Reflection Coefficients of Permeant Molecules in Human Red Cell Suspensions

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ABSTRACT The Staverman reflection coefficient, $\sigma$ for several permeant molecules was determined in human red cell suspensions with a Durrum stopped-flow spectrophotometer. This procedure was first used with dog, cat, and beef red cells and with human red cells. The stopped-flow technique used was similar to the rapid-flow method used by those who originally reported $\sigma$ measurements in human red cells for molecules which rapidly penetrate the red cell membrane. The $\sigma$ values we obtained agreed with those previously reported for most of the slow penetrants, except malonamide, but disagreed with all the $\sigma$ values previously reported for the rapid penetrants. We were unable to calculate an "equivalent pore radius" with our $\sigma$ data. The advantages of our equipment and our experimental procedure are discussed. Our $\sigma$ data suggest that $\sigma$ is indirectly proportional to the log of the nonelectrolyte permeability coefficient, $\omega$. Since a similar trend has been previously shown for log $\omega$ and molar volume of the permeant molecules, a correlation was shown between $\sigma$ and molar volume suggesting the membrane acts as a sieve.

The osmotic pressure for a molecule which exerts its full osmotic effect is given by van't Hoff's classical equation, $\pi_{theory} = CRT$, where $\pi$ is osmotic pressure, $C$ is concentration, and $R$ and $T$ have their usual meanings. This relationship holds for molecules which do not penetrate the membrane. Permeant molecules do not exert their full osmotic effect, as reported by Staverman (1) who defined the reflection coefficient, $\sigma$, as being equal to the observed osmotic pressure, $\pi_{obs}$, divided by $\pi_{theory}$. The reflection coefficient can also be shown (2) to be:

$$\sigma = \frac{C_i}{C_o}. \quad (1)$$

Here $C_i$ is the concentration of the red cell isotonic buffer and $C_o$ is the extracellular permeant molecule concentration at which no cell volume change occurs. Values for $\sigma$ usually range between zero for molecules which are as permeable as water and unity for solutes to which the membrane is impermeable.
able. Negative σ values can occur if the solute is more permeant than the solvent (3). Therefore, reflection coefficients directly indicate the ability of a membrane to distinguish between solvent and solute molecules.

Goldstein and Solomon previously determined σ with a rapid-flow technique (2). They obtained changes in red cell volume as a function of time by varying the length of tubing between the mixing and light-scattering chambers. The red cell volume was determined at 40, 90, 140, and 190 ms after mixing an isosmolar red cell suspension with a set of permeant molecule solutions, including either 0.2, 0.3, and 0.4 M nonelectrolyte or 0.3, 0.4, and 0.5 M nonelectrolyte, where the latter set was used with nonelectrolytes which rapidly penetrated the cells. Goldstein and Solomon (2) interpreted their results with a "zero-time method" by plotting the slope of a cell volume vs. time graph as a function of permeant solute concentration and interpolating to zero volume change to find Cσ. Their Cσ values were considerably higher than the isosmolar buffer concentration, Cb, and this resulted in σ being much less than unity.

The rationale for repeating the earlier σ work of Goldstein and Solomon (2) and of Sha'afi et al. (4), using the stopped-flow technique of Rich et al. (5) is that recent articles by Stein (6), Lieb and Stein (7), Forster (8), and Eilam and Stein (9) have questioned the consistency of these earlier σ results.

**Experimental Methods**

Red cells were obtained by venipuncture from healthy human donors. The heparinized blood was centrifuged to separate red cells from plasma, washed twice with isosmolar buffer, and resuspended in buffer to make a red cell volume fraction of about 0.2%. The isosmolar buffer had a pH of 7.4 and contained 4.7 mM KH₂PO₄, 12 mM Na₂HPO₄, and enough NaCl (~135 mM) to give a 300 mosmol/kg solution. All of the nonelectrolytes were reagent grade. The osmolality of the nonelectrolyte solutions was measured with an Advanced Digimatic Osmometer, model 3D (Advanced Instruments, Inc., Needham Heights, Mass.), which had a precision of about ±1%.

