Water Permeability of the Fetal Erythrocyte

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ABSTRACT The rate constant for the diffusion of tritiated water across the fetal erythrocyte membrane has been measured as 0.056 ± 0.013 msec⁻¹. The equivalent pore radius calculated by the method of Paganelli and Solomon for the fetal erythrocyte is 3.9 Å. The effect of a normal distribution of channel sizes within the erythrocyte membrane is discussed and the theoretical effect of variation of the distribution on the diffusion to bulk flow ratio is evaluated. Since membrane channels of 4 Å characterize a variety of membranes, it is suggested that permeability differences may be associated with slight variations in the statistical distribution of the channel sizes within the membrane. The limits of ±0.5 Å standard deviation that qualitative experiments place on the expected variability of channel size will not account for the lower rate of water diffusion in the fetal cell.

There is experimental evidence indicating that the erythrocytes associated with intrauterine and neonatal life possess membrane permeability characteristics that differ from those found in the adult erythrocyte (1). The present experiments were undertaken to measure the rate of diffusion of tritiated water across the membrane of the fetal red blood cell. Using a molecule such as water, which is small relative to the molecular structure of the membrane and which moves passively across the membrane, subtle alterations in the membrane structure can be explored and presumably will be reflected in an altered rate of water diffusion.

EXPERIMENTAL PROCEDURE

Materials and Methods

To determine the kinetic curve for diffusion of tritiated water across the erythrocyte membrane, the method of Paganelli and Solomon (2) was used. In this system blood and tritiated water (THO) labeled buffer are rapidly mixed and forced at a velocity of about 900 cm per sec. along a tube of precisely known diameter. Provision is made for the collection of cell-free samples of the extracellular water at measured distances from the point of mixing. From the velocity of flow of the blood-buffer mixture, the cross-sectional area of the tube, and the distances separating the sampling ports, the duration of THO diffusion for each sample can be calculated relative to the time
of mixing. The device used in these experiments was modified from the apparatus described by Paganelli and Solomon to allow measurements to be made on 50 ml samples of blood. The critical dimensions that were altered are as follows: The mixing chamber was constructed with opposed jets 0.159 cm in diameter with the central mixing chamber 0.318 cm in diameter and 0.159 cm in depth. The tube along which the samples were collected was 0.159 cm in internal diameter and had ten collection ports positioned 1.5 cm apart. Compressed gas at 100 psi was used to drive the blood and buffer through the system, and at this pressure the velocity of flow was approximately 900 cm per sec. The components of the original system were changed to allow a tenfold reduction in the volume of blood required for an experiment. With these changes the following studies were carried out to insure that adequate mixing would occur in the new system:

(a) The velocity at which turbulent flow could be expected was estimated at 875 cm per sec. by Reynolds' equation. In all the experiments the velocity of flow was in excess of 900 cm per sec. at 100 psi. (b) The plot of the pressure–volume flow relations for the new system was linear for flow rates below 750 cm per sec.; above 750 cm per sec. a linear relationship was obtained when flows were plotted against the square root of the applied pressure. (c) In two experiments a sodium-labeled buffer was used. The mixing of blood and buffer, sample collection, and counting were done in the usual manner. The slope of the plot of specific activity of the extracellular fluid against time was essentially zero, thereby indicating homogeneous mixing along the collecting system. (d) A comparison was made of the adult erythrocyte diffusion rate constant as determined on the rapid flow system with a mixing chamber of the original dimensions of Paganelli and Solomon, and the rate constant measured on the modified device used in the present experiments. In a series of seven experiments with the original equipment (Biophysical Laboratory, Harvard Medical School), the adult erythrocyte diffusion constant was 0.087 ± 0.024 msec⁻¹ (4). In ten experiments using the present miniaturized device the adult erythrocyte diffusion rate constant for water was 0.091 ± 0.022 msec⁻¹. These values for the adult erythrocyte diffusion constant differ from the value reported in Reference 2. In the experiments reported in Reference 2, the initial specific activity at time zero was calculated from values of the hematocrit before and after mixing. The inconstancy of hematocrit determinations led to a spurious elevation in the computed value of the zero time specific activity, and ultimately in k/vq. In the present experiments a value for the zero time specific activity is obtained by extrapolation from the ten individually determined points.

