Patterns of Nonelectrolyte Permeability in Human Red Blood Cell Membrane

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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).

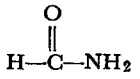
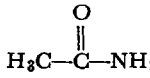
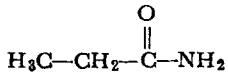
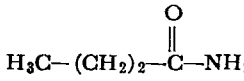
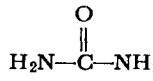
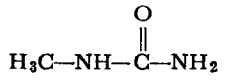
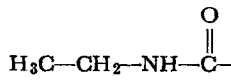
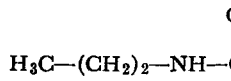
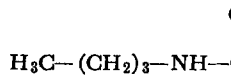
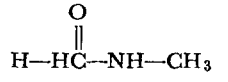
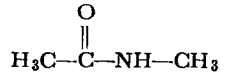
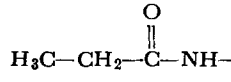
K_{ether} has been chosen because its value is known for more solutes than the values for any other partition coefficients. In addition, we have found empirically as has been reported earlier, that the use of k_{ether} gives a better fit to our data. Ideally, one would like to know the value of the partition 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

TABLE II

VIOLATION OF OVERTON'S RULE BY THE SMALLEST MEMBER OF A HOMOLOGOUS SERIES

Name	Formula	$P \times 10^{-5}$ cm/s	k_{ether}
Amide series			
Formamide		8.05	0.0014
Acetamide		4.20	0.0025
Propionamide		3.80	0.013
Butyramide		4.88	0.058
Urea series:			
Urea		23.87	0.00047
Methyl urea		1.83	0.0012
Ethyl urea		0.25	0.0041
Propyl urea		0.62	—
Butyl urea		1.69	—
Alcohol series			
Methanol	H_3C-OH	11.35	0.14
Ethanol	H_3C-CH_2-OH	8.76	0.26
Propanol	$H_3C-(CH_2)_2-OH$	6.35	1.9
Butanol	$H_3C-(CH_2)_3-OH$	4.12	7.7
Methyl-substituted amide series			
Methyl formamide		11.35	—
Methyl acetamide		3.18	—
Methyl propionamide		6.24	—
Terminal diol series			
Ethylene glycol	$HO-CH_2-CH_2-OH$	3.38	0.0053
1,3-Propanediol	$HO-(CH_2)_3-OH$	0.91	0.012
1,4-Butanediol	$HO-(CH_2)_4-OH$	1.5	0.029
1,5-Pentanediol	$HO-(CH_2)_5-OH$	1.64	0.055
1,6-Hexanediol	$HO-(CH_2)_6-OH$	2.26	0.12

chain length. Partition coefficients have been shown to increase regularly with the length of the hydrocarbon chain (7). It is clear that for the urea series, amide series, methyl-substituted amides, and terminal diol series, the relative permeability coefficient decreases up to the second or third member of each series, and only thereafter increases in accordance with Overton's rule. In the alcohol series, the permeability coefficient decreases regularly with increasing chain length from methanol to butanol in spite of a significant increase in k_{ether}. This behavior is contrary to what would be expected from Overton's rule. It is evident then that for small molecules of molecular weight under 75 the partition coefficient is not a good index of permeability and that Overton's rule is systematically violated.

Molecular Size

Fig. 1 shows the variation of ln $P_{\text{rel}}/k_{\text{ether}}$ with molecular weight. According to irreversible thermodynamic consideration the permeability coefficient for a solute is given as follows:

$$P = \omega RT = k_s/\Delta x(f_{sw} + f_{sm}), \tag{1}$$

in which ω is the permeability coefficient and has units of moles/dyne·second, R is gas constant, T is absolute temperature, K_s is the partition coefficient of the solute between membrane and external solution, and Δx is the path length through the membrane. Solute-water and solute-membrane frictions are denoted by f_{sw} and f_{sm}. Assuming k_{ether} to serve as a qualitative indicator of K_s, the ratio P/k_{ether} should be inversely proportional to the sum of the frictional coefficient, Δx being assumed constant. There are four important conclusions that can be drawn from Fig. 1:(*a*) Steric hindrance has a consistent effect on the entire series including both hydrophilic and lipophilic molecules. (*b*) As evident from the slopes of the lines, the dependence on molecular weight varies considerably among various series. (*c*) Chemical factors are of great importance since each series falls on an entirely different curve. (*d*) The ratios $P_{\text{rel}}/k_{\text{ether}}$ of molecules that differ in chemical structure show no correlation with molecular weight when pooled together. The fact that we have plotted $P_{\text{rel}}/k_{\text{ether}}$ instead of P/k_{ether} does not change any of these conclusions. In fact we have deliberately chosen P_{rel} instead of P to eliminate Δx.

We have also plotted the results using molar volume instead of molecular weight. Molar volume is a parameter which includes geometrical factors being equal to the molecular weight divided by the density of the pure compound. The molecular weight may be construed as a measure of molecular size based on a spherical model. Division by the density modifies the strictly geometrical interpretation by introduction of hydrogen-bonding ability because, as Pimentel and McClellan (11) have pointed out, hydrogen bonding generally increases the density and lowers the molar volume. The correlation of hydro-

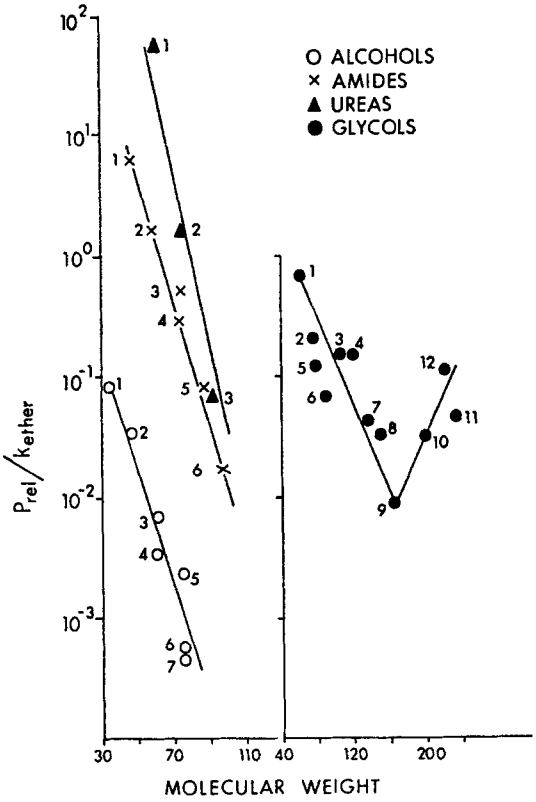


FIGURE 1. Relation among relative permeability coefficient, partition coefficient and molecular weight for a series of alcohols, amides, ureas, and glycols in human red cells. The permeability coefficient is expressed relative to water. The code for the solutes is given below.

Number	Glycols	Alcohols	Amides	Ureas
1	Ethylene glycol	Methanol	Formamide	Urea
2	Ethylene glycol monomethyl ether	Ethanol	Acetamide	Methyl urea
3	Diethylene glycol	Isopropanol	Dimethyl formamide	Ethyl urea
4	Diethylene glycol monomethyl ether	*n*-Propanol	Propionamide	—
5	Propylene glycol (1,2-propanediol)	*tert*-Butanol	Butyramide	—
6	Ethylene glycol monoethyl ether	*n*-Butanol	Isovaleramide	—
7	Dipropylene glycol	Isobutanol	—	—
8	Triethylene glycol	—	—	—
9	Diethylene glycol monobutyl ether	—	—	—
10	Tetraethylene glycol	—	—	—
11	Triethylene glycol diacetate	—	—	—
12	Tetraethylene glycol diacetate	—	—	—

gen-bonding ability with density is illustrated particularly effectively by the butanediol series in which the ability to form hydrogen bonds with other molecules decreases as the hydroxyl groups move closer and become able to form intramolecular hydrogen bonds. In these diols the density decreases as the ability to form hydrogen bonds with other molecules decreases. Substitution of molecular weight by molar volume does not alter any of the previous conclusions.