A Durrum stopped-flow spectrophotometer (Durrum Instrument Corp., Palo Alto, Calif.) with a mixing time of about 5 ms was utilized to monitor the relative intensity of 550-nm light scattered at 90°. A diagram of our system is shown in Fig. 1 and operation has been previously described (10).

Under normal operating conditions, a stopped-flow apparatus has a mixing artifact sufficiently large to preclude accurate measurement of relative cell volume changes before 150 ms after mixing (11). Blum and Forster (12) have even reported mixing artifacts lasting 300 ms upon mixing isotonic buffer with a red cell suspension in a stopped-flow rapid-reaction apparatus. Solomon's group typically subtracts out "mixing controls," which includes mixing an equal volume of isosmolar red cell suspension with an isosmolar glucose solution (4). They reported complicated but reproducible changes in scattered light in their mixing controls, but not enough data
were given to make a direct comparison with our mixing artifact. Subtracting mixing controls was not considered a satisfactory procedure by Jain et al. (13) who concluded that using a syringe ratio of 10 vol of a permeant molecule: 1 vol of a red cell suspension and enlarging the smaller syringe nozzle diameter to 1.5 mm greatly reduced the stopped-flow mixing artifact. We have used a syringe ratio of approximately 8:1 by using the “C” syringe (~3.1-mm ID) from Durrum-Gibson, Inc., kit no. 13838 for the red cell suspension along with the standard syringe (~8.7-mm ID) for the nonelectrolyte solution. This arrangement worked well for our experiments as shown by the relatively level “control curves” in Figs. 2 a, 3 a, and 4 a.

RESULTS AND DISCUSSION

Our mixing artifact consistently occurred during the initial ~20 ms when nonelectrolyte was mixed with red cells (Fig. 2 a). This implies that our artifact is not a phenomenon intrinsically related to the rapid stopping of a flowing red cell suspension, but it is probably due to a nonelectrolyte-cell interaction, e.g., possibly a change in cell surface index of refraction. Only a slight mixing artifact was detected when nonelectrolyte was mixed with isotonic buffer solution without red cells.

Reflection Coefficients for Slowly Penetrating Molecules

Table I shows that our average \( \sigma \) value of 0.88 for glycerol precisely agrees with the value reported by Goldstein and Solomon (2) from their zero-time method. Malonamide penetrates red cells much more slowly than glycerol, e.g., from recent hemolysis data (15) the permeability coefficients, \( \omega \), for malonamide and glycerol are \( 0.004 \times 10^{-14} \text{ mol/dyn-s} \) and \( 0.23 \times 10^{-16} \text{ mol/dyn-s} \), respectively. It has recently perplexed investigators (7, 8) that malonamide should have a \( \sigma \) of 0.83 compared to a \( \sigma \) for glycerol of 0.88 (2), since one would expect \( \sigma \) to approach unity for practically impermeant molecules such as malonamide. Yet DiPolo et al. (16) did not find it surprising that
Figure 2. (a) The effect of different malonamide concentrations on the rate of change in intensity of 90° scattered light from a red cell suspension, \( dV/dt \), where \( V \) is the response of the photomultiplier tube in volts. The light-to-dark voltage was 6 V, and therefore the 2% calibration mark corresponds to 120 mV. Since red cell volume is indirectly proportional to the amount of 90° scattered light (14, 15), we use photomultiplier voltage as an index of cell volume, i.e., \( \Delta \) voltage \( \propto \) cell volume or swelling, and \( \Delta \) voltage \( \propto -\Delta \) cell volume or shrinking. The control trace represents the rapid mixing of ~8 volumes of isotonic buffers with 1 vol of an isotonic red cell suspension. (b) The negative of the slopes of the tangents drawn to the curves in a between approximately 20–150 ms plotted as a function of malonamide concentration. The line drawn through the data points is a least-squares line. From Eq. 1, \( \sigma \) in this representative experiment is equal to 305/287 or 1.07, where 305 mosmol/kg is the concentration of the red cell isotonic buffer and 287 mosmol/kg is from interpolation at zero-voltage derivative or relative volume change.

