PROBABILITY DENSITY FUNCTION OF THE RED CELL MEMBRANE PERMEABILITY COEFFICIENT

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ABSTRACT The distribution of a random variable is determined by the probability density functions (PDF) of all other random variables with which the variable in question is jointly distributed. If the PDF of the random variable of interest is normal, or skewed normal, then the distributions with which it is jointly distributed determine its mean and standard deviation. In the case described here (where hemolysis time of the red blood cell is a function of the permeability coefficient and geometric variables of the cell) the mean and standard deviation of the permeability coefficient and the known distributions of the geometric variables on which the hemolysis time depends determine a predicted distribution of hemolysis time. An observed distribution of the hemolysis time is obtained spectrophotometrically. By choosing the mean and standard deviation of the permeability coefficient so that the predicted PDF of the hemolysis time matches the observed PDF best by least-squares criterion, the complete distribution of the permeability coefficient is determined.

INTRODUCTION

The classic test for red blood cell (RBC) membrane permeability to various substances has been to place cells in a hypotonic medium and to observe the increase in hemolysis (generally observed as a decrease in opacity of the cell suspension) with time (Jacobs, 1934; 1952). The percent hemolysis vs. time curves being sigmoid in shape, the time to hemolysis was characteristically taken to be the time at which a given fraction (e.g. 75%) of the total number of cells had hemolyzed. From a hemolysis time obtained in this way, the permeability of the cell membrane to a particular substance was calculated.

The fact that the curves are sigmoid in shape (rather than step functions) implies that in a population of RBC there exists a distribution of hemolysis times, which in turn implies that there are differences in the properties of individual cells. Canham and Burton (1968) in fact have observed distributions of various size and shape factors of the RBC.

In this paper we make explicit the concept that a given type of membrane has a distribution of permeability coefficients to a given substance. We have derived an expression for the membrane permeability of an individual RBC as a function of hemolysis time as measured in experiments done in our laboratory. Using known probability density functions (PDF) for some of the variables in the expression along with observed percent hemolysis vs. time data we calculate the PDF of permeabilities in samples of red cells.

PERMEABILITY RELATION FOR A SINGLE CELL

We first derive a relation between membrane permeability and the various cellular parameters affecting it. The system which our derived relation represents is an RBC placed in a medium which initially is at iso-osmotic concentration in some foreign permeant. The permeant follows its concentration gradient into the cell; water follows the resulting osmotic gradient and the cell eventually hemolyzes.

A reasonable starting point in the derivation is Fick's law of diffusion:

$$dc/dt = (PA/V)(c_o - c), \tag{1}$$

where $c = c(t)$ is the concentration of foreign permeant inside the cell, c_o is the concentration of foreign permeant outside the cell, P is the membrane permeability coefficient for the foreign permeant, A is the cell membrane area, $V = V(t)$ is the osmotically active volume of the cell and t is time.

The assumptions we made in using the above form of Fick's law are: (*a*) there is no rectification of flux across the membrane, (*b*) the membrane area (A) is constant, (*c*) the concentration of foreign substrate outside the cell (c_o) is constant, and (*d*) foreign substrate is well mixed within the cell.

The volume (V) is not constant. If we assume that the rate-limiting step for volume flux into the cell is the entry of foreign substrate, we may also assume that substrate enters the cell at its external concentration. That is, osmotic pressure differences set up by diffusion of substrate are immediately compensated for by water flux. This condition is stated by:

$$V = V_o + (S/c_o) = V_o + (Vc/c_o), \tag{2}$$

where $V_o = V(0)$ is initial osmotically active intracellular volume, $S = S(t) = Vc$ is the number of moles of foreign substrate inside the cell. Solving Eq. 2 for V gives:

$$V = V_o c_o/(c_o - c). \tag{3}$$

Substituting Eq. 3 into Eq. 1 eliminates V:

