and less able to deform. Viscosity measurements of hypertonic red cell suspensions show that, at high shear rates, red cell viscosity is increased (15, 16). Losses in the ability to deform may serve to impede red cell movement through larger vessels (greater than 40μ) as well as through smaller (3-8μ) vessels. Fahraeus and Lindqvist (17) were the first to demonstrate that the movement of whole blood in glass tubes with diameters between about 40 and 100μ is accompanied by migration of the red cells to the center of the stream where the movement of fluid is more rapid. A similar phenomenon is observed in vivo. Because the red cells have a greater velocity than the suspending medium and are restricted to a smaller volume within the vessels, labeled red cells traverse vascular beds more rapidly than does labeled plasma (18, 19). Goldsmith (20) and others have cited both theoretical and experimental evidence.
that this movement of red cells towards the axial stream of 65–200μ vessels will not occur unless the red cells are relatively deformable.

The passage of red cells through smaller vessels is also dependent, in large part, on the normal ability of red cells to deform. Capillary diameters are often significantly smaller than red cell diameter (7.5μ). Red cell movement through these capillaries requires considerable red cell deformation: the normal biconcave shape is lost and the red cell assumes a parachute shape which permits traversal of these capillaries (21–25). In vitro methods have been used to relate the ability of red cells to deform to the movement of these cells through graded filters. Gregersen et al. (26) found that under normal circumstances red cells will pass through polycarbonate sieves with pore diameters of 3.0μ (less than half the red cell diameter). Once they have been hardened (with acetaldehyde) and are no longer able to deform, they are unable to cross similar sieves with 6.8μ pores. Similarly, red cell movement through graded Millipore filters is slowed in hypertonic media (27).

These observations suggest that loss of red cell deformability due to dehydration is responsible for impeding red cell transit through the lungs. It should be anticipated that red cells “hardened” by hypertonic dehydration would tend to exhibit diminished axial migration in larger vessels. Further, they would be unable to enter smaller vessels or on entering them would be unable to alter their shape and thereby slip ahead of the plasma at the capillary wall. Visual evidence by high-speed cinemicrography that hypertonic radiopaque solutions impair (1) the ability of red cells to deform and (2) their entry into smaller mesenteric vessels has been provided by Brown et al. (8) who indicated that following injection of these substances the red cells look like “ping pong balls” bouncing against the arteriolar walls.

Red cell clumping following hypertonic injections has also been observed, but its role in slowing red cell transit through the lungs is less certain. In vitro viscometric studies at low shear rates indicate that the adherence of red cells to one another is diminished rather than augmented by hypertonic solutions (15, 18, 28, 29). It has been suggested that the assumption of a crenated spherical shape actually diminishes the opportunity for adherence between red cells (15). Morphologic evidence of clumping may simply reflect a pile up of red cells unable to traverse the smaller vessels, perhaps with subsequent aggregation.

At high hematocrits and normal osmolality, some slowing of red cells relative to plasma protein through skeletal muscle capillaries has been reported by Groom (30). The impairment of red cell transit in these experiments may be due to the tendency of red cells to agglutinate at high hematocrits.

Although red cells transiently exposed to hypertonic solutions of sucrose and sodium chloride traversed the pulmonary vascular bed relatively slowly, recovery of these cells, as judged from the recovery of radioactive red cell and plasma protein labels, appeared to be complete. The proportional outflow of red cells from the lungs rose above that of T-1824-labeled plasma protein at a time when serum osmolality was still close to maximal values. It is possible that the delayed rise in the outflow of these cells from the lungs was increased by vasodilation, which may be the primary effect of hypertonic solutions on the pulmonary vessels (4). Read et al. (31) found that increasing left atrial pressure by infusion of dextrans, plasma, or blood or by constricting the aorta protected dogs from the pulmonary hypertensive effect of rapid intravenous injections of 15% NaCl. They concluded that elevation of left atrial pressure resulted in pulmonary vasodilation permitting passage of red cells.

The failure of rapid injections of hypertonic urea to slow the passage of red cells relative to that of plasma protein through the pulmonary vascular bed is probably related to the rapid entry of urea into red cells (half-time approximately 1/3 second) (32). Red cells maintained in hypertonic urea solutions are not dehydrated, and rapid injections of urea fail to increase the apparent pulmonary vascular resistance (6).
The sudden decline in blood pressure to precarious levels (33), which can follow injections of hypertonic angiographic media, has been attributed to loss of the ability of red cells to deform with a consequent rise in pulmonary vascular resistance and a marked fall in cardiac output. A similar loss of red cell deformability has been blamed for local tissue ischemia in patients with red cells containing hemoglobin S (34, 35). Recently, a decline in the ability of red cells to deform has been described in red cells containing malarial parasites, and it has been suggested that the clinical manifestations and pathological findings of “cerebral” malaria may be attributed to the failure of red cells to traverse the cerebral capillaries, with consequent vascular obstruction and ischemia (36). The failure of rigid red cells to negotiate the sinusoids of the spleen has been held responsible for splenic sequestration of spherocytes or red cells containing abnormal hemoglobin (37).

It is quite clear that blood must be considered to be a biphasic (or multiphasic) fluid and that hemodynamic studies must consider selective alterations in the viscosity and other physical properties of each phase. Indeed, the flows of the red cell and the plasma phases through the pulmonary vasculature were temporarily dissociated in the present study. Sucrose and sodium chloride injections resulted in an initial decline in the relative flow of red cells followed by a rise above base-line levels. These observations indicate that plasma can continue to move between red cells which are trapped within or moving slowly through the pulmonary vessels.

Acknowledgment

I wish to thank Kathleen Marold, Ruth Dumpys, and Gerald Green for the fine technical assistance they provided in these studies and Louise Robinson and Barbara McDonald for their secretarial help. I would also like to express my gratitude to Dr. Francis P. Chinard and Dr. William Perl for their encouragement, support and suggestions throughout the course of these studies.

References


Circulation Research, Vol. XXXI, October 1972


Impairment of Red Cell Transit through the Canine Lungs following Injections of Hypertonic Fluids
Richard M. Effros

Circ Res. 1972;31:590-601
doi: 10.1161/01.RES.31.4.590
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/31/4/590