Efllux and Influx of Erythrocyte Water

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ABSTRACT Rabbit erythrocytes were washed in buffered NaCl solutions isotonic with rabbit serum ($\Delta t = -0.558^\circ C$.) and suspended in buffered NaCl solutions of tonicity equidistant from intracellular tonicity ($\Delta t = -0.558^\circ C$ ± 0.112$^\circ C$) of varying pH and incubated at varying temperatures. After incubation, the freezing point depression ($\Delta t$) was measured on the supernatant. Change in the $\Delta t$ measured change in the water content of the extracellular solutions—water being withdrawn by erythrocytes ($W_x$) from the hypotonic solutions and added ($W_r$) to the hypertonic solutions. $W_m$ was always less than $W_x$ and was inversely proportional to the pH in the range 6.5–8.0. $W_r$ was significantly increased by lowering the temperature of the cell suspension to 4°C. $W_t$ was increased by raising or lowering the pH or raising the temperature of the cell suspension. $W_x \times W_r \approx k$. $W_m$ and $W_r$ were affected differently by changes in pH and temperature. It was concluded that $W_x$ and $W_r$ were probably under different physicochemical control.

INTRODUCTION

Influx of erythrocyte water from hypotonic solutions, reviewed by Ponder (1, 2), has been studied more extensively than efflux of erythrocyte water into hypertonic solutions. This is due, in part, to the fact that while hemolysis acts as an accurate end point for water influx, no similar end point is available to measure water efflux. Furthermore, while hematocrit methods of determining cell volume are accurate for measuring distended erythrocytes, similar methods are not accurate for measuring volumes of shrunken erythrocytes.

A method has been proposed (3) for measuring water efflux from erythrocytes by changes in the freezing point depression ($\Delta t$) of a standardized extracellular NaCl solution in which the erythrocytes are suspended. This method is based on the premise that although there is no rigid intra- and extracellular partition of cations in the cell suspension (4–11), exchanges of sodium and potassium between the cells and the surrounding medium take place milliosmol for milliosmol either along or against their respective concentration

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gradients. Consequently, if the ionic content of the extracellular solution remains constant in number, although not necessarily in kind, any change in the $\Delta^t$ of the standardized extracellular solution must be the result of a change in the water content of the solution—water passing from erythrocytes into hypertonic solutions and being taken up by erythrocytes from hypotonic solutions.

The purpose of this paper is to quantitate and compare the efflux and influx of erythrocyte water under varying conditions of temperature and pH.

**Figure 1.** Dilution curve for buffered NaCl solutions ($-0.670^\circ$C).

**Materials and Methods**

Five solutions of phosphate-buffered NaCl were prepared with pH 6.5, 7.1, 7.4, 7.7, 8.0.\(^1\) Osmolarity\(^2\) of these solutions was adjusted by suitable dilutions with water until three sets of five solutions were obtained with the above pH range but varying freezing point depression:

Solution A = $-0.670^\circ$C.

Solution B = $-0.558^\circ$C.

Solution C = $-0.446^\circ$C.

Four ml. of solutions A was diluted with water by micropipette and the $\Delta^t$ determined after each dilution (Fig. 1). The $\Delta^t (y)$ was a linear function of the amount of

\(^1\) pH measured with Beckman model G pH meter.

\(^2\) Osmolarity measured with Fiske osmometer.
water \( (x) \) added and, consequently, the amount of water added to 4 ml. of any of solutions A may be determined by

\[
x = \frac{0.668 - y}{0.151}
\]

Coefficient of variation of this equation was 0.99.

Four ml. of phosphate-buffered NaCl solution of \( \Delta t = -0.486^\circ C \) was diluted with measured amounts of water to \( \Delta t \) of \( -0.446^\circ C \). (Fig. 2). Again the \( \Delta t \) \((y)\) of the dilution was a linear function of the amount of water \((x)\) added and the amount of water added to 4 ml. of these solutions may be determined by

\[
x = \frac{0.485 - y}{0.098}
\]

Similarly the amount of water removed from these solutions may be determined by

\[
x = 0.40 - \left[ \frac{0.485 - y}{0.098} \right]
\]

Coefficient of variation of these equations was 0.99.

Heparinized rabbit heart blood was centrifuged at 1400 g and the supernatant and buffy coat removed. Cells were washed in solutions B \((\Delta t = -0.558^\circ C)\) and centrifuged at 1400 g for 15 minutes. The supernatant was again removed and the process...
of washing and centrifuging repeated twice. The cells were then suspended in solutions B and centrifuged for one-half hour at 1400 g. The supernatant was removed and its $\Delta t$ determined. This was found to be $-0.558^\circ C \pm 0.001^\circ C$ on each occasion. The surface of the packed cells was blotted lightly with a cotton applicator. One ml. of packed erythrocytes was pipetted into 4 ml. of each of the test solutions (solutions A and C), mixed by gentle swirling, and placed in a water bath for one-half hour. Following this the cell suspensions were centrifuged briefly and about 2 ml. of the supernatant decanted for determination of the $\Delta t$.

