Osmotic Water Permeability of the Human Red Cell

HENRY J. MLEKODAY, RICHARD MOORE, and DAVID G. LEVITT

From The Departments of Physiology and Radiology, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT The osmotic permeability coefficient ($P_f$) was measured with a stopped-flow light-scattering technique. There is an artifactual light-scattering signal produced by the initial mixing that decays with a half-time of $\sim 0.2$ s. This seriously interferes with the measurement of the osmotically induced change in cell volume, which has a similar half-time. This “injection artifact” is associated with the biconcave shape of the cells. It is negligible for cells that have been made nearly spherical by swelling them in 160 mosmol. The dependence of this artifact on the cell volume may explain the previously observed dependence of $P_f$ on the cell volume. When cells are made echinocytic (and therefore spherically symmetric), this injection artifact becomes negligible at all cell volumes and $P_f$ can be accurately measured. The $P_f$ of echinocytic cells was nearly constant, varying by $<10\%$ with the direction of flow and the medium osmolarity (160–360 mosmol). The average value of $P_f$ was $2.0 \times 10^{-2}$ cm/s ($T = 23^\circ$C).

INTRODUCTION

The hydraulic (osmotic) permeability ($P_f$) characteristics of the red cell membrane are a subject of considerable controversy. Rich et al. (1968) and Blum and Forster (1970) originally suggested that the hydraulic conductivity ($P_f$) varied as a function of the medium osmolarity, while Farmer and Macey (1970) concluded that $P_f$ varied with the direction of water flow (rectification). This variation in $P_f$ probably cannot be explained by changes in membrane area or unstirred layer effects (Macey, 1979). Thus, if $P_f$ were not a constant, it would have important theoretical and experimental implications. Theoretically, it would imply that the structure of the red cell membrane is more complicated than is usually assumed. For example, rectification would seem to require a small unstirred compartment associated with the membrane.
where solute polarization could develop. Experimentally, in order to determine the permeability or reflection coefficient using a light-scattering technique, it is necessary to know how \( P_f \) varies with the experimental conditions.

When we reinvestigated this problem, we found that there was a large injection artifact that had roughly the same time course as the volume change and could therefore produce large errors in the determination of \( P_f \). Since this artifact varied with the cell volume, it suggested to us that the apparent variation in \( P_f \) may have been caused by variations in this injection artifact. The purpose of these experiments was to eliminate this artifact and then reinvestigate the dependence of \( P_f \) on osmolarity and direction of flow with particular emphasis on determining the value of \( P_f \) that should be used in our measurement of the reflection coefficient (Levitt and Mlekoday, 1983). While this work was in progress, Terwillinger and Solomon (1981) determined \( P_f \) using a nonlinear relationship between the cell volume and light scattering and concluded that \( P_f \) was a constant, independent of osmolarity and direction of flow.

**METHODS**

The red cells were freshly drawn (usually from donor H.L.M.) in heparin and washed three times in the 220-mosmol suspension medium with removal of the buffy coat. The suspension medium consisted of 8 mM KCl, 10 mM Tris (pH 7.28) plus NaCl to produce the desired osmolality (measured with an Osmette freezing point osmometer [Precision Systems, Waltham, MA]). To make the cells echinocytes, 10 \( \mu \)l of a solution of egg lecithin (United States Biochemical Corp., Cleveland, OH) in methanol (700 mg/ml) was added to 100 ml of the cell suspension before addition of the cells. The lecithin was added to either the cell suspension and the challenging solution together or to just the cell suspension, with identical results. Although lecithin is insoluble at this concentration and the solutions are slightly cloudy, this did not affect the light-scattering measurements. The shape of the cells was directly checked by microscopic observation of a drop of the cell suspension using Sigmacote-treated (Sigma Chemical Co., St. Louis, MO) slides and coverslips. The control cells were always biconcave in shape and the treated cells were always echinocytes.

Light scattering from the red cells was measured with an Aminco-Morrow stopped-flow apparatus (American Instrument Co., Silver Spring, MD) (Reich, 1971; Morrow, 1970). The mixing time for this apparatus, determined by following the pH change when HCl and NaHCO₃ are mixed, is ~10 ms. The red cell suspension (hematocrit of 0.5%) was mixed with an equal volume (~100–180 \( \mu \)l) of the challenging solution, and the change in transmitted light (590 nm) was recorded on a storage oscilloscope. All the experiments were run at room temperature (21–25°C).