The samples of extracellular water were collected in capillary pipettes positioned over the ten ports in the flow tube. The initial sample of extracellular water was discarded in each experiment before the capillary pipettes were positioned, and collection was accomplished after the blood-buffer had reached its terminal velocity. The samples collected were slightly in excess of 10 μl. 10 μl samples were transferred directly into scintillation counting vials using a single Misco constriction pipette of the type manufactured by the Microchemical Specialities Company. The THO specific activity was assayed on a Packard Tri-Carb liquid scintillation spectrometer.

Blood for these experiments was obtained at the time of delivery of apparently
normal full term infants. Immediately after delivery of the infant, the placental
blood was drained into a sterile flask containing 0.01 ml of heparin (sodium heparin,
Lederle, 10 mg per ml) per 1.0 ml of blood. This blood was filtered and the THO
diffusion measured with the cells suspended in their own plasma. Freezing point
measurements of the plasma and buffer insured that the plasma and buffer osmo-
larities would not be different. The composition of the isotonic buffer used as the
carrier for the THO label is given in Reference 5.

<table>
<thead>
<tr>
<th>Fetal erythrocytes</th>
<th>Adult erythrocytes</th>
</tr>
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<tr>
<td>Experiment No.</td>
<td>Rate constant ( \left( \frac{k}{v_q} \right) ) msec.(^{-1} )</td>
</tr>
<tr>
<td>1</td>
<td>0.081</td>
</tr>
<tr>
<td>2</td>
<td>0.035</td>
</tr>
<tr>
<td>3</td>
<td>0.043</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
<td>0.052</td>
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<tr>
<td>10</td>
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<td>13</td>
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</tr>
<tr>
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</tr>
<tr>
<td>16</td>
<td>0.070</td>
</tr>
<tr>
<td>17</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Mean ± sd = 0.056 ± 0.013 Mean ± sd = 0.091 ± 0.022

**EXPERIMENTAL DATA AND RESULTS**

Reference 2 discussed the differential equations and the associated assumptions for the use of these equations to describe the diffusion of water into the interior of the erythrocyte. The solution of the diffusion equations has the form:

\[
\frac{P_t}{P_\infty} - 1 = \left( \frac{P_0}{P_\infty} - 1 \right) e^{-\frac{k}{v_q} P_\infty t}
\]

(1)

The symbols are defined as follows:

- \( t \) = time in milliseconds,
- \( k \) = proportionality constant,
- \( P \) = the specific activity of the THO in the suspension medium,
- \( v_q \) = volume of the intracellular water.
The subscripts 0 indicating values at time zero; \( n \) indicating values at time \( t \); and \( \infty \) indicating equilibrium values.

In the experiments the decrease in specific activity of the extracellular water is plotted on a semilog plot against the time in milliseconds. The resulting slope and the extrapolated zero time value for the specific activity of the extracellular water allow calculation of the rate constant \( k/v_q \) for the diffusion of water across the erythrocyte membrane.

**Figure 1.** Kinetic curves for the exchange of tritiated water across the membranes of the human adult and fetal erythrocytes.

In the present experiments the rate of water entrance into the erythrocyte is calculated as follows: The rate constant \( k/v_q \) is increased by 14 per cent to correct for the isotopic difference in the diffusion rate of ordinary water and the tritium-labeled molecule (6). This corrected value of the diffusion rate constant is multiplied by the water content of the cell to give \( (\bar{m}) \), the rate of water entrance by diffusion.

The results of the experiments on the diffusion of water across the fetal and adult erythrocyte membrane as measured in the present experiments are shown in Table I.

Fig. 1 shows the plotted data of single experiments with adult and fetal erythrocytes. It has been possible with the present equipment to extend the time scale for diffusion measurements to 18 msec, showing data consistent with two compartment kinetics operating over this time scale.
DISCUSSION

Sjölin (1) has shown that the fetal erythrocyte as represented in the cord blood taken at delivery has a membrane permeability that differs from that of the adult erythrocyte. His measurements were made allowing water to enter the cell under an osmotic gradient, with the rate of change of cell volume indicative of the rate of entrance of water. Permeability coefficients for adult and fetal erythrocytes calculated from his data show $P_w$ of 0.21 cm$^4$-osm$^{-1}$-sec.$^{-1}$ for the adult and $P_w$ of 0.11 cm$^4$-osm$^{-1}$-sec.$^{-1}$ for the fetal cells. Thus, there is a twofold permeability difference to the bulk flow of water in comparing the adult and fetal erythrocytes. When the adult and fetal cells are washed and suspended in buffer these permeability differences remain; hence, it is presumed that the cell membrane rather than the suspending plasma produces these differences and that the permeability to water is intimately associated with the structure of the membrane.

