Patterns of Nonelectrolyte Permeability in Human Red Blood Cell Membrane

P. NACCACHE and R. I. SHA'AFI

From the Department of Physiology, The University of Connecticut School of Medicine, Farmington, Connecticut 06032

ABSTRACT The permeability of human red cell membrane to 90 different molecules has been measured. These solutes cover a wide spectrum of nonelectrolytes with varying chemical structure, chain length, lipid solubility, chemical reactive group, ability to form hydrogen bonds, and other properties. In general, the present study suggests that the permeability of red cell membrane to a large solute is determined by lipid solubility, its molecular size, and its hydrogen-bonding ability. The permeability coefficient increases with increasing lipid solubility and decreasing ability to form hydrogen bonds, whereas it decreases with increasing molecular size. In the case of small solutes, the predominant diffusion factor is steric hindrance augmented by lipid solubility. It is also found that replacement of a hydroxyl group by a carbonyl group or an ether linkage tends to increase permeability. On the other hand, replacement of a hydroxyl group by an amide group tends to decrease the permeability coefficient.

INTRODUCTION

Recently, the permeability coefficients of a series of amide, ureas, and diols have been measured on human red cells (1). Based on these studies, it was postulated that there are three important variables which need to be considered separately in understanding the permeation process across human red cell membranes. The first is a parameter describing lipid solubility, the second a parameter depending on molecular size, and the third a parameter which is concerned with the chemical nature of the solute. Although this conclusion is in general agreement with earlier studies of nonelectrolyte permeations in red cells, particularly by Jacobs and Höber and Ørskov (*See* Danielli [2]), it is based only on the measurements of the permeability of human red cell membranes to 14 solutes. In order to extend this further and to gain a better understanding of the parameter which is concerned with the chemical nature of the solute, we have measured the permeability of human red cell membranes to 90

molecules. These molecules cover a wide spectrum of nonelectrolytes with varying chemical structure, chain length, degree of branching, type of bond, chemical reactive group, position of reactive group, lipid solubility, ability to form hydrogen bonds, and other properties. In selecting among the various solutes we were guided by the excellent study of Wright and Diamond which deals with the measurement of the reflection coefficients of various nonelectrolytes in rabbit gallbladder (3).

MATERIALS AND METHODS

Human blood obtained by venipuncture was used throughout this study, with EDTA as an anticoagulant. The blood was kept refrigerated at 4°C for at most 48 h. Both the isotonic buffer (whose composition in millimoles/liter was: NaCl, 150; KCl, 5.0; $MgCl_2$, 1.0; $CaCl_2$, 0.25; NaH_2PO_4, 1.0; Na_2HPO_4, 5; pH = 7.4) and the test solutions were prepared on the day of the experiment. The experiments were carried out at room temperature (19–24°C) and at pH 7.4. The solutes were obtained from Fluka (Fluka, AG, Basel, Switzerland), Merck (Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.), Fisher (Fisher Scientific Co., Pittsburgh, Pa.), and Eastman Kodak (Eastman Kodak Co., Rochester, N. Y.).

The rate of water entry into the cells was measured by a modification of the hemolysis and stop-flow technique (4, 5). Changes in cell volume were measured by spectrophotometry at 540 nm using a Beckman Model B Spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) which was connected to a Grass DC amplifier (Grass Instrument Co., Quincy, Mass.) and paper recorder. The red cells were diluted 200 times in an isotonic phosphate buffer just before the start of each experiment. 0.2 ml of this suspension was then injected into the observation tube which contained 2.5 ml of the test solution under study. The time-course of the change in light transmission at 540 nm was recorded. The test solution contained 0.3 M solute under study. The final mixture in the observation tube contained 0.04% red cells, 0.277 M test solute, and 0.024 M NaCl. The base line, determined with the isotonic buffer as a test solution, was checked every three runs. 5–10 control runs in distilled water were carried out at the start of each experiment and at various times during the course of the experiment. Permeability coefficients were calculated using the equations derived initially by Jacobs and later summarized by Stein (4, 6).