$$dc/dt = (PA/V_o c_o)(c_o - c)^2. \tag{4}$$

The boundary conditions for integration of Eq. 4 are

$$c(0) = 0; \quad c(t_h) = c_h ,$$

where t_h is the time after addition of permeant solution at which the cell hemolyzes. Integrating Eq. 4 with the above boundary conditions and solving for P gives:

$$P = \frac{V_o}{At_h}\left[\frac{c_o}{c_o - c_h} - 1\right]. \tag{5}$$

The concentration of substrate inside the cell at hemolysis (c_h) is experimentally indeterminable. Using Eq. 2 at $t = t_h$ and solving for c_h yields:

$$c_h = c_o[1 - (V_o/V_h)], \tag{6}$$

where V_h is the osmotically active volume at hemolysis. A sufficiently close estimate of V_h is the volume of a sphere having the surface area (A) of the RBC minus the osmotically inactive volume. If we estimate the initial water volume to be approximately 0.7 of the initial total cell volume (Altman, 1961), the nonwater volume is then 0.3 of the initial total volume or 0.3/0.7 of the initial water volume. Recognizing that many authors have produced evidence and arguments for the existence of intracellular bound water, we assume for convenience that all of the intracellular water is osmotically active (Solomon, 1971). The osmotically inactive volume is then (0.3/0.7) $V_o = 0.4\ V_o$, and

$$V_h = (\pi d^3/6) - 0.4\ V_o = (Ad/6) - 0.4\ V_o , \tag{7}$$

where d is the diameter of the spherical RBC at hemolysis. Using Eq. 7 to eliminate V_h from Eq. 6 and using Eq. 6 to eliminate c_h from Eq. 5 results in:

$$P = (1/t_h)[(d/6) - 1.4\ (V_o/A)]. \tag{8}$$

Eq. 8 is the relation we desire. It gives the permeability coefficient of the RBC membrane to a permeable, foreign substrate as a function of initial water volume (V_o), critical diameter (d), cell membrane area (A), and time to reach hemolysis (t_h). The terms on the right in the equation—t_h, d, and V_o/A—are ones for which PDF are available or experimentally observable. Here we have taken the fraction of the cell volume which is osmotically inactive (solids, nonsolvent water) to have a single value, 0.3, rather than a distribution with variation about a mean value. For purposes of the following derivation we will henceforth refer to the volume-to-area ratio, V_o/A, as r.

METHOD OF DERIVATION OF THE PDF OF PERMEABILITY

Having derived a relation for the permeability of the membrane of a single cell to hemolysis time and cell parameters, we now use it to determine the PDF of perme-

ability for a population of cells. Since we do not know *a priori* the form of the PDF of permeability we must assume a particular type of PDF, determine the values of the parameters of the PDF most consistent with observation and examine the consistency of those values in light of known biological or physical laws. In the present study we determined the permeability coefficients of the red cell membrane for four permeants. Our assumption of a normal PDF of permeability resulted in reasonable distribution parameters (mean and standard deviation) for only one of the four. Going a step further and assuming a skewed PDF of permeability—specifically, a logarithmic-normal (ln-normal) PDF—resulted in reasonable distribution parameters for all four permeants. We will describe how each PDF, normal and ln-normal, is derived.

Normal PDF of Permeability

Given our assumption of a normal PDF of permeability and the observations that cell diameter (d) and volume-to-area ratio (r) are each normally distributed, we then assume that they are jointly normally distributed. The joint normal PDF for n variables (see textbook on probability theory such as Parzen, 1962) is:

$$f(x_1, x_2, \cdots x_n) = \frac{1}{(2\pi)^{n/2}|K|^{1/2}} \exp\left[-\frac{1}{2}\sum_{i,j=1}^{n}(x_i - \bar{x}_i)K^{ij}(x_j - \bar{x}_j)\right], \quad (9)$$

where x_i, x_j are the jointly distributed variables and $\bar{x}_i$, $\bar{x}_j$ are the respective mean values of the variables. K is the n by n matrix with each term K_{ij} equal to the covariance of x_i and x_j. Thus,

$$K = [K_{ij}]$$
$$= [\text{cov}\,(x_i, x_j)] = [\rho_{x_i,x_j}\,\sigma_{x_i}\,\sigma_{x_j}],$$

where ρ is the correlation coefficient of the subscripted variables and σ is the standard deviation of the subscripted variable. The K^{ij} are the terms of K^{-1}.