The rate constants for water diffusion of the adult and fetal cells from the present experiments, and the permeability coefficients calculated from the data of Sjölin (1) are shown in Table II. The data may be used to calculate an equivalent pore radius for the membrane of the fetal erythrocyte of 3.9 A and 4.1 A for the adult erythrocyte. The equations relating the water diffusion and bulk flow through the membrane to an equivalent pore size are due to Pappenheimer et al (7). These equations were applied to the calculation of a channel size in the adult membrane by Paganelli and Solomon (2) and are used in the same form and with the same values for the physical constants in the present calculations. Inherent in these calculations is the assumption that Poiseuille's law describing the rate of laminar flow through cylindrical channels can be corrected to apply to molecular channels. Durbin (8)

<table>
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<tr>
<th>Area X 10$^8$ cm$^2$</th>
<th>Volume X 10$^8$ cm$^3$</th>
<th>Per cent water</th>
<th>$\frac{A}{Q}$</th>
<th>$P_w^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>163</td>
<td>87</td>
<td>70</td>
<td>0.091</td>
<td>0.21</td>
</tr>
<tr>
<td>187†</td>
<td>106†</td>
<td>65</td>
<td>0.056</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* $P_w$ values for adult and fetal erythrocytes calculated from the data of Sjölin (1).
has demonstrated that this equation will apply to water flow through artificial membranes when the channel radii are of the order of 15 to 20 Å.

Two methods independent of the diffusion and bulk flow calculations have been used to estimate the channel radii. These methods have given results consistent with the equations of Pappenheimer et al. Giebel and Passow (9) have measured the rate of exchange of mono- and dicarboxylic acids across the beef erythrocyte and have made pore estimates on the basis of the steric hindrance experienced by these organic acids in moving across the cell membrane. Their results of 3.8 to 4.5 Å are in agreement with channel radii of 4.1 Å in beef erythrocytes calculated using the equations of Pappenheimer as reported by Villegas, Barton, and Solomon (5).

Goldstein and Solomon (10) have evaluated the Staverman reflection coefficient \( \sigma \) with various permeant molecules penetrating the erythrocyte membrane. Evaluating \( \sigma \) has enabled them to relate this coefficient to the equivalent pore radius for the membrane using the relation of Durbin, Frank, and Solomon (11).

\[
[1 - \sigma] = \frac{A_{sf}}{A_{wf}} \tag{2}
\]

where \( \sigma \) = the Staverman reflection coefficient,
\( A_{sf} \) = the effective area for solute filtration,
\( A_{wf} \) = the effective area for water filtration.

Their method has given a value of 4.2 Å for the equivalent pore radius of the adult human erythrocyte.

Since the channel radius penetrating the fetal erythrocyte membrane is calculated to be the same as in the adult membrane, the approximately twofold difference in permeability can be explained by (a) an increased thickness in the fetal erythrocyte membrane, or (b) the presence of a set of channels of variable size through the membrane, but distributed about some mean value. The lower fetal permeability would reflect more sharply peaked distribution of channel sizes in the latter case.