RESULTS AND DISCUSSION

Table I gives the values of the permeability coefficients for all the 90 solutes which have been studied along with the molecular weight, and the ether:water partition coefficient (k_{ether}). For each molecule the value of at least nine determinations on three different blood samples is given. The values of k_{ether} are taken from Collander (7). The molecules are ordered according to increasing number of carbon atoms. It is important to point out here that the present values of the permeability coefficients of water and the 14 molecules which have been previously determined are smaller than those reported earlier (1). This is due to difference in experimental method since the present technique

TABLE I

VALUES OF PERMEABILITY COEFFICIENTS FOR VARIOUS NONELECTROLYTES IN HUMAN RED CELL MEMBRANES

Name	Partition coefficient (k_{ether})	Permeability coefficient ($P \times 10^{-5}$ cm/s)
Water	0.003	915±15
Sulfamide	—	0.01
Methanol	0.14	11.35±0.41
Formamide	0.0014	8.05±0.66
Nitromethane	—	6.15±0.28
Urea	0.00047	23.87±1.14
Thiourea	0.0063	0.07±0.03
Two carbon atoms		
Ethanol	0.26	8.76±0.34
Ethylene glycol	0.0053	3.38±0.07
Dimethyl sulphoxide	—	1.30±0.09
Acetonitrile	0.60	4.58±0.38
Acetamide	0.0025	4.20±0.29
Thioacetamide	—	3.39±0.18
2-Iodoacetamide	—	3.87
Methyl formamide	—	11.35±0.61
Methyl urea	0.0012	1.83±0.05
Three carbon atoms		
Acetone	0.62	9.75±0.51
n-Propanol	1.9	6.35±0.18
IsoPropanol	0.64	4.38±0.21
Ethylene glycol monomethyl ether	0.061	12.15±0.62
1,2-Propanediol	0.018	1.79±0.10
1,3-Propanediol	0.012	0.91±0.04
Glycerol	0.00066	0.58±0.04
Dimethyl formamide	0.024	11.90±0.94
Ethyl formamide	—	5.02±0.33
Methyl acetamide	—	3.18±0.42
Propionamide	0.013	3.80±0.28
Acrylamide	—	3.66±0.33
Ethyl carbamate	0.64	8.34±0.82
Ethyl urea	0.0041	0.25±0.02
Malonamide	0.00030	0.01
Four carbon atoms		
Tetrahydrofuran	—	6.99±0.19
n-Butanol	7.7	4.12±0.14
Isobutanol	6.9	2.81±0.11
tert-Butanol	2.2	4.65±0.30
Diethyl ether	10	11.17±1.00
Dioxane	—	11.94±0.58
Ethyl acetate	8.5	5.54±.33
1,3-Butanediol	0.042	2.17±0.10
1,4-Butanediol	0.029	1.15±0.04
2-Butene-1,4-diol	—	0.79±0.08
2-Butyne-1,4,diol	—	1.33±0.13
Ethylene glycol monoethyl ether	0.20	12.82±0.80
1,2,4-Butanetriol	—	0.24±.03
Diethylene glycol	0.004	0.63±.04
Thiodiglycol	—	1.70±0.15
3-Methoxy-1,2-propanediol	0.019	1.00±0.04
2,3-Dioxanediol	—	0.01
n-Butyramide	0.058	4.88±0.08
Isobutyramide	—	2.85±0.12