For our particular case, the joint PDF is $f(P, d, r)$. In calculations using this function we have made the simplifying assumption that correlations between the functional parameter P and the geometric parameters d and r are zero. The validity of this assumption will be discussed later. Using this assumption, the matrix K becomes:

$$K = \begin{bmatrix} \sigma_P^2 & 0 & 0 \\ 0 & \sigma_d^2 & \rho_{d,r}\,\sigma_d\,\sigma_r \\ 0 & \rho_{d,r}\,\sigma_d\,\sigma_r & \sigma_r^2 \end{bmatrix}.$$

Since all correlation coefficients except $\rho_{d,r}$ are zero, we will henceforth designate $\rho_{d,r}$ by ρ. The determinant of K is:

$$|K| = \sigma_P^2(\sigma_d^2\sigma_r^2 - \rho^2\sigma_d^2\sigma_r^2).$$

The terms K^{ij} are obtained from:

$$K^{-1} = \begin{bmatrix} \frac{1}{\sigma_P^2} & 0 & 0 \\ 0 & \frac{1}{\sigma_d^2(1-\rho^2)} & \frac{\rho}{\sigma_d \sigma_r(\rho^2-1)} \\ 0 & \frac{\rho}{\sigma_d \sigma_r(\rho^2-1)} & \frac{1}{\sigma_r^2(1-\rho^2)} \end{bmatrix}.$$

Then, taking into account our assumptions, the joint PDF of P, d, and r is:

$$f(P, d, r) = B \exp\left[-\frac{1}{2}\left[\frac{(P-\bar{P})^2}{\sigma_P^2} + \frac{(d-\bar{d})^2}{\sigma_d^2(1-\rho^2)} + \frac{2\rho(d-\bar{d})(r-\bar{r})}{\sigma_d \sigma_r(\rho^2-1)} + \frac{(r-\bar{r})^2}{\sigma_r^2(1-\rho^2)}\right]\right], \quad (10)$$

where $B^{-1} = (2\pi)^{3/2}\sigma_P\sigma_d\sigma_r(1-\rho)^{1/2}$.

At this point we eliminate P as a variable from Eq. 10 while retaining the parameters of its own normal PDF, $\bar{P}$ and σ_P. This transformation is made by substitution for P using Eq. 8. The joint PDF becomes:

$$f(t_h, d, r) = |J|^{-1} f[(1/t_h)(d/6 - 1.4\,r), d, r], \quad (11)$$

where J is the Jacobian (see Parzen, 1960):

$$J = \begin{vmatrix} \frac{\partial t_h}{\partial P} & \frac{\partial t_h}{\partial d} & \frac{\partial t_h}{\partial r} \\ \frac{\partial d}{\partial P} & \frac{\partial d}{\partial d} & \frac{\partial d}{\partial r} \\ \frac{\partial r}{\partial P} & \frac{\partial r}{\partial d} & \frac{\partial r}{\partial r} \end{vmatrix} = \begin{vmatrix} -\frac{1}{P^2}\left(\frac{d}{6} - 1.4\,r\right) & \frac{1}{6P} & -\frac{1}{P} \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{vmatrix}$$

$$= -\frac{1}{P^2}\left(\frac{d}{6} - 1.4\,r\right) = -t_h^2\left(\frac{d}{6} - 1.4\,r\right)^{-1}.$$

A PDF for t_h alone is obtained by integrating Eq. 11 over all values of d and r, that is,

$$f(t_h) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(t_h, d, r)\delta d\,\delta r. \quad (12)$$

The double integration above was done numerically—specifically, by double application of Simpson's rule, over reasonable finite limits.

