Efflux of Red Cell Water into Buffered Hypertonic Solutions

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Abstract Buffered NaCl solutions hypertonic to rabbit serum were prepared and freezing point depressions of each determined after dilution with measured amounts of water. Freezing point depression of these dilutions was a linear function of the amount of water added. One ml. of rabbit red cells was added to each 4 ml. of the hypertonic solutions and after incubation at 38°C. for 30 minutes the mixture was centrifuged and a freezing point depression determined on the supernatant fluid. The amount of water added to the hypertonic solutions by the red cells was calculated from this freezing point depression. For each decrease in the freezing point of -0.093°C. of the surrounding solution red cells gave up approximately 5 ml. of water per 100 ml. of red cells in the range of -0.560 to -0.930°C. Beyond -0.930°C. the amount of water given up by 100 ml. of red cells fits best a parabolic equation. The maximum of this equation occurred at a freezing point of the hypertonic solution of -2.001°C. at which time the maximum amount of water leaving the red cells would be 39.9 ml. per 100 ml. of red cells. The data suggest that only about 43 per cent of the red cell water is available for exchange into solutions of increasing tonicity.

Introduction

Many studies are available concerning the swelling of erythrocytes in hypotonic solutions (reviewed by Ponder (1-2)). A similar extensive literature on the shrinking of erythrocytes in hypertonic media is not available. The reason for this is twofold: (a) while hematocrit methods are quite accurate in measuring red cell swelling (influx of water), these same methods are inaccurate in measuring red cell shrinkage (efflux of water) and (b) difficulty in accurate correction for trapped intercellular fluid following centrifugation in hypertonic solutions.

The purpose of this study is to propose a method of measuring efflux of red cell water based on the change in freezing point depression of the hypertonic media in which the cells are suspended and to determine the quantity of water leaving the red cell in response to measured increases in the tonicity of the surrounding solutions.

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Materials and Methods

One and one-half to 2.5 kg. New Zealand female rabbits were used in these experiments. Heart blood was drawn and the mean serum freezing point depression ($\Delta t$) in thirty rabbits was found to be $-0.558^\circ$C. ± 0.008.

A stock solution containing $Na_2HPO_4$ 13.65 gm., $NaH_2PO_4$ 1.87 gm., and $NaCl$ 90.00 gm. (3) was made up to 1 liter with distilled water. Distilled water was then added to each of 8 aliquots of the stock solution until the final freezing point depressions were exactly $-0.651$, $-0.744$, $-0.837$, $-0.930$, $-1.023$, $-1.209$, $-1.395$, $-1.581^\circ$C. The final pH of these solutions was 7.43 ± 0.04. (A series of 4 ml. of aliquots was taken from each of the eight solutions and distilled water was added to each by means of a micropipette in amounts of 0.10, 0.20, 0.30, 0.40 ml. per 4 ml. of solutions and the $\Delta t$ determined in each of the final dilutions (Table I).

Ten to 20 ml. of heparinized rabbit heart blood was centrifuged at 1400 g for $\frac{1}{2}$ hour and the serum and buffy coat removed. The cells were then washed with an

\[ \begin{array}{c|cccccc}
\text{Solution No.} & \text{0.00 ml.} & \text{0.10 ml.} & \text{0.20 ml.} & \text{0.30 ml.} & \text{0.40 ml.} & \text{Regression equation} \\
\hline
1 & -0.651 & -0.633 & -0.618 & -0.605 & -0.594 & \frac{x}{0.649 - y} \\
2 & -0.744 & -0.725 & -0.711 & -0.692 & -0.678 & \frac{x}{0.743 - y} \\
3 & -0.837 & -0.814 & -0.795 & -0.776 & -0.759 & \frac{x}{0.833 - y} \\
4 & -0.930 & -0.906 & -0.884 & -0.865 & -0.846 & \frac{x}{0.928 - y} \\
5 & -1.023 & -0.997 & -0.974 & -0.953 & -0.931 & \frac{x}{1.020 - y} \\
6 & -1.209 & -1.113 & -1.070 & -1.011 & -0.961 & \frac{x}{1.190 - y} \\
7 & -1.395 & -1.308 & -1.236 & -1.169 & -1.110 & \frac{x}{1.385 - y} \\
8 & -1.581 & -1.484 & -1.400 & -1.328 & -1.261 & \frac{x}{1.568 - y} \\
\end{array} \]

TABLE I
RELATIONSHIP OF ADDED H$_2$O TO $\Delta t$ OF BUFFERED NaCl SOLUTIONS

\[^1\] Freezing point depressions were determined with the Fiske osmometer.
\[^4\] pH was measured at 38°C. with a Beckman model G pH meter.
TABLE II

<table>
<thead>
<tr>
<th>Solution No.</th>
<th>Freezing point of solution</th>
<th>Mean efflux (\text{H}_2\text{O}/100 \text{ml. R.B.C.} )</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.551</td>
<td>5.0</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>-0.744</td>
<td>9.8</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>-0.837</td>
<td>14.8</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>-0.930</td>
<td>19.3</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>-1.023</td>
<td>22.1</td>
<td>0.40</td>
</tr>
<tr>
<td>6</td>
<td>-1.209</td>
<td>28.5</td>
<td>1.10</td>
</tr>
<tr>
<td>7</td>
<td>-1.395</td>
<td>32.9</td>
<td>1.21</td>
</tr>
<tr>
<td>8</td>
<td>-1.581</td>
<td>36.7</td>
<td>0.37</td>
</tr>
</tbody>
</table>

equal volume of isotonic buffered NaCl (\(\Delta^t - 0.560^\circ\text{C.}, \text{pH} 7.34\)) and centrifuged again for 15 minutes. The supernatant fluid was removed and the cells were resuspended in an equal volume of buffered NaCl and centrifuged for 30 minutes at 1400 g. The supernatant fluid was removed and exactly 1 ml of red cells was transferred by pipette to each 4 ml of the hypertonic solutions. These solutions containing red cells in the hypertonic media were placed in a water bath at 38°C for 30 minutes, re-

\[Y=-33.73+73.54X-18.37X^2\]

\[Y=-27.52+50.3X\]

FIGURE 1. Efflux of red cell water (ordinate) related to increasing tonicity of surrounding solutions (abscissa). Each point represents the mean of twelve determinations.
moved, centrifuged briefly, and about 2 ml. of the supernatant fluid decanted into tubes for determination of $\Delta t$.