Two molecules with different \( \omega \) did not have different \( \sigma \) values in their cellulose acetate membranes. They pointed out that \( \sigma \) discriminated between solute and solvent, whereas \( \omega \) measures solute flux. It might be equally plausible to expect that there is a direct relationship between solute-solvent interactions and solute permeability, and as a molecule becomes less permeant \( \sigma \) would approach unity. A similar suggestion was discussed by Forster (8).

The effect of rapidly mixing a series of malonamide solutions with a red cell suspension is shown in Fig. 2 a. The amount of 90° scattered light, as measured by the response of the photomultiplier tube in units of volts, is related to the relative cell volume (4, 13). It is interesting to note that red cells scatter more light when they shrink and less light when they swell as...
Table I

| Molecule       | Mean ± SEM | No. of exp | σ*     | Mean ± SEM | No. of exp | Mean ± SEM | No. of molec. radius | 10⁻¹⁵ mol/dyn-s | No. of exp |
|----------------|------------|------------|--------|------------|------------|------------|----------------------|-----------------|------------|
| Sodium chloride| 0.98 ± 0.02 (4) | —         | —      | —          | —          | —          | —                    | —               | —          |
| Malonamide     | 1 ± 0.04   | (3)        | 0.83 ± 0.04 (4) | 2.57      | 0.008%     | (4)        | 2.03                 | 11 ± 1          | (4)        |
| Urea           | 0.79 ± 0.02 | (7)        | 0.62 ± 0.02 (3) or 2.05 | 11 ± 1 | 0.008%     | (4)        | 2.03                 | 11 ± 1          | (4)        |
| Glycol         | 0.88 ± 0.02 | (5)        | 0.88 ± 0.02 (5) | 2.74      | 0.46%      | (4)        | 2.03                 | 11 ± 1          | (4)        |
| Methylurea     | 0.85       | (1)        | 0.80 ± 0.02 (5) | 2.18      | 3.2 ± 1.1 | (2)        | 2.03                 | 11 ± 1          | (4)        |
| Acetamide      | 0.80 ± 0.03 | (6)        | 0.56 ± 0.03 (6) | 2.03      | 2.9 ± 0.4 | (4)        | 2.03                 | 11 ± 1          | (4)        |
| Ethylene glycol| 0.96 ± 0.03 | (6)        | 0.63 ± 0.03 (6) | 2.24      | 2.4 ± 0.5 | (3)        | 2.03                 | 11 ± 1          | (4)        |
| Thiourea       | 0.91 ± 0.01 | (2)        | 0.85 ± 0.02 (5) | 2.18      | 0.6 ± 0.01 | (4)        | 2.03                 | 11 ± 1          | (4)        |
| Propionamide   | 0.84       | (1)        | 0.80 ± 0.03 (4) | 2.31      | 2.9 ± 0.4 | (4)        | 2.03                 | 11 ± 1          | (4)        |
| 1,2-Propanediol| 0.85       | (1)        | 0.85 ± 0.04 (3) | 2.61      | 1.1±1     | (4)        | 2.03                 | 11 ± 1          | (4)        |

SEM denotes the standard error of the mean.
* This paper.
† Goldstein and Solomon (2).
‡ Galey et al. (24).
§ From Naccache and Sha'afi (15), but multiplied by two to approximately correct the hemolysis data. This appears reasonable when their other ω values are compared with older and more reliable ω values (23, 24).
¶ Sha'afi et al. (4).
** Owen (unpublished data).
†† Calculated by assuming a 50% increase ω = 0.73 ± 0.2 × 10⁻¹⁵ mol/dyn-s for 1,3-propandiol which is the approximate trend reported (23) for 1,3 butanediol, ω = 2.0 ± 0.3 × 10⁻¹⁵ mol/dyn-s, and 2,3-butanediol, ω = 3.1 ± 0.8 × 10⁻¹⁵ mol/dyn-s.

Previously reported (10, 14). To determine the malonamide concentration at which no cell volume change occurs: (a) the slopes of the tangents to the light-scattering curves, dV/dt, (Fig. 2 a) were drawn from about 20–150 ms, (b) these slopes were multiplied by (−1) to convert relative voltage change into relative volume change and plotted as a function of malonamide concentration (Fig. 2 b); and (c) the concentration where the slope −dV/dt was zero, C0, was found by interpolation to be 287 mosmol/kg, a σ = 1.07 was calculated from Eq. 1.