Having the PDF for t_h, we apply an iterative procedure to find $\bar{P}$ and σ_P such

that the derived PDF matches the PDF for t_h obtained by differentiation of the empirical percent hemolysis vs. time curve. On obtaining $\bar{P}$ and σ_P, the normal PDF for the permeability coefficient has been established:

$$f(P) = \frac{1}{\sqrt{2\pi}\sigma_P} \exp\left[-\frac{1}{2}\left[\frac{P - \bar{P}}{\sigma_P}\right]^2\right]. \quad (13)$$

ln-normal PDF of Permeability

In assuming an ln-normal PDF of permeability we assume that the logarithm (base e) of P is normally distributed. The PDF analogous to Eq. 13 is then (Hald, 1952):

$$f(P) = \frac{1}{\sqrt{2\pi}P\sigma_{\ln P}} \exp\left[-\frac{1}{2}\left[\frac{\ln P - \ln \xi}{\sigma_{\ln P}}\right]^2\right], \quad (14)$$

where $\sigma_{\ln P}$ is the standard deviation of the logarithm of P and ξ is the median value of P.

Since we are assuming stochastic independence between any function of P and the geometric parameters d and r, we may simply write the joint normal PDF of $\ln P$, d, and r as the product of the PDF $f(P)$, given by Eq. 14, and the bivariate PDF of d and r, $f(d, r)$ given by Eq. 9. Thus,

$$\begin{aligned} f(P, d, r) &= f(P)\cdot f(d, r) \\ &= \frac{1}{(2\pi)^{3/2}(1 - \rho)^{1/2}\sigma_{\ln P}\,\sigma_d\,\sigma_r P} \exp\left[-\frac{1}{2}\left[\frac{(\ln P - \ln \xi)^2}{\sigma_{\ln P}^2}\right.\right. \\ &\quad \left.\left. + \frac{(d - \bar{d})^2}{\sigma_d^2(1 - \rho^2)} + \frac{2\rho(d - \bar{d})(r - \bar{r})}{\sigma_d\,\sigma_r(\rho^2 - 1)} + \frac{(r - \bar{r})^2}{\sigma_r^2(1 - \rho^2)}\right]\right]. \end{aligned} \quad (15)$$

Substituting for P using Eq. 8, dividing by the same Jacobian as in Eq. 11 and integrating overall values of d and r we obtain a new version of Eq. 12. Whereas previously we altered $\bar{P}$ and σ_P to obtain a fit of $f(t_h)$ to an empirical PDF of t_h, for the present case we alter ξ and $\sigma_{\ln P}$ to obtain the fit. When ξ and $\sigma_{\ln P}$ are so obtained, the log-normal PDF of P as given by Eq. 14 is determined.

APPLICATION OF THE MODEL

Experimental PDF of Hemolysis Times

Fig. 1 schematically indicates the steps taken in developing an experimental PDF of hemolysis times. On placing a small volume (10μl) of whole blood in a spectrophotometer cuvette, swiftly adding a relatively large volume (3.0 ml) of hemolytic solution (50 mosM in salt and buffer, pH 7.4; 260 mosM in nonelectrolyte) and observing the change in percent transmittance of light (700 nm wavelength), we observe a curve like the lower solid curve in Fig. 1.