The hydraulic permeability (\( P_f \) in units of centimeters per second) was determined using the following theoretical derivation. The volume flux (\( J_v \)) across the red cell can be described by:

\[
J_v = \frac{dV_c}{dt} = \bar{V}_w P_f A_c (C - C^m),
\]

where \( C^m \) and \( C \) are the osmotic activity of the impermeants in the medium and cell, respectively, \( \bar{V}_w \) is the molar volume of water, \( A_c \) is the cell surface area, and \( V_c \) is the cell volume. \( C \) is defined as equal to the amount of impermeants in the cell divided
by the "effective" osmotic volume ($V_{\text{os}}$). Since the amount of impermeants is a constant:

$$CV_{\text{os}} = C^m V_{\text{os}}^\infty,$$  \hspace{1cm} (2)

where the superscript $\infty$ indicates the equilibrium value when $C$ must equal $C^m$. It is assumed that the cell volume can be related to the "osmotic" volume by the relation:

$$V_c = V_{\text{os}} + b V_c^{\text{iso}},$$  \hspace{1cm} (3)

where $b$ is a constant and $V_c^{\text{iso}}$ is the isotonic (290 mosmol) cell volume. The following relations can be derived from Eqs. 2 and 3:

$$V_c^{\text{iso}} = [(C^{\text{iso}}/C^m)(1 - b) + b] V_c^{\text{iso}};$$  

$$V_{\text{os}}^\infty = (C^{\text{iso}}/C^m)(1 - b) V_c^{\text{iso}}.$$  \hspace{1cm} (4)

Thus, given $b$ and the isotonic cell volume, the equilibrium cell and osmotic volume at any other osmolarity ($C^m$) can be determined. The values of the cell surface area ($A_c$) and the isotonic cell volume ($V_c^{\text{iso}}$) were assumed to be 137 $\mu$m$^2$ and 104 $\mu$m$^3$ (Jay, 1975).

The value of $b$ was determined by the following procedure. Solving Eq. 4 for $b$:

$$b = (V_c^{\text{iso}}/V_{\text{os}}^{\infty} - C_c^{\text{iso}}/C^m)/(1 - C_c^{\text{iso}}/C^m).$$  \hspace{1cm} (5)

Since the dried volume ($V_d$) of the cell is independent of the total cell volume, Eq. 6 can be rewritten as:

$$b = [(V_c/V_d)^{\text{iso}}/(V_c/V_d)^{\text{iso}} - C_c^{\text{iso}}/C^m)]/(1 - C_c^{\text{iso}}/C^m),$$  \hspace{1cm} (6)

where $V_c/V_d$ is the fractional dry volume of the cell. Cells were equilibrated in solutions of various osmolalities ($C^m$) and then packed in a low-speed centrifuge. Microhematocrit capillary tubes were filled with the packed cells and the cells were further packed in a microhematocrit centrifuge for 5 min. The capillary tube was broken in the cell region and the cells were blown out onto a coverslip, weighed, dried at 90°C for 48 h, and reweighed to determine $V_d/V_c$. It is assumed in these calculations that the dried cell has the same density as hemoglobin (1.35 gm/ml). Small corrections were made for the microhematocrit trapped volume ($\approx 2.9\%$) measured with $[14]$C]sucrose. This procedure also provides a direct measure of the true nonwater (dried) fraction of the isotonic cell volume ($f = V_d/V_c^{\text{iso}}$). In six experiments of this type we found an average $b = 0.46 \pm 0.01$ (SEM) and $f = 0.39 \pm 0.01$.