Recently, Lieb and Stein (12) have suggested that cell membranes should be treated as homogeneous membranes in which the permeability coefficient may be computed from an equation in which the only variables are molecular weight and the oil-water partition coefficient. In the case of bovine red cells, they have fitted permeability data obtained from hemolysis measurements to the equation

$$P = P_o \beta^n \text{ mol wt}_{\text{rel}}^{-p}, \tag{2}$$

in which P is the permeability coefficient and P_o, n, and p are adjustable constants, β the oil-water partition coefficient, and mol wt_{rel} the molecular weight, relative to methanol. Lieb and Stein obtained values of 1.4 for n and 6.0 for p. The least-squares fit to our data on human red cells in Table I was extremely poor. About 50 molecules were used for this analysis. These were the molecules which have known k_{ether}. The correlation is poor, as shown not only by the correlation coefficient of <0.4, but also by the very great scatter when the values predicted according to Eq. 1 are compared with the experimentally determined values. This is not surprising since Lieb and Stein's hypothesis has been already scrutinized and rejected by Sha'afi et al. (1), Smulders and Wright (13), and Dickson and Diamond (14).

The lack of uniformity among solutes in Fig. 1 and the anomalous behavior of small hydrophilic solutes indicate quite clearly that no unitary hypothesis will serve to account for the behavior of all the solutes we have studied. The simplest and most straightforward explanation for these observations is to postulate that the red cell membrane behaves operationally as a mosaic structure containing both lipid- and polar-region pathways. Neither pathway is exclusive, and for small lipophilic molecules such as methanol, both pathways are open. Steric factors are important to permeation by either route but not sufficient to account for all observations.

The finding which appears to be most inconsistent with this general hypothesis is the observation reported by Macey and Farmer (15). These authors have shown that the compound phloretin significantly decreases the permeability coefficients for small hydrophilic solutes and exercises no effect on water transfer in human red cells. This would be quite inconsistent with the preceding hypothesis which postulates that small hydrophilic solutes permeate the membrane by the same polar-region pathways used by water. This ques-

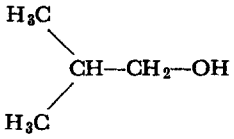
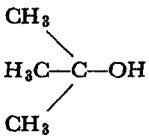
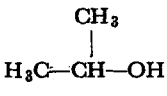
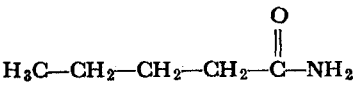
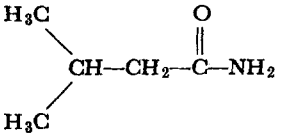
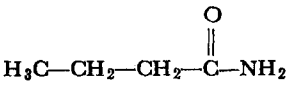
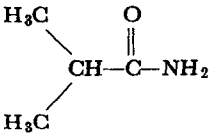
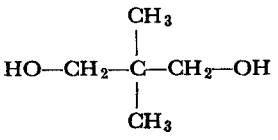
tion has been recently investigated by Owen and Solomon (16), who have shown that phloretin exercises a general and far-reaching effect on the permeability coefficients for both hydrophilic and lipophilic solutes. The rate of transfer of lipophilic solutes is increased by this compound, whereas the permeation of hydrophilic solutes is inhibited. In other words, the lipid solubility of a molecule determines whether its rate of permeation is inhibited or accelerated by phloretin. They have shown further that based on the k_{ether} for water, one would predict, in agreement with their finding, that phloretin should slightly increase the permeability coefficient to water. Accordingly, the initial finding of Macey and Farmer (15) is consistent with the two-pathway concept for red cell membranes.