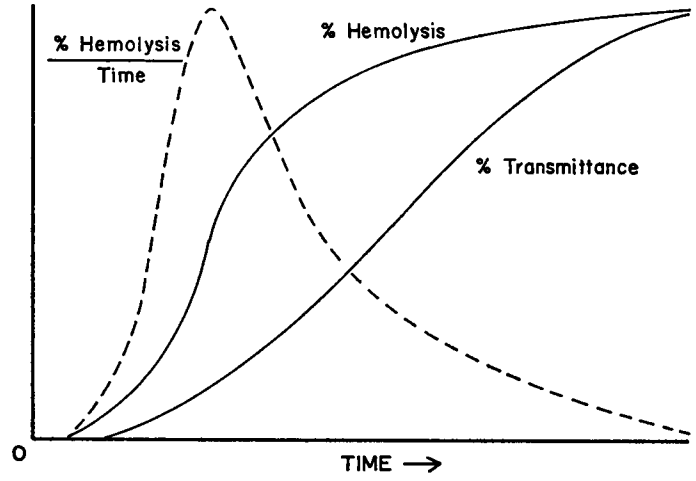

FIGURE 1 Schematic representation of steps taken in converting the percent transmittance vs. time curve, occurring during hemolysis of a suspension of red cells, to a relative PDF of time to hemolysis.

Percent transmittance may be converted to percent hemolysis by determination of the correspondence between transmittance and percent hemolysis as follows: (*a*) we make a series of buffered NaCl solutions varying in osmolarity; (*b*) add blood to each of the series of solutions in the same volumetric proportions as in the above described procedure; (*c*) read the percent transmittance of each suspension at 700 nm wavelength, having set the 100% reference by adding blood to H_2O; (*d*) centrifuge out the intact cells and read the absorbance of each supernatant solution at 580 nm wavelength (peak absorption wavelength for hemoglobin) to estimate the amount of hemoglobin released, which is proportional to the percent of cells hemolyzed. Use of the resulting correspondence in the form of a curve (Fig. 2) will convert the percent transmittance vs. time curve to a percent hemolysis vs. time curve which resembles the upper solid curve in Fig. 1. Note that use of buffered NaCl solutions for calibration of the curves for nonelectrolyte hemolysis is valid since none of the nonelectrolytes tested interfered with readings at either 580 or 700 nm wavelengths.

Numerical differentiation of the percent hemolysis vs. time curve yields a curve resembling the broken line in Fig. 1. This curve differs from a PDF of hemolysis times only by a proportionality constant.

Fitting the Theoretical Curve to the Experimental Data

After having generated an experimental PDF of hemolysis times from the percent transmittance vs. time curve as just described, we fit the theoretical PDF to it by choosing $\bar{P}$ and σ_P or ξ and $\sigma_{\ln P}$ appropriately. The parameters of the PDF of critical diameter d, the initial volume-to-area ratio r, and their correlation coefficient ρ (all

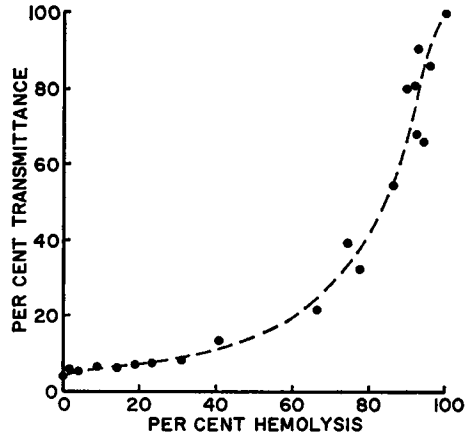

FIGURE 2 Calibration of percent hemolysis to percent transmittance of a red cell suspension. See text for procedure.

required for Eq. 11) were supplied by Dr. P. B. Canham on reanalysis of data from his paper (Canham and Burton, 1968). The values are as follows: $\bar{d} = 6.62$ μm, $\sigma_d = 0.426$ μm, $\bar{r} = 0.533$ μm, $\sigma_r = 0.0267$ μm, $\rho = 0.599$. The values of $\bar{r}$ and σ_r used are the actual geometric ratios given us by Dr. Canham multiplied by 0.7 as a correction for nonwater volume.