RESULTS

The efflux of red cell water into each of the eight hypertonic solutions was calculated for cells from twelve rabbits (Table II).

The efflux of red cell water is a linear function of the tonicity of the media for $\Delta t$ between $-0.560$ and $-0.930^\circ$C. (A-B, Fig. 1), the equation being $y = -27.52 + 50.3x$. In this range for each 0.093°C. decrease in the freezing point of the surrounding solution there is an efflux of about 5 ml. of water per 100 ml. of red cells. The remainder of the curve (B-C, Fig. 1) fits best a parabola $y = 33.73 + 73.54x - 18.37x^2$. The experiment cannot be continued accurately beyond $-1.581^\circ$C. because hemolysis developing in the tubes is great enough to affect the $\Delta t$.

DISCUSSION

The concept of the mammalian red cell acting as a simple osmometer changing its shape in response to change in water content has undergone important modifications in the last two decades. With the demonstration that the red cell membrane was permeable to cations it was shown that a constant exchange of intracellular potassium for extracellular sodium occurs along their respective diffusion gradients (4-5). This rate of exchange is influenced by time, temperature, addition of lysins (6), and many other factors. A different kind of ion exchange occurs against a diffusion gradient and depends upon energy derived from cellular metabolism (7-12). Ponder (1) sums up this work by stating that the exchange of sodium and potassium between the cells and the surrounding medium usually takes place milliosmol for milliosmol and that this is true whether the ions are exchanging in the cold along, or at 37°C. against, their concentration gradients. Further, equal numbers of anions and cations need not be taken up by the cell but the exchange of ion for ion must be equal if electrical potential is to be maintained.

Freezing point depression, being a colligative property of a solution, would change with the addition or subtraction of ions or water to or from a solution. If the ionic content of the hypertonic solution in which the red cells are suspended remains constant in number (although not necessarily in kind) the $\Delta t$ of the solution after suspension of red cells would reflect only the change in water content of the solution.

Very exact measurement of efflux of red cell water into hypertonic solutions would require a correction for the trapped buffered NaCl after the last centrifugation (see Materials and Methods) before transfer of the red cells to the hypertonic solutions. Buffered NaCl has a lower specific gravity
than plasma and it is improbable that more than 5 per cent was trapped by centrifuging at 1400 g for 30 minutes (13). This would be equivalent to adding 0.05 ml. of buffered NaCl to 4 ml. of the hypertonic media. This amount would raise the Δf of the hypertonic solutions only about 0.002°C. which in the regression equations would be equivalent to about 1 ml. H₂O per 100 ml. red cells. This correction factor would become progressively less with increasing tonicity of the test solutions.

The shape of the curve (Fig. 1) suggests that not all the available red cell water exchanges into the hypertonic media. The maximum of the parabolic regression \( y = 33.73 + 73.54x - 18.37x^2 \) is at \( x = -2.00^\circ\)C. and \( y = 39.9 \) ml. H₂O per 100 ml. of red cells. Rabbit erythrocytes contain about 70 ml. of water per 100 ml. of red cells; hence, if it is justifiable to extend the parabola to its maximum only 57 per cent of the total red cell water is “free” to be exchanged into the surrounding hypertonic medium and 43 per cent remains “bound” inside the cell. However, the figure 43 per cent is not precise, for although the parabola gives a good fit for the data at high tonicities, solutions 6 and 7 (Table II) have comparatively high standard errors which makes the placement of their true means in some doubt when compared with the other solutions.

Previous studies on the amount of bound water in red cells show considerable variation in results depending on the investigative method used. Hill (14) using vapor pressure methods gives the amount of bound water as 2 to 3 per cent. Drabkin (15) using spectrophotometric methods on crystalline hemoglobin gives a value of 16 per cent of the water as bound, while Parpart and Shull (16) analyzing the distribution of ethylene glycol, glycerol, and urea between the suspending media and the erythrocytes concluded that about 30 per cent of the cell water was bound. Gough (17) and Krevisky (18) centrifuging cells in hypertonic solutions and applying appropriate correction factors concluded that 30 to 35 per cent of the cell water was bound and not osmotically transferable. The comparatively high figure of 43 per cent bound water found in the present study probably results from the fact that as water leaves the red cell a change of state of the hemoglobin occurs. The hemoglobin may become gelatinous or crystallized, two states which bind water firmly and make impossible the further removal of the water by osmotic methods.

CONCLUSIONS

A method is proposed for measuring the efflux of red cell water into buffered hypertonic solutions based on the change in the freezing point depression of the surrounding medium.

By this method it appears that the red cells give up water at a rate of
about 5 ml. per 0.093°C. depression of the freezing point of the surrounding solution in the range of -0.560 to -0.930°C. Beyond -0.930°C. the relationship between further increase in tonicity and efflux of red cell water fits best a parabolic curve.

Within the limits of this experimental method the data suggest that about 43 per cent of the red cell water is bound and, as such, is not available to exchange across the cell membrane in response to increasing tonicity of the surrounding solution.

REFERENCES