TABLE I—*Concluded*

Name	Partition coefficient (k_{ether})	Permeability coefficient ($P \times 10^{-5}$ cm/s)
Dimethyl acetamide	—	14.73±0.37
Methyl propionamide	0.031	6.24±0.21
Succinimide	0.031	1.73±0.05
Ethyl acetamide	—	8.34±0.24
N-2-hydroxyethyl acetamide	—	0.01
Succinonitrile	0.32	3.48±0.07
n-Propyl urea	—	0.62±0.08
Isopropyl urea	—	0.40±0.04
Five carbon atoms		
Isoamyl alcohol	19	7.06±0.21
3-Pentanol	—	1.75±0.04
Furfural	—	6.66±0.40
Furfuryl alcohol	—	5.87±0.73
Tetrahydrofurfuryl alcohol	—	9.23±0.13
2,2-Dimethyl-1,3-propanediol	—	1.81±0.05
1,5-Pentanediol	0.055	1.64±.10
Diethylene glycol monomethyl ether	0.037	4.94±0.16
Monoacetin	0.041	0.79±0.08
Pyridine	1.2	36.44±1.89
Diethyl formamide	—	7.69±0.77
Dimethyl propionamide	—	8.87±0.60
n-Valeramide	—	4.02±0.16
Isovaleramide	0.17	4.14±0.16
Glutaronitrile	—	4.89±0.08
Butyl urea	—	1.69±0.05
Asymmetrical diethyl urea	0.019	1.77±0.05
Six carbon atoms		
Cyclohexanol	—	4.44±0.23
Cathechol	—	0.01
1,4-Cyclohexanedione	—	2.31±0.16
2,5-Hexanedione	0.45	2.51±0.06
1,6-Hexanediol	0.12	2.26±0.06
2,5-Hexanediol	—	3.25±0.14
2-Methyl-2,4-pentanediol	0.51	4.52±0.08
Pinacol	0.43	4.91±0.08
Ethylene glycol, monobutyl ether	—	4.10±0.37
Dipropylene glycol	0.035	1.54±0.05
Triethylene glycol	0.0031	0.10±0.03
Diethyl acetamide	—	21.0±0.50
Dimethyl butyramide	—	5.82±0.30
Nicotinamide	—	1.22±0.08
Seven carbon atoms		
2,2-Diethyl-1,3-propanediol	—	2.66±.06
Monobutyrin	—	19.80±0.70
Diacetin	0.22	1.11±0.18
Diethyl propionamide	—	6.53±0.43
Diethylene glycol monobutyl ether	1.1	9.76±0.12
Tetraethylene glycol	0.0024	0.07±0.01
Diethyl butyramide	—	4.00±0.54
Nine or more carbon atoms		
Triacetin	1.4	4.65±0.47
Tetraethylene glycol dimethyl ether	0.061	6.79±0.19
Triethylene glycol diacetate	0.52	25.6±0.87

tends to underestimate the values of permeability coefficients. We were quite aware of this and have discussed in previous papers the reasons behind this expected difference in the methods (8). It is only fair to say that it would have been an overwhelming task to measure the permeability coefficients of all these solutes by any other methods available. Moreover, the present technique does not change the order of permeation of the various molecules relative to each other (1). From consideration of each homologous series such as amides, ureas, and others, it appears that there are at least three important variables which need to be considered separately in understanding the permeation process of these solutes. The first is a parameter describing lipid solubility, the second a parameter dependent on molecular size, and the third a parameter which is concerned with the chemical nature of the solute. As has been pointed out earlier by Sha'afi et al. (1), this model is perforce empirical and its specific properties depend upon the exact nature of each of the parameters that has been selected. In order to have an overview of these factors affecting permeation, we have chosen ether:water partition coefficient to reflect the lipid solubility parameter along with molecular weight to reflect molecular size.

Lipid Solubility

It is evident from Table I that at least to a first approximation permeability coefficients increase with increasing k_{ether}. For example, in a given homologous series, aside from the first members, increasing the number of CH_3 groups results in an increase of both the permeability coefficients and k_{ether}. This phenomenon, usually referred to as Overton's rule, has been observed in other systems and was one of the earliest indications of the lipid nature of cell membranes and of the key role of lipids as a diffusion barrier (3, 9).

K_{ether} has been chosen because its value is known for more solutes than the values for any other partition coefficients. In addition, we have found empirically as has been reported earlier, that the use of k_{ether} gives a better fit to our data. Ideally, one would like to know the value of the partition coefficient between water and membrane lipids in order to minimize experimental errors. The partition coefficients of nonelectrolytes have been studied by Hansh et al. (10) who found that aqueous solubility was the primary determinant of partition between water and a wide variety of organic solvents. They also showed that virtually any monofunctional organic liquid would serve equally well to represent the lipid phase in partition experiments with water. Since we are interested only in relative rates of permeation, k_{ether} will thus be a good index of the partition coefficient between water and membrane lipids.

Violation of Overton's Rule

Table II gives the chemical formula, the permeability coefficients, and k_{ether} for a few homologous series in which the only variable is the hydrocarbon

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