The integration indicated by Eq. 12 was done numerically by double application of Simpson's rule (Hildebrand, 1956) over ranges of d and r corresponding to the mean $\pm 3\sigma$. The values of $\bar{P}$ and σ_P (when a normal PDF of permeability was assumed) or ξ and $\sigma_{\ln P}$ (when a ln-normal PDF was assumed) were altered to obtain the best possible fit of the theoretical PDF, $f(t_h)$, to the experimental PDF. Specifically, the fitting procedure involved (*a*) setting the peak (maximum probability density) of each curve equal to one, (*b*) altering $\bar{P}$ (or ξ) to match the positions of the curves on the abscissa, and (*c*) altering σ_P (or $\sigma_{\ln P}$) to yield the best fit by least-squares criterion. Fitting was facilitated by use of the Control Data Corporation 6400 digital computer at the Data Centre of The University of Calgary.

Results

An example of the fitting procedure is illustrated in Fig. 3 for hemolysis of normal adult RBCs in a hypotonic solution of propylene glycol. The dots represent points on the experimental, differentiated percent hemolysis vs. time curve, in essence an observed PDF for hemolysis time. The broken curve represents the best fit of the theoretical PDF for hemolysis time to the observed PDF for hemolysis time when a normal PDF for permeability is assumed. The root mean square (RMS) error of the fit illustrated in Fig. 3 is 0.049 or 4.9% of the maximum probability density. For the four permeants tested, whether a normal or a ln-normal PDF of permeability was assumed, the largest RMS error was 11.5% of the maximum probability density (see Table I).

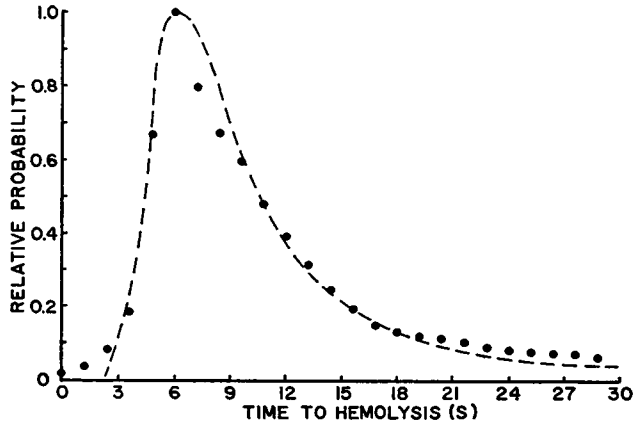

FIGURE 3 Relative PDF of hemolysis time obtained theoretically (broken line) matched to that observed (dotted curve) for hemolysis in a solution of propylene glycol. A normal PDF of the probability coefficient was assumed. The root mean square (RMS) error for this particular case was 0.049 of the maximum probability density.

TABLE I

PARAMETERS OF TWO DERIVED PDF OF PERMEABILITY AND SOME LITERATURE VALUES OF PERMEABILITY COEFFICIENTS

Solute	Literature values of P*	Normal PDF			ln-normal PDF		
		$\bar{P}$	σ_P	RMS error‡	$\bar{P}$§	σ_P§	RMS error‡
	10^{-7} cm/s	10^{-7} cm/s			10^{-7} cm/s		
Ethylene glycol	800 (J) 320 (S) 160 (HØ)	70	73	0.070	110	63	0.115
Propylene glycol	110 (S)	43	24	0.049	47	20	0.089
Glycerol	83 (S) 26 (J) 4.1 (HØ)	14	8.5	0.041	16	6.9	0.056
Thiourea	18 (S) 5.7 (J) 4.1 (HØ)	5.0	0.57	0.048	4.2	0.17	0.036

* All literature values reported here were calculated from Fig. 3.14 in Stein (1967), which is a compilation of values from the studies of (J), Jacobs et al. (1935); (HØ), Höber and Ørskov (1933); and (S), Stein (1956, 1962).

‡ RMS error is the root mean square error of the fit of the theoretical PDF to the observed PDF of hemolysis time (example, Fig. 3). The value for each case is given as the fraction of the maximum probability density.

§ The values of $\bar{P}$ and σ_P for the ln-normal PDF were calculated from the values ξ and $\sigma_{\ln P}$ by use of Eqs. 16 and 17.