The value of $P_f$ is determined as follows. For the small volume changes used here (10%), it is assumed that the change in photocell signal ($S$) is proportional to the cell volume change (Levitt and Mlekoday, 1983):

$$S - S^\infty = D(V_c - V_c^\infty) = D(V_{\text{os}} - V_{\text{os}}^\infty).$$  \hspace{1cm} (7)

From Eq. 3:

$$\frac{dV_c}{dt} = \frac{dV_{\text{os}}}{dt}.$$  \hspace{1cm} (8)

Substituting Eqs. 2 and 9 into Eq. 1 yields a differential equation that can be integrated from 0 to $t$ to obtain (using Eq. 8):

$$\ln |S - S^\infty| + k(S - S^\infty) = -\alpha t + [\ln |S^\infty - S^\infty| + k(S^\infty - S^\infty)];$$  \hspace{1cm} (9)

$$k = 1/(DV_{\text{os}}^\infty);$$  \hspace{1cm} (10)

$$\alpha = \frac{V_c}{P_f A_c C^m/V_{\text{os}}^\infty}.$$
The value of $P_f$ can be determined from the slope of a plot of the left-hand side of Eq. 10 vs. $t$. The calibration constant ($D$) cannot be accurately determined directly from the light-scattering data because the injection artifact obscures the $t = 0$ data (when $V_c$ is known). To determine $P_f$, $D$ was first estimated from the $t = 0$ and $t = \infty$ light-scattering data; then Eq. 10 was plotted and extrapolated to $t = 0$ to obtain a better value for $D$, which was used to repeat the process. Because the second term on the left is much smaller than the first, $P_f$ has only a very weak dependence on $D$ and it is not necessary to measure $D$ accurately. For example, the value of $P_f$ determined from the rough estimate of $D$ and the best estimate of $D$ differed by $<2\%$.

**RESULTS**

**Injection Artifact**

Fig. 1A shows the light-scattering signal when cells equilibrated in 230 mosmol are challenged with an identical solution. Since there is no volume change in this experiment, the observed signal must be regarded as an injection artifact. This artifact seriously interferes with the $P_f$ measurement because it has about the same time course as the cell volume change (Fig. 1D). When the mixing chamber was examined microscopically during an injection, it was observed that the discoid cells became aligned in vortices set up by the injection. This suggested that the artifact could be reduced if the cells were spherical so that the light scattering would not depend on their spatial orientation. As predicted, the artifact was significantly reduced when the cells were swollen by equilibrating them at 160 mosmol (Fig. 1B).

We needed a procedure that would eliminate the artifact at all osmolarities, not just 160 mosmol. As shown in Fig. 1C, when the cells were treated with lecithin to make them echinocytes, the injection artifact was greatly reduced at 230 mosmol (and all osmolarities between 400 and 160 mosmol). This is expected since echinocytes are spherically symmetrical.

To test whether the lecithin treatment itself had any effect on $P_f$, we compared the $P_f$'s of lecithin-treated and untreated cells, both of which were swollen in 160 mosmol. The $P_f$'s for the treated and untreated cells differed by $<2\%$. The $P_f$'s could not be compared at other osmolarities because the injection artifact of the untreated cells interfered with the measurement. However, it seems unlikely that lecithin treatment would only affect the $P_f$ of unswollen cells.

**$P_f$ Measurements**

Fig. 2 shows a typical plot of the left-hand side of Eq. 10 vs. time. The osmotic permeability ($P_0$) is determined from the slope of this line. The experimental data points were measured directly from the photograph of the oscilloscope recording of the photocell output. It can be seen that the data can be fit nearly perfectly by a straight line, as predicted by Eq. 10. Any nonlinearity in the relation between cell volume and light scattering (Eq. 8) should show up as a deviation from the straight line in Fig. 2. No such deviation was detected at any of the osmolarities that were investigated. This implies that Eq. 8 is valid, at least over the volume changes used in this study. The total volume change
was always <10% and the volume change represented by data points in Fig. 2 is \( \sim 60\% \) of the total volume change.

Fig. 3 shows the \( P_f \) of lecithin-treated cells for different osmolarities and direction of flow (shrinking or swelling experiments). Each point represents the average (and standard deviation) of about four measurements made on the same blood sample. The data represent a total of 12 different blood donations carried out over a period of \( \sim 6 \) mo. All the points in Fig. 3 are from blood from one donor (H.L.M.). Measurements on blood from two other donors were within the standard deviation of the data in Fig. 3. There is no significant correlation between the \( P_f \) for swelling experiments and the osmo-