An alternate explanation which has been proposed by Stein (6) for the observed lack of uniformity and the anomalous behavior of small hydrophilic solutes is that the red cell membrane is homogeneous and that all molecules permeate by dissolving in the membrane fabric. The permeability coefficient for a given molecule is determined by its partition coefficient and molecular weight. According to this, the observed anomalies would then be the results of specialized membrane transport systems, such as facilitated transport (6). Even though there is some evidence derived from red cell studies which supports this idea of facilitated transport systems for solutes such as glycerol and possibly even for urea (17, 18), we are unaware of any evidence to support this idea for all the deviant small molecules. The main argument Stein advances in support of his hypothesis is to show on quantitative grounds that the presence in human red cell membranes of aqueous channels of average radius 3.5 Å will not account for the observed permeability coefficients. Using restricted-diffusion analysis formulated by Renkin (19), and assuming that the postulated channels have no special affinity to water molecules, Stein showed that permeability coefficients for nonelectrolytes calculated on the basis of 3.5 Å for the average pore radius would be much higher than those observed experimentally. Granted that actual calculation of average radius, as pointed out earlier (by Sha'afi and Gary-Bobo (8)), may be completely invalid, this does not invalidate the concept of a polar route for solute permeation. In other words, Stein has merely shown that the observed permeability coefficients for various solutes in human red cells are inconsistent with a radius of 3.5 Å, but this does not justify rejection of the polar-route concept.

Another important geometrical factor which can be related to molecular size is the degree of branching in the molecules. The partition coefficients and permeability coefficients of isomers are summarized in Table III. Although there is no clear-cut apparent correlation between the degree of branching and the permeability coefficient, there are, however, two points which can be made. First, in the case of lipophilic solutes, the permeability coefficient decreases with branching. It is conceivable that the observed decrease in perme-

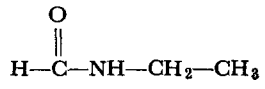
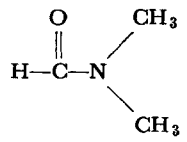
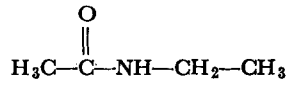
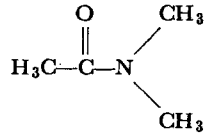
TABLE III

EFFECT OF BRANCHING ON THE PERMEABILITY COEFFICIENT (P) FOR NONELECTROLYTES IN HUMAN RED CELLS

Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
n-Butanol	$H_3C-CH_2-CH_2-CH_2-OH$	7.7	4.12
Isobutanol		6.9	2.81
tert-Butanol		2.2	4.65
n-Propanol	$H_2C-CH_2-CH_2-OH$	1.9	6.35
Isopropanol		0.64	4.38
n-Valeramide*		—	4.02
Isovaleramide		0.17	4.14
n-Butyramide		0.058	4.88
Isobutyramide		—	2.85
1,5-Pentanediol	$HO-CH_2-CH_2-CH_2-CH_2-CH_2-OH$	0.055	1.64
2,2-Dimethyl-1,3-propanediol		—	1.81

* The permeability coefficient for *n*-valeramide is much too low.