When a normal PDF of permeability is assumed, the values of $\bar{P}$ and σ_P giving the best fit of theoretical PDF to observed PDF of hemolysis time define a PDF of the form given by Eq. 13. Those values of $\bar{P}$ and σ_P so obtained for four different permeants are given, along with the RMS error of the fit, in columns 2, 3, and 4 of

Table I. It is evident that assuming a normal PDF of permeability leads to small RMS errors in fitting the theoretical PDF to the observed PDF of hemolysis times, but that the values of $\bar{P}$ and σ_P obtained for three permeants define a PDF having a large fraction of negative permeability values. Since this is clearly nonsense we sought to describe the distribution of permeabilities in terms of a PDF which does not allow negative values of permeability.

When a ln-normal PDF of permeability is assumed, the values of ξ and $\sigma_{\ln P}$ giving the best fit of theoretical PDF to observed PDF of hemolysis time define a PDF of the form given in Eq. 14. We have not reported the values of ξ and $\sigma_{\ln P}$ directly but have transformed them to the parameters $\bar{P}$ and σ_P and entered them in columns 5 and 6 of Table I to facilitate comparison with variables of the normal distribution. The transformations were made by the relations (Parzen, 1960):

$$\sigma_P^2 = \exp [2 (\ln \xi) + 2 \sigma_{\ln P}^2] - \exp [2 (\ln \xi) + \sigma_{\ln P}^2] \quad (16)$$

$$\bar{P} = \exp [\ln \xi + \sigma_{\ln P}^2/2]. \quad (17)$$

Fig. 4 illustrates graphically the ln-normal PDF of permeability obtained for the four substances tested and the normal PDF obtained for thiourea permeability, the one case where assuming a normal PDF did not yield a distribution with a large population of negative permeability coefficients.

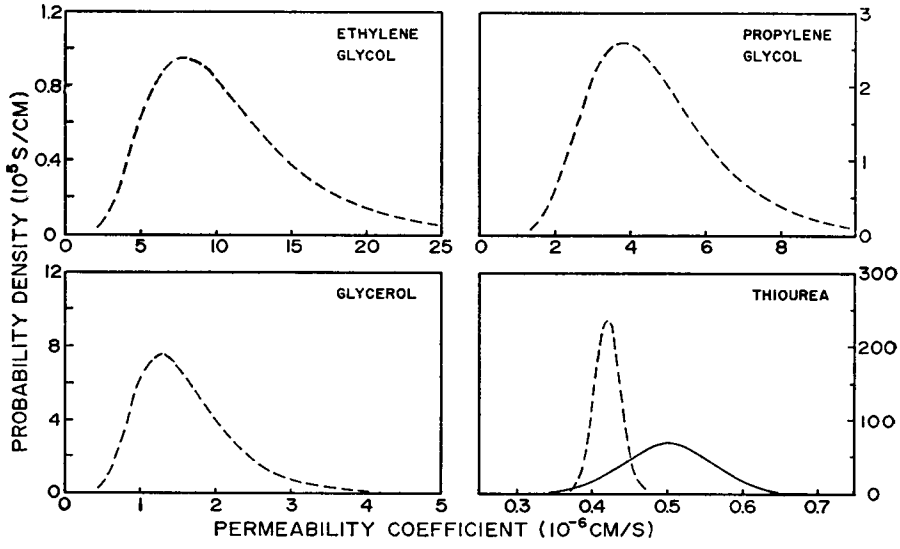

FIGURE 4 Derived PDF of the red cell membrane permeability coefficient for four permeants. The broken curves are ln-normal PDF and were determined by Eq. 14 after ξ and $\sigma_{\ln P}$ were obtained from fitting the theoretical PDF to the observed PDF of hemolysis time. The solid curve for thiourea is a normal PDF determined by Eq. 13 after matching the theoretical PDF to the observed PDF of hemolysis time.

