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APPARENT VISCOSITY OF HUMAN RED CELL MEMBRANES

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Summary

The apparent diffusion coefficient of methanol in the human red cell membrane may be computed from the permeability coefficient of the membrane to methanol, and the membrane-buffer partition coefficient for methanol. The apparent methanol diffusion coefficient in the human red cell membrane is $5.1 \cdot 10^{-8}$ $cm^2 \cdot s^{-1}$ at approx. 21°C. Using the Stokes—Einstein relation, this diffusion coefficient is equivalent to an apparent membrane viscosity of 1.7 P.

The apparent transverse viscosity of human red cell membranes may be computed from the diffusion coefficient of methanol across the human red cell membrane by using the Stokes—Einstein equation. The permeability coefficient, ω, of human red cells to methanol is $131 \pm 1 \cdot 10^{-15}$ moles$\cdot$dyne$^{-1} \cdot s^{-1}$ as given by Savitz and Solomon [1]. The membrane diffusion coefficient, D, may be calculated by the equation $D = \omega RT\Delta x/k$ in which k is the red cell membrane-buffer partition coefficient for methanol; Δx is membrane thickness, and R and T have their usual meanings. It is also necessary to know what fraction of the resistance to permeation is attributed to transfer across the buffer—membrane interface and what fraction to diffusion within the membrane.

One advantage of this method of computing viscosity is that the concentration of the methanol probe in the membrane is very much lower than that of the probes used in electron spin resonance measurements of diffusion. Since the methanol permeability measurements were made using radioactive tracers, the membrane concentration of methanol was about $5 \cdot 10^{-5}$ moles/mole lipid, very much smaller than the mole fraction of 0.2 to 0.5 for the spin-label analog of phosphatidylcholine used by Scandella et al. [2] in their measurements on diffusion in sarcoplasmic reticulum vesicles. Therefore, the perturbation introduced into the red cell membrane by the methanol probe is minimal, particularly since methanol itself is a very small molecule.

The membrane—buffer partition coefficients for human red cells have

been determined by Seeman et al. [3] for the series of monohydric alcohols from pentanol through decanol. When ΔG^0, the standard free energy change for partition, is plotted against chain length, a linear relationship is found, as shown in Fig. 1. The line has been drawn by the method of least squares and the correlation coefficient is 0.997. The octanol—water partition coefficients for the monohydric alcohols from methanol through butanol, and also octanol, are given by Leo et al. [4]; ΔG^0 for these coefficients is also plotted in Fig. 1. The correlation coefficient for the least squares line through these data is 0.999. Hence, it seems justifiable to determine the red cell membrane—buffer partition coefficient for methanol by extrapolating from the data of Seeman et al. which leads to $k = 0.029$ at room temperature. Each methylene group makes a contribution of -695 cal·mole^{-1} to ΔG^0 for membrane—buffer partition which indicates that methanol solubility in the membrane is favored over that of water. Hence solution in the lipid phase should be the major component of the membrane permeation process for methanol. Methanol is also many times more soluble in ether than water is, as shown by Collander's [5] measurement of an ether—water partition coefficient of 0.14 for methanol. This is very much greater than the ether—water partition coefficient for water of 0.003 given by Gary-Bobo (personal communication).

PARTITION COEFFICIENTS FOR MONOHYDRIC ALCOHOLS

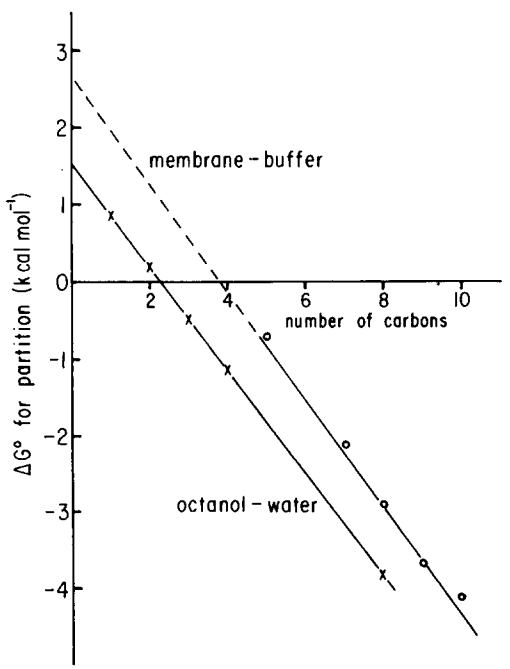

Fig. 1. Relation of ΔG^0 for partition to chain length for monohydric alcohols.

A quantitative evaluation of the importance of the hydrophilic and hydrophobic routes through the red cell membrane may be obtained from the data of Savitz and Solomon [1]. They point out that the red cell methanol permeability coefficient is much higher than the value expected for permeation through the equivalent pore in the red cell membrane. Gary-Bobo and Solomon [6] have shown that, for hydrophilic permeation of the human red cell membrane, the natural logarithm of the permeability coefficient is linearly related to solute molar volume. The permeability coefficient attributable to methanol entrance by the hydrophilic route can therefore be computed from the data of Savitz and Solomon [1] as $22 \cdot 10^{-15}$ moles·dyne^{-1}·s^{-1}. Subtracting this figure from the total permeability coefficient, which is the sum of both routes, leaves $109 \cdot 10^{-15}$ moles·dyne^{-1}·s^{-1} for the hydrophobic route.