Reflection Coefficients for Rapidly Penetrating Molecules

The data for urea and acetamide are shown in Figs. 3 and 4, respectively, and σ values for these molecules as well as ethylene glycol are listed in Table I. The data for these molecules represent dramatically different results from those reported by Goldstein and Solomon (2) and Sha'afi et al. (4). Our σ values for urea, acetamide, and ethylene glycol were approximately 0.8, whereas the previously reported (2, 4) σ values were about 0.6.

Reflection Coefficients for Other Molecules

Table I shows that σ data for methylurea, thiourea, propionamide, and 1,2-propanediol, which have ω values intermediate between the slow penetrant
Figure 3. (a) Same as Fig. 2 a, except urea instead of malonamide. Also, it was necessary to use a shorter time range (~20–130 ms) to determine the red cell light-scattering slopes due to water movement, since urea begins to penetrate the cells at about 130 ms. (b) Same as Fig. 2 b, except urea instead of malonamide and $\sigma = 305/404 = 0.75$.

malonamide and the rapid penetrant urea. There was good agreement between our $\sigma$ data (Table I) and the previously reported $\sigma$ results for these molecules (2).

Analysis of Reflection Coefficient Data

Goldstein and Solomon (2) plotted their $\sigma$ data as a function of the permeant molecule radius, and using the Renkin equation (2, 17) they fitted a curve to data corresponding to an equivalent pore radius of 4.2 Å (see their Fig. 5 [2]). Although this procedure is apparently valid for membranes with large pores (~20 Å) (18), the soundness of such a treatment for red cells has been questioned by others (6, 19). Also, their Fig. 5 (2) is essentially two clusters of widely dispersed data points. One group of points in their Fig. 5 represents a $\sigma$ of about 0.85 for glycerol, thiourea, malonamide, 1,3-propanediol, propionamide, and methylurea and the second group represents $\sigma$ of approximately 0.6 for urea, acetamide, and ethylene glycol.
Fig. 5 is a plot of our $\sigma$ data against molecular radii previously reported by Goldstein and Solomon (2). They calculated mean molecular radii by measuring along three perpendicular axes of molecular models while the models were in their largest and smallest steric configurations. We do not feel there
is sufficient reason to accept the Renkin treatment for our red cell reflection coefficient data, as exemplified by our scatter-gram of \((1 - \sigma)\) vs. radius of permeant molecule. Instead of relating \(\sigma\) to the molecular radius, which was determined by several approximating steps, we decided to relate \(\sigma\) to another experimentally measured quantity. Fig. 6 shows our plot of \((1 - \sigma)\) as a function of the log of the permeability coefficient, \(\omega\). As \(\omega\) increases, \((1 - \sigma)\) also increases, indicating that the more the permeant molecule interacts with water the more rapidly it penetrates the red cell membrane. Further speculation concerning the existence or nonexistence of membrane pores would not be supported by our experimental data.

\[ y = 0.060x - 0.041 \]

**Figure 6.** A plot of the data in Table I of \((1 - \sigma)\) as a function of log \(\omega\). The line \((y = 0.060x - 0.041)\) was drawn by the method of least squares.

*Calculation of \(\sigma\) for Urea from the Data of Sha’afi et al. (4)*

Forster (8) recently pointed out that \(\sigma\) for urea at the red cell minimum volume point could be estimated from the data of Sha’afi et al. (4). Forster reported that by “back-calculating from the average intramembrane concentration, \(\bar{C}_i\), which Sha’afi et al. gave in their article... the value of \(\sigma\) obtained is closer to unity than to 0.55, the value reported elsewhere in the same article resulting from interpolation of initial rates of volume change as a function of extracellular urea concentration.” Further examination\(^1\) of the

\(^1\)Kedem and Katchalsky (26) give the following equation for solvent and uncharged solute flow across membranes:

\[ J_V = -L_p\Delta\pi_i + L_{pd}\Delta\pi_s. \]  

\(J_V\) is the volume flow per unit area of membrane. \(\Delta\pi_i\) and \(\Delta\pi_s\) are the osmotic pressures due to the impermeant and the permeant solutes, respectively. \(L_p\) is the hydraulic conductivity of the membrane. \(L_{pd}\) is the cross-coefficient for the volume flow.
Sha'afi et al. data gives a $\sigma$ for urea of approximately 0.8 which agrees with our experimentally observed $\sigma$ value for urea (Table I and Fig. 3), but our calculation involves several approximations as also pointed out by Forster in his treatment (8).