**Comparison of the Adult versus the Fetal Erythrocyte Membrane Thickness**

The values given for the erythrocyte membrane thickness vary from 50 to 5,000 Å depending on the method used in measurement of the membrane thickness. Consequently, an absolute value for the thickness of the membrane is not available. Assuming a uniform mosaic of channels penetrating the membrane, the diffusion data from the present experiments can be used to calculate the ratio of adult to fetal membrane thickness if this is the factor operating to give a lower value for the fetal permeability. If the pores are uniformly distributed within the matrix of the cell membrane, we can solve
the diffusion equation for $\Delta x$(adult) and $\Delta x$(fetal), the membrane thickness of the adult and fetal cells respectively. Forming their ratio:

$$\frac{\Delta x \text{ (adult)}}{\Delta x \text{ (fetal)}} = \left[\frac{\frac{A_{pd}}{m} \times \text{cell area}}{\frac{A_{pf}}{m} \times \text{cell area}}\right]_{\text{adult}}$$

Insertion of the values for the cell surface area, the experimentally determined values for the rate of water diffusion, and the effective pore areas $A_{pd}$ in the right side of equation (3) gives a ratio of approximately (0.75) for the relative thickness of the adult to fetal erythrocyte membrane.

**Pore Distribution**

The concept of a membrane perforated by uniform right circular cylindrical pores is offered only as a model by which the cell permeability to non-lipid soluble solutes may be described. Pappenheimer (12) discusses the effect of the distribution of pores on the bulk flow of solute, indicating that the effective pore radius will be greater than the arithmetical mean of any distribution of pore sizes when the relative frequency of each channel size is introduced into the calculation. Ferry (13) has studied the statistical consideration of channel size as it relates to molecular sieving in ultrafiltration experiments. In these discussions if the membrane is not isoporous a weighted value must be used to represent accurately the contribution of the channels of various sizes to both the diffusion and bulk flow. It is possible then to examine this idealized structure further and to determine the effect of a normal distribution of pores on the bulk flow and diffusion processes.

The total cross-sectional area of the channels will be the sum of the areas of the individual channels.

$$A_p = \pi n_1 r_1^2 + \pi n_2 r_2^2 + \cdots + \pi n_\infty r_\infty^2 = \pi \sum_{i=0}^{\infty} n_i r_i^2$$

where $A_p =$ the total geometric area of the pores,

$n_i =$ the number of discrete pores of radius $r_i$,

$r_i =$ radius of the $i$th channel.

In a normally distributed population the relative frequency of $r_i$ will be given by the values of the ordinate of the normal curve. The number of channels $n_i$ with radius $r_i$ will be

$$n_i = n_{f(r_i)} = \frac{n}{\sqrt{2\pi}} \exp \left(\frac{(r_m-r_i)^2}{2\sigma^2}\right)$$
\[ n = \sum_{i=1}^{\infty} n_i \] is the total number of discrete pores,

\[ f(r_i) \] = relative frequency of occurrence of \( r_i \) in a normally distributed population,

\[ \bar{r} = \text{the mean of the values } r_i, \] and

\[ s = \text{the standard deviation of the group of pores}. \]

The total area of the channels penetrating the membrane will be:

\[ A_p = n\pi \sum_{i=1}^{\infty} f(r_i)r_i^2 \] (6)

Renkin's (14) equations define the ratios \( A_p/A_d \) and \( A_p/A_f \) as polynomials expanded about \( \left( \frac{a}{r} \right) \). \( A_p/d \) and \( A_p/f \) are the effective pore areas for diffusion and filtration respectively. \( \left( \frac{a}{r} \right) \) is the ratio of the radius of the penetrating molecule to the radius of the membrane pore. With a given class of pores with radii distributed about a mean value, there will be pores with radii greater than the mean value; these larger pores will be fewer in number but will allow increased filtration or diffusion. Using Renkin's equations the effective pore area for diffusion can be formulated as follows:

\[ A_{pd} = n\pi \sum_{i=1}^{\infty} f(r_i)r_i^2 g \left( \frac{a}{r_i} \right) \] (7)

where \( g \left( \frac{a}{r} \right) \) is the Renkin factor relating effective pore area to the geometric pore area.