This computation has been made on the assumption that the entire surface area of the human red cell membrane is available for permeation by dissolution in the membrane. Engelman [7] has computed that the lipid area of the cell membrane is sufficient to account for 80—90% of the total surface area and Branton and Deamer [8] have computed that the lipids occupy 67% of the area by a different method which requires more assumptions. Taking 80% as a reasonable estimate, ω_{lipid} becomes $136 \cdot 10^{-15}$ moles·dyne^{-1}·s^{-1}.

The next consideration concerns the contribution of interface permeation to the permeability coefficient. Galey et al. [9] have studied the permeation of a homologous series of lipophilic amides and have found that relative interface resistance decreases with chain length. At 25°C the interface resistance for propionamide was only 6% of the total membrane resistance as compared to 53% for valeramide. A similar relation is obtained for the monocarboxylic acids. Hence, the contribution of the interface resistance to methanol permeation should be very small and we have neglected it. Finean [10] has estimated that the thickness of the red cell membrane lipid layer is 45 Å. Using these figures, the methanol diffusion coefficient in the human red cell membrane is $5.1 \cdot 10^{-8}$ cm^2·s^{-1}. According to the Stokes—Einstein relation, $\eta = RT/6\pi D N_{Av} r_s$. The solute radius, r_s, may be computed from the partial molar volume, $\bar{V}_s$, as $r_s = (3\bar{V}_s/4\pi N_{Av})^{1/3}$ leading to $\eta = 1.7$ P for the apparent membrane viscosity.

This value of D applies to transverse diffusion across the membrane from one face to the other. Thus the diffusion coefficient is an average of the diffusion coefficients across both the ordered region near the interface and the less ordered region in the membrane core (see Jost et al. [11], Rigaud et al. [12], Lange et al. [13] and Seelig and Seelig [14]). It is interesting to compare our value for D_{methanol} with the diffusion coefficient for lateral diffusion of rhodopsin in the disc membrane of the visual receptor, which is given by Poo and Cone [15, 16] as $3.9 \cdot 10^{-9}$ cm^2·s^{-1} at room temperature and that of Scandella et al. [2] of $1.8 \cdot 10^{-8}$ cm^2·s^{-1} for the spin-label analog of phosphatidylcholine in sarcoplasmic reticulum vesicles. Since the radius of rhodopsin is almost exactly ten times greater than that of methanol, the agreement of $D_{\text{rhodopsin}}$ with D_{methanol} is surprisingly good. Rhodopsin penetrates deeply into the disc membrane and so its lateral diffusion probably averages

transverse differences across the membrane, though the very much greater cholesterol content of the human red cell membrane might be expected to make $D_{methanol}$ smaller than $D_{rhodopsin}$.

In the case of sarcoplasmic reticulum vesicles there are two opposing factors to take into account in making the comparison with the red cell membrane. The vesicle water content is high which should increase the lateral diffusion coefficient (see Lange et al. [13]) whereas the probe molecule is much larger than methanol, which should reduce its diffusion coefficient. Consequently,the agreement of the phosphatidylcholine diffusion coefficient with $D_{methanol}$ seems reasonable, notwithstanding the possibility that the structure is perturbed by the probe.

Since the value of the diffusion coefficient depends strongly on the size and shape of the probe, comparisons in terms of apparent viscosity may be more meaningful. Poo and Cone [15, 16] and Cone [17] give 2 P for the viscosity of the disc membrane, which, as stated above, agrees surprisingly well with our value of 1.7 P for the apparent viscosity of the human red cell membrane. Calculations [13] from the data of Scandella et al. [2] give 0.9 P for the apparent viscosity of sarcoplasmic reticulum vesicles at 20°C. Rudy and Gitler [18] have determined the microviscosity of red cell ghosts from the fluorescence anisotropy of perylene. Computation from their data gives 1.7 P for the ghost membrane microviscosity at 20°C. The agreement of this figure with the present value may be fortuitous since Rudy and Gitler based their results on a fluorescence lifetime obtained on rabbit red cell membranes and computed the microviscosity by comparison with the viscosity of a white oil. Shinitzky et al. [19] have shown that this comparison procedure is justifiable for detergent micelles whose core they have shown to be isotropic. However, it is much more difficult to justify such a comparison with the core of the red cell membrane particularly in view of Seelig and Seelig's [14] observation that order persists further down the lipid chain in bilayers than had previously been thought. Aloni et al. [20] studied the fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) which behaves like a rotating rod, whereas perylene is a flat disc shaped molecule. Though Aloni et al. [20] measured the fluorescence lifetime directly they also determined their microviscosity by comparison with a reference oil. Aloni et al. give a microviscosity of 4.8 poise for red cell ghosts at 20°C and 7.2 poise for red cell membranes at 20°C. The value for the red cell ghost does not agree well with that of Rudy and Gitler, nor does that for the whole red cell membrane agree with our finding.

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