At zero volume change, which is the condition for which $\sigma$ is determined, Eq. a becomes:

$$\frac{L_{pd}}{L_p} = \frac{\Delta \pi_1}{\Delta \pi_s}.$$  \hspace{1cm} (b)

From the definition of $\sigma = -L_{pd}/L_p$ (26) and Eq. b

$$\sigma = -\frac{\Delta \pi_1}{\Delta \pi_s}.$$ \hspace{1cm} (c)

Applying van't Hoff's equation $\Delta \pi = \Delta CRT$ to Eq. c

$$\sigma = \frac{C_i^{\Delta x} - C_i^0}{C_s^0 - C_s^{\Delta x}}.$$ \hspace{1cm} (d')

$\Delta x$, $\sigma$, $i$, and $v$ denote intracellular, extracellular, impermeant, and permeant concentrations, respectively. $C_i^0$ is determined by adding the external buffer concentration to the NaCl concentration in the permeant molecule solution (4).

The average distribution of the permeant solute across the membrane, $C_s$, is defined (26) as:

$$C_s = \frac{C_s^0 - C_s^{\Delta x}}{\ln \frac{C_i^{\Delta x}}{C_i^0}}.$$ \hspace{1cm} (e)

If one chooses a minimum volume time of 0.15 s for the time-course of the cell volume change in the presence of urea (Fig. 3 of Ref. 4), as Forster similarly chose (8), then $C_i$ becomes 340 mosmol/kg according to Fig. 4 of Ref. 4. Using $C_i^{\Delta x} = 306$ mosmol/kg from Eq. $e$, $C_i^{\Delta x} = 270$ mosmol/kg (44) (average value from their range of 260–280 mosmol/kg), $C_i = ([270/2] + [100/2])$ mosmol/kg, and $C_s^{\Delta x} = 750/2$ mosmol/kg, where the division by two is due to the mixing with an equal volume of a red cell suspension, Eq. d yields:

$$\sigma = 1.2.$$ \hspace{1cm} (d'')

There is sufficient scatter in the data points so it is equally plausible to choose a minimum volume time of 0.13 s (Fig. 3 of Ref. 4). Also, a minimum volume time of 0.13 s would be consonant with our minimum volume time from our urea light-scattering curves in Fig. 3 a (see the bottom trace of Fig. 3 a; this represents a urea concentration of 913 mosmol/kg which, as far as osmolality best mimics the 750 mosmol/kg urea plus 100 mosmol/kg NaCl solution used in Fig. 3 of Ref. 4). This yields a $C_i = 320$ mosmol/kg (Fig. 4 of Ref. 4) and a $C_i^{\Delta x} = 270$ mosmol/kg from Eq. $e$; so Eq. d gives

$$\sigma = 0.8.$$ \hspace{1cm} (d''')

One can conclude that a calculated value for $\sigma$ is highly dependent upon the choice of $C_i$, which in turn is dependent upon the minimum volume time. In order to arrive at a calculated $\sigma$ for urea which would agree more closely with the previously reported experimental $\sigma$ values of 0.62 (2) and 0.55 (4), a minimum volume time of about 0.10 s would be necessary.
Possible Reasons for Discrepancies Between Our σ Data and the σ Data of Goldstein and Solomon²