DISCUSSION

A comparison of the mean permeability values obtained from this work and permeability values reported in the literature is given in Table I. The method described here was found to provide mean permeability values which are reasonably close to reported values, but improves on prior methods by providing an estimate of the whole distribution of permeabilities.

The method gives an estimate of the probability that a randomly chosen cell will have a given permeability coefficient and concurrently provides an additional parameter by which RBC may be characterized, namely the standard deviation of the permeability coefficient. The introduction of parameters in addition to $\bar{P}$ suggests the possibility of interesting applications. For example, a specific pathological state of the RBC may be associated with a change in σ_P without any change in $\bar{P}$. In such a case the diagnostic criterion is lost with the usual method. But in addition to a diagnostic criterion the determination of σ_P may offer insights into the underlying characteristic of the pathological state.

Various assumptions were made in the building of the present model. The assumptions made, we feel, provide a reasonable starting point for our model as is indicated by the fits of the curves in Fig. 3 (which is characteristic of all four substances tested) and by general agreement with values of permeability from other laboratories. The assumptions may be reconsidered to the ends that (*a*) a more uniformly close fit of the curves might be obtained and (*b*) new information might be obtained from additional parameters required by less restrictive assumptions.

We assumed Fick's law of diffusion and entry of permeant into the cell at a concentration equal to that in the medium. Use of these assumptions precludes any characterization of the membrane in terms of the PDF of the reflection coefficient or of the hydraulic water permeability. Descriptive equations approaching those of Kedem and Katchalsky (1958) would improve the model presented, yet would introduce additional parameters for which PDF are at present unknown.

A further consequence of the above assumption, namely that an approximate osmotic balance exists across the membrane during entry of permeant, is a general inapplicability of the method for rapid permeants. As the permeability of the permeant approaches that of water, the rate limitation of water passage in determination of the hemolysis time becomes significant. An attempt to apply the model in its present form to urea vividly illustrated this fact. The comparison of our $\bar{P}$ with literature values in Table I indicates that $\bar{P}$ for ethylene glycol and propylene glycol may be suffering from this limitation. The low permeabilities of glycerol and thiourea make them the safest candidates for the assumption of osmotic balance. Thus, in a strictly quantitative sense, the method requires improvement for rapid permeants, but as a diagnostic or comparative tool it retains utility in its present form, even for urea.

Another step in the derivation requires the choice of a given type of PDF for the

permeability coefficient. We first chose to represent the permeability with a normal PDF. This proved inadequate in that the normal PDF derived allowed for a large proportion of negative permeability coefficients for all but thiourea. We corrected for this problem by assuming that a function of P which does not allow for negative values, the logarithm of P, was normally distributed. This gave us what we consider to be reasonable PDF for all permeants tested. More accurately stated, the ln-normal PDF are ones for which we have no *a priori* grounds for dismissal. The same holds true for the normal PDF for thiourea. For the case of thiourea we in fact have no criterion by which to choose one PDF over the other except for the goodness of fit of the theoretical PDF to the observed PDF of hemolysis time. In that case the ln-normal PDF for thiourea prevails with an RMS error of 0.036 (as against 0.048).

We further assumed there was a zero correlation between permeability and the geometric parameters. The fact that there is a dependence of geometric parameters as well as of membrane chemical composition on cell age (van Gastel et al., 1965) may well indicate closer examination of this assumption. Elimination of this assumption, as with the previous ones, would require additional experimental measurements.

In summary, the general method we have illustrated is as follows: (*a*) choose or derive a descriptive equation of the problem such as Eq. 8, (*b*) choose a joint PDF such as Eq. 9 which is based on the parameters of the descriptive equation, (*c*) eliminate variables with unknown PDF by substitution and variables with known PDF by integration, and (*d*) fit the resultant theoretical PDF to an empirical or known PDF to obtain parameters of any unknown PDF. While we have applied the method to the determination of one particular variable, it is likely to be useful in many cases where the parameter to be determined is considered a random variable.

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