RESULTS

Table I shows the water efflux ($W_s$) compared with the water influx ($W_t$) at 38°C. and pH 7.4. Although the osmolarities of the hypertonic and hypotonic solutions, A and C respectively, are equidistant ($0.112^\circ C$) from cellular isotonicity ($-0.558^\circ C$), the influx of water from the hypotonic solutions was about twice the efflux of water into hypertonic solutions.

Table I also indicates that the efflux of erythrocyte water into the hypertonic solutions was inversely proportional to the pH of these solutions in the range of pH 6.5-8.0. Nearly twice as much water was transferred from the erythrocytes into the hypertonic solutions at pH 6.5 as was transferred at pH 8.0.

The influx of erythrocyte water from the hypotonic solutions was not so constant as the efflux of water into the hypertonic solutions as shown by the relatively large standard errors in the magnitudes of $W_t$ as compared with $W_s$. However, $W_t$ was significantly increased over that of pH 7.4 by both lowering (6.5) or raising (8.0) the pH ($p < 0.01$).

Lowering the temperature of the cell suspension during the incubation period to 4°C. significantly increased ($p < 0.01$) $W_s$ when compared with $W_s$ at 38°C. There was a significant increase in $W_t$ at 45°C. when compared with $W_t$ at 38°C. ($p < 0.01$).
DISCUSSION

After washing the rabbit erythrocytes three times in buffered NaCl at $\Delta t = -0.558^\circ C$, no water was exchanged between the cells and the extracellular solutions as indicated by the fact that the $\Delta t$ of the extracellular solutions after the third washing was $-0.558^\circ C$. (see Materials and Methods). If the interior of the erythrocyte was hypertonic to the extracellular solution at this time, there should be some point at which, by increasing the tonicity of the extra-

cellular solution, intracellular and extracellular tonicity would be equal and no water exchanges occur. Fig. 3 shows that no such point exists. Changing the tonicity of the extracellular solution by increasing increments of as little as $-0.038^\circ C$ still causes water shift from the cells as indicated by the rise in $\Delta t$ of the solutions following addition of washed erythrocytes. This is in agreement with recent studies by Williams et al. (12) showing that human erythrocytes are isotonic with serum and NaCl solutions between $-0.391^\circ C$ and $-0.966^\circ C$. (expressed in the units of this paper).

The hypertonic and hypotonic solutions in this study were made equidistant from cellular tonicity ($-0.558^\circ C \pm 0.112^\circ C$); hence, if water in the system was free to move in both directions across the cell membrane, $W_s = W_t$. This was not the case for all instances $W_s \neq W_t$. Apparently, a restriction of movement is placed on $W_s$ compared with $W_t$. When all the data relating to pH

![Figure 3](image-url)
and temperature (Tables I and II) are considered, $W_x$ and $W_t$ are not reciprocally related for $W_x \times W_t = k$. For these reasons it would appear that $W_x$ and $W_t$ are under different physicochemical control.

If the rabbit erythrocyte is isotonic with the extracellular solutions between $-0.446°C$ and $-0.670°C$ as is the human erythrocyte, it is clear that this osmotic equilibrium is reached between erythrocytes and the hypertonic solutions with less efflux of water than the influx of water necessary to reach osmotic equilibrium in the hypotonic solutions.

### TABLE II

**EFFECT OF TEMPERATURE ON EFFLUX AND INFLUX OF ERYTHROCYTE WATER AT PH 7.4**

<table>
<thead>
<tr>
<th>Temperature°C</th>
<th>No. tested</th>
<th>Mean $W_x$ ml./100 ml. R.B.C.</th>
<th>S.E.</th>
<th>Mean $W_t$ ml./100 ml. R.B.C.</th>
<th>S.E.</th>
<th>$W_x/W_t$</th>
<th>$W_x \times W_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>20</td>
<td>8.47 0.18</td>
<td></td>
<td>13.11 0.28</td>
<td></td>
<td>0.645 111.0</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>20</td>
<td>6.98 0.12</td>
<td></td>
<td>14.55 0.39</td>
<td></td>
<td>0.480 101.6</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>20</td>
<td>6.75 0.16</td>
<td></td>
<td>16.66 0.32</td>
<td></td>
<td>0.405 112.4</td>
<td></td>
</tr>
</tbody>
</table>

*S.E. = standard error of the mean.

**CONCLUSIONS**

1. Measurement was made of efflux and influx of rabbit erythrocyte water into hypertonic and from hypotonic buffered NaCl solutions of tonicity equidistant from intracellular tonicity.

2. Water efflux into the hypertonic solutions was always less than water influx from the hypotonic solutions.

3. Water efflux into the hypertonic solutions was inversely proportional to the pH of the cell suspension within the range of pH 6.5-8.0. Water efflux into hypertonic solutions was significantly increased by lowering the temperature of the cell suspension to 4°C.

4. When compared with water influx at 38°C and pH 7.4, water influx from the hypotonic solutions was increased by raising or lowering the pH or raising the temperature of the cell suspension.

5. The product of water influx and water efflux was not a constant under the experimental conditions and water efflux and influx were affected differently by changes in pH and temperature. It is probable that water efflux and water influx from the rabbit erythrocyte are each under different physicochemical control.

**REFERENCES**