The rapid-flow apparatus used by Goldstein and Solomon (2) had two crucial restrictions: (a) the earliest possible cell volume data point could not be taken until 40 ms, and (b) the time interval between data points was extremely long, i.e. 50 ms. In case of a rapidly permeant molecule such as urea, cell volume data points due to water movement cannot be taken beyond 130 ms, since urea permeability begins to predominate (see Fig. 3 a). If data points beyond 130 ms were included in the zero-time slope measurements with rapidly permeant molecules like urea, then the resulting slopes would be higher and lower for hypo- and hypertonic nonelectrolyte solutions, respectively. This would yield a higher Cᵣ value and consequently a lower σ value. If this was not the case, it suggests only two cell volume data points were used by Goldstein and Solomon in the original urea σ experiments. Several data points, i.e., at 40, 90, 140, and 190 ms, could be taken with slowly permeant molecules (see their Fig. 2 [2], as well as our Fig. 2 a). Even with slowly permeant molecules such as glycerol and malonamide, the determination of the zero-slope is still somewhat subjective, e.g. the slope for the 0.5 M glycerol-red cell suspension curve in Fig. 2 of Goldstein and Solomon (2) appears to be drawn through only one point. This might be a possible source of error with the very slowly permeating molecule malonamide, although it did not cause a discrepancy in our σ for glycerol as compared with the previously reported glycerol σ value (2).

In the original determination of σ for red cell permeant molecules (2), only three different nonelectrolyte concentrations were mixed with the red cell suspension. These concentrations were either 0.2, 0.3, and 0.4 M, or 0.3, 0.4, and 0.5 M, chosen so that the first solution would cause the red cells to swell, the second would cause no cell volume change, and the third would shrink the cells. Normally, one would wish to have more than three points to characterize a phenomenon, and choosing three points so close to the concentration at which no cell volume change occurs reduces the amplitude of the zero-time slope and could decrease the accuracy of the σ data.

Possible Reasons for Discrepancies between Our σ Data and the σ Data of Sha’afi et al. (4)

Although Sha’afi et al. (4) did three separate σ experiments with as many as eight different urea concentrations, different σ values can be obtained from their data depending upon which data points are analyzed. Fig. 7 represents

² When this paper was being reviewed Levitt (1974. Biochim. Biophys. Acta. 373:115-131) reported similar discrepancies on the basis of his computer simulation of the σ data of Goldstein and Solomon (2).
Reflection Coefficients in Human Red Cell Suspensions

| MEDIUM (mosmol/kg) | UREA (mosmol/kg) |
|-------------------|-----------------|
| (AFTER MIXING)    | (BEFORE MIXING) |
| 224               | 109             |
| 323               | 307             |
| 420               | 501             |
| 519               | 699             |
| 607               | 875             |
| 702               | 1065            |

Figure 7. Light-scattering data of Dr. G. T. Rich (used with her kind permission) from which Sha'afi et al. (4) reported a urea \( \sigma \) of 0.55. Their zero-time slopes are represented by dashed lines and our slopes of their data are shown as solid lines. See Discussion in text concerning the discrepancies in these slopes. The traces for 699 and 1,065 mosmol/kg urea are at a relative computer of average transients (CAT) reading sensitivity which is twice as high as the other traces. Up in this figure is towards increasing cell volume although decreasing not increasing, scattered light intensity or CAT reading would normally be observed, as pointed out previously (10).
the previously unpublished data for experiment 17H-12/8/66 (used with the kind permission of Dr. G. T. Rich), from which they obtained a urea \( \sigma \) of 0.55 (Table III of Ref. 4). Fig. 8 shows their slope data from Fig. 7 plotted as a function of medium concentration (dashed line) which is similar to their data (open circles) in Fig. 7 of Ref. 4. They drew their zero-time slopes through the data points in approximately the initial 25 ms, as shown by their dotted lines in Fig. 7. Their slopes were drawn in such a manner that it appears considerably more emphasis was placed on the first few data points in the traces obtained with hypotonic urea solutions than with hypertonic solutions. The first 10 ms of most of their traces in Fig. 7 show a large bi-

![Figure 8](image)

**FIGURE 8.** The zero-time slope data of Fig. 8 plotted as a function of medium concentration. A urea \( \sigma \) of 0.55 was obtained from their interpretation of their data (dashed line) and a urea \( \sigma \) of 0.8 was calculated from our interpretation of their data (solid line) by using Eq. 1.

phasic phenomenon, suggesting that this portion of the traces should not have been considered to be due to osmotic changes in cell volume by Sha'afi et al. (4). This biphasic effect also manifests itself in their published hypertonic urea-red cell shrinking and swelling time-course trace (Fig. 3 of Ref. 3).