Insertion of the expression for the effective pore area in the diffusion equation:

\[ \dot{m}_{H_2O} = -D_{H_2O} \frac{A_{pd}}{\Delta x} = -D_{H_2O} \cdot n\pi \sum_{i=1}^{\infty} r_i^2 f(r_i) g \left( \frac{a}{r_i} \right) \] (8)

where

\[ \dot{m}_{H_2O} = \text{water flow in ml/sec.}, \]

\[ D_{H_2O} = \text{water diffusion coefficient}, \]

\[ \Delta x = \text{path length for diffusion}. \]

Similarly, the expression for the effective pore area for filtration inserted in Poiseuille's equation is:

\[ \dot{M}_{H_2O} = \frac{r^2 A_{pf}}{8\eta\Delta x} = \frac{n\pi}{8\eta\Delta x} \sum_{i=1}^{\infty} r_i^4 f(r_i) G \left( \frac{a}{r_i} \right) \] (9)
where

\[ \dot{M}_{H_2O} = \text{volume rate of water flow per unit pressure difference}, \]

\[ G\left(\frac{a}{r}\right) = \text{Renkin's factor relating } A_{pf} \text{ to the pore area, and} \]

\[ \eta = \text{viscosity of water}. \]

Solution of the diffusion and filtration equations gives the expression:

\[ \frac{m}{M} = \frac{8\eta D_{H_2O}}{\sum_{\delta} r_\delta^2 f(r_\delta) G\left(\frac{a}{r_\delta}\right)} \tag{10} \]

The right side of equation (10) cannot be integrated in closed form; however, it can be integrated numerically for values of \( r_m \) and various standard deviations. Fig. 2 shows the computed values from equation (10) plotted against \( r_m \).

**Figure 2.** The ratios of \( \frac{m}{M} \) predicted by the equations of Poiseuille, Fick, and Renkin are calculated for channels of radius \( r_m \) (standard deviation). The horizontal lines represent the experimentally determined values of \( \frac{m}{M} \) for the membranes of the adult and fetal erythrocytes.

As the family of curves \( s = 0.0, 0.5, 1.0, \) and \( 1.5 \) A are plotted, they show the expected change in the ratio of diffusion to bulk flow in a normally distributed set of channels. It should be noted that the solution in closed form of the equations for an equivalent pore size is equivalent to the set of channels with zero distribution from the mean \( r_m \).

From Fig. 2 it can be seen that a distribution of the channel radii penetrating the membrane has the effect of reducing the mean value of the channel radius. The horizontal lines in Fig. 2 represent the experimentally determined ratios of the water diffusion to bulk flow across the erythrocyte membrane for the adult and fetal cells. The intersecton of the horizontal line with the family of curves allows the determination of a mean value of the pore radius for any distribution that will be consistent with the experimental data. Referring to Fig. 2 and following the horizontal line across, a membrane with channels of \( 2.8 \pm 1.0 \) A or \( 3.6 \pm 0.5 \) A would be indistinguishable from a membrane with uniform pores of \( 3.9 \) A as calculated for the fetal erythrocyte membrane. Any of these membranes would be expected to give
diffusion and osmotic measurements consistent with the measurements experimentally obtained.

Examination of condensed systems of large molecules, notably lecithin and cephalin, has shown a common short spacing reflection of 4.2 Å, corresponding to the close packing of parallel hydrocarbon chains, and interchain linkages at 7.2 Å spacing (15). Solomon (16) has explored the dimensions of pores in a number of somatic cells, the adult erythrocyte, HeLa cells grown in tissue culture, and mucosal cells of the rat intestine, using the method of Goldstein and Solomon (10) for calculating values of the pore radius. He has consistently found pore radii of 3.5 to 4.0 Å. The geometric area of a channel with such a radius corresponds well with the area of the interlattice spacings noted in the lipid systems.

The close limits such a pore distribution might have are made clear by qualitative experiments measuring the entrance of solutes into the erythrocyte interior. As a minimum size the dimensions of the water molecule would serve as a limit, and at the upper end the glucose molecule at 4.5 Å radius will not enter the erythrocyte by diffusion. Considering that in a normally distributed population the elements are virtually contained within the limits of ±3 SD, the standard deviation in channel size for the erythrocyte membrane would not be expected to exceed ±0.5 Å.

The relatively slow diffusion of water across the fetal erythrocyte membrane is not entirely explained by suggesting the existence of a set of sharply peaked, normally distributed channels penetrating the membrane. Qualitative experiments suggest limits of ±0.5 Å as an expected standard deviation for the channels of the adult erythrocyte membrane. The present calculations indicate that a normally distributed set of channels, varying no more than ±0.5 Å from the mean, would not increase water diffusion in the adult erythrocyte by a factor of 1.6. In the adult cell a shorter path length or higher pore density must account for the enhanced water diffusion.

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