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**Figure 1.** Photocell signal as a function of time (abscissa) photographed directly from the storage oscilloscope. The sweep is initiated when the flow is stopped. For each pair of traces, the horizontal trace is the equilibrium value. For all four pictures, the scale on the abscissa is 0.2 s/div. The scale on the ordinate is either 5 or 20 mV/div. A. Cells (no lecithin) in 230 mosmol challenged with an identical solution. Four separate experiments are shown. B. Cells (no lecithin) in 160 mosmol challenged with an identical solution (three experiments). C. Lecithin-treated cells (echinocytes) in 230 mosmol challenged with an identical solution (four experiments). D. Lecithin-treated cells in 180 mosmol challenged with an equal volume of 260 mosmol (final osmolarity of 220 mosmol).
larities. There is a small but significant \((P < 0.01)\) correlation between the shrinking \(P_f\) and the osmolarity. This correlation depends on the single point at 395 mosmol. If this point is not included, the correlation is no longer significant \((P > 0.05)\). Grouping the data, the mean and standard error of \(P_f\) are \(2.03 \pm 0.025\) (SEM) \(\times 10^{-2}\) cm/s \((n = 19)\) for the shrinking data and \(1.93 \pm 0.033 \times 10^{-2}\) \((n = 12)\) for the swelling data. This difference is significant at the \(P = 0.02\) level. The data shown in Fig. 3 are for the absolute value of \(P_f\). If the data for each set of experiments on the same cells are normalized for the \(P_f\) at a standard osmolarity (shrinking experiments at \(\sim 217\) mosmol), then the spread in the values is reduced by \(\sim 50\%\).

**Figure 2.** Plot of the left-hand side of Eq. 10 vs. time. The line is the least-squares fit to the data points (*). The photocell signal \((S)\) is measured in inches on the photograph of the oscilloscope record.

**DISCUSSION**

Although it seems clear from the results of Fig. 1 that it is the spherical asymmetry that is responsible for the injection artifact, the exact mechanism is uncertain. One mechanism that can definitely be ruled out is that the cells are aligned by the injection and then the light-scattering changes as the cells become randomly oriented. As shown theoretically and experimentally by Oster and Zulusky (1974), the time for oriented discoid red cells to become randomly oriented is 1–2 min, much longer than the time scale of the signal in Fig. 1A (\(\sim 1\) s). A more probable mechanism is that the signal is associated
with the cells becoming oriented in the vortices set up by the injection. Another possibility is that the flow pattern changes during this time period, changing the alignment of the cells in the light path. This injection artifact has roughly the same time constant and is ~25% as large as the signal associated with a 10% volume change during a Pf experiment (compare Figs. 1A and D). Thus, this artifact could produce a large error in the Pf determination. Since the size of the artifact decreases as the cell becomes more spherical, this could explain, at least in part, the variation of Pf with osmolality or direction of flow that has been observed. In the recent measurements of

Terwilleger and Solomon (1981), this artifact was corrected for by subtracting the control (no volume change) from the experimental light-scattering curve. When this artifact is eliminated by treating the cells with lecithin to make them echinocytes, we find that Pf has only a small, if any, dependence on osmolality or direction of flow (Fig. 3). The accuracy of the light-scattering method used here is limited by at least two effects. First, there may be a small (5%) nonlinearity in the relation between light scattering and cell volume that could not be detected in these experiments. This nonlinearity would tend to introduce errors of opposite sign into the shrinking and swelling experiments.
Second, the assumption (see Eqs. 2 and 3) that is used to determine the cell volume as a function of the osmolarity is only an approximation and its validity should be a function of cell volume. This uncertainty (5–10%) in the measurement of \( P_f \) is an inherent limitation of the light-scattering method. Since these errors are systematic, they cannot be reduced by increasing the number of data points. Thus, the observed 5% variation in \( P_f \) with osmolarity and direction of flow over the range of 165–395 mosmol is within the limits of accuracy of this method. The most one can say with certainty is that \( P_f \) is nearly constant for the human red cell, varying by no more than 10% as a function of the direction of flow (shrinking/swelling) or the cell volume. This conclusion is in general agreement with the recent results of Terwilliger and Solomon (1981). The average \( P_f \) is \( 2.0 \times 10^{-2} \) cm/s. This value is for cells from a single donor (H.L.M.) and is well within the normal range of Terwilliger and Solomon (1981).

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