The dead time, or the time which elapses between the mixing and the arrival of the mixed red cells and nonelectrolyte at the observation chamber, was about 11 ms for their stopped-flow apparatus (20). They routinely subtracted mixing control consisting of 0.3 M glucose to reduce the initial "noise" due to mixing. Yet this does not necessarily ensure this initial net result was due to osmotic cell volume change, especially if volume change was greatly dominated by some other effect. Also, the experimental scatter in the nonelectrolyte data and the mixing control data is large with their
experiment, so the difference results in a very scattered set of initial data points (Owen, unpublished data, 1972).

For the reasons outlined above, we did not use the initial 10 ms of data points when we interpreted their exp. 17H. Our zero-time slopes for their data in Fig. 7 are indicated by the solid lines, which are least-square lines drawn through the 10-40- and 10-30-ms data points for the swelling and shrinking traces, respectively. This procedure appeared to give the largest slope which was due to water movement across the cell membrane. Fig. 8 represents our slope data from Fig. 7 plotted as a function of total medium concentration (solid line). This conforms to the procedure used by Sha'afi et al. (4), and it is similar to our previous figures except the nonelectrolyte as well as the buffer concentration is plotted. Our interpretation of their data gives a urea $\sigma$ of 0.8, which agrees with our results in Fig. 3 and Table I.

**Mechanism of Red Cell Nonelectrolyte Permeation**

At least six reasons can be listed as "proof" that hydrophilic channels or aqueous "pores" exist in red cell membranes which are different from lipophilic pathways: (a) The ratio of the osmotic permeability coefficient to water diffusion permeability coefficient is greater than 1 (21). (b) The activation energy for osmotic permeability is similar to the activation energy value for the bulk flow of water in free solution (22). (c) The reflection coefficients for small nonelectrolytes are less than $\sigma$ values for larger permeant molecules (2). (d) Small nonelectrolytes penetrate the red cell membrane according to the molar volume of the permeant molecule (23). (e) Small hydrophilic nonelectrolytes have lower temperature coefficients than larger lipophilic molecules (24). (e) Phloretin at 0.25 mM inhibits permeation of hydrophilic molecules, but it enhances the permeability of lipophilic nonelectrolytes (25, 10).

Since our results summarized in Fig. 6 indicate a correlation exists between log $\omega$ and $(1 - \sigma)$ and it has previously been shown (23) that a similar correlation exists for log $\omega$ and molar volume of small hydrophilic molecules (formamide, urea, acetamide, methylurea, and dimethylurea), a general trend would be expected for $(1 - \sigma)$ and molar volume. Table II shows that for most hydrophilic molecules the value for $\sigma$ decreases as the molar volume of the permeant molecules decreases. This trend could, therefore, be thought of as a combination of observations $c$ and $d$ listed above, and might be considered additional support for aqueous pores, although we are unable to calculate dimensions of such pores with our $\sigma$ data.

We wish to thank reviewer no. 2 for suggesting that a correlation might exist between our $\sigma$ data and the molar volume of the permeant molecules.

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TABLE II

COMPARISON OF HUMAN $\sigma$ WITH MOLAR VOLUME

| Nonelectrolyte     | $\sigma$ (Table I) | Molar volume | $k_{ether}$ |
|--------------------|---------------------|--------------|-------------|
| Urea               | 0.79±0.02           | 45           | 0.00047     |
| Acetamide          | 0.80±0.03           | 59.1         | 0.0025      |
| Ethylene glycol    | 0.86±0.03           | 56           | 0.0053      |
| Methylurea         | 0.85                | 61.5         | 0.0012      |
| Propionamide       | 0.84                | 70.1         | 0.013       |
| 1,2-Propanediol    | 0.85                | 73.2         | 0.018       |
| Glycerol           | 0.88±0.02           | 73.1         | 0.00066     |
| Thiourea           | 0.91±0.01           | 54.2         | 0.0063      |
| Malonamide         | 1±0.04              | —            | 0.00030     |

The values for $k_{ether}$ were from Collander (27).

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