TABLE III—*Concluded*

Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
Ethyl formamide		—	5.02
Dimethyl formamide		0.024	11.9
Ethyl acetamide		—	8.34
Dimethyl acetamide		—	14.73

ability coefficient is the result of a decrease in the partition coefficient. There is no doubt that this is partially responsible for the observed differences, but not all, because (*a*) the solute isovaleramide has a much higher partition coefficient than *n*-butyramide and yet it is still less permeant and (*b*) the difference in permeability coefficients between the first pair of isomers is much higher than would be predicted on the basis of differences in partition coefficients. In water, Gary-Bobo and Weber (20) have found very little difference between the diffusion coefficients of butyramide and isobutyramide. Coupled with the finding that solubility in lipid solvent, as indicated by the partition coefficient, is not very sensitive to branching of a molecule, the above observation indicates that the discriminatory power of the red cell membrane is much greater than either bulk lipid solvent or bulk water. It appears, therefore, that the lipids in cell membrane are very much less fluid than lipid solvents or water and must be held in an organized structure so that they are less free to bend around a solute. This can be so if the hydrocarbon tails of membrane fatty acid residues are aligned in parallel and closely packed to each other. Second, in the case of the last two pairs, branching seems to increase permeability coefficients. This increase cannot be due to differences in k_{ether} or molecular size since ethylformamide is certainly as lipid soluble as dimethyl formamide. This is also true for ethyl acetamide and dimethyl acetamide. For example, ethyl urea has a value for k_{ether} of 0.0041 whereas the corresponding value for dimethyl urea is 0.0031. The situation is quite clear when one examines the first three

isomers in the table. The solute *tert*-butanol obviously has a much lower partition coefficient than the other two isomers and yet it shows a higher permeability coefficient. One possible explanation for this behavior is that the presence of the dimethyl groups in the case of the amide series tends to decrease the ability of the latter group to form hydrogen bonds with external acceptors. As will be discussed later, the permeability coefficient for a molecule increases with decreasing ability to form hydrogen bonds. A similar explanation can be used in the case of *tert*-butanol. If the density of a solute in a given series can be taken as an index of the hydrogen-bonding ability of this solute, then such an explanation is quite reasonable. One piece of evidence which supports this idea is that the density of *tert*-butanol is less than that of *n*-butanol. Also the densities of dimethyl formamide and dimethyl acetamide are less than the densities of ethyl formamide and ethyl acetamide, respectively.

Hydrogen-Bonding Ability

The first systematic analysis of the possible role of solute hydrogen-bonding ability and its relation to permeation in membranes was carried out by Stein (6). In his book, he tried to correlate permeability coefficients for solutes to the number of hydrogen bonds, N_H, the solute is able to form with water, and he found, in general, that when N_H increases, the rate of penetration decreases. In addition, Diamond and Wright presented further evidence to support this concept (21). The results in the present study confirm this general hypothesis and show further that extreme care must be exercised when dealing with the effect of hydrogen-bonding ability of a solute on its rate of permeation. As will be discussed later, this interdependence is invariably violated in the case of small molecules both hydrophilic and lipophilic. In addition, this interdependence is often complicated by the dependence of permeability coefficients on lipid solubility since in general the smaller the N_H the higher the partition coefficient. However, a true dependence of the permeability coefficient on N_H does indeed exist. To show this, it is instructive first to compare the behavior of the molecule 1,3-propanediol with that of propionamide. These two molecules have very similar physical properties and the order of permeability coefficient is propionamide > 1,3-propanediol. The major difference lies in the altered hydrogen-bonding ability of the solute which results from the presence of a single amide group rather than two hydroxyls.

Hydrogen-bonding ability is somewhat greater for amides than alcohols as illustrated by differences in N_H. Franks and Ives (22) give this number as 2 for the alcohol group, whereas the most likely value for the amide group is 3 (23). Gary-Bobo et al. (24) have shown that a series of amides experiences greater friction than an analogous series of alcohols when diffusing across a nonporous cellulose acetate membrane; furthermore, the ratio of the frictions is about 3:2. If simple additivity of hydrogen-bonding ability is assumed as a

first approximation, it is apparent that the N_H of the diols should be greater than that of amides. The density of 1,3-propanediol is also slightly greater than that of propionamide, although density differences are less significant among solutes having different, rather than similar, reactive groups. Since the only apparent dissimilar characteristic between these two molecules is their hydrogen-bonding ability, the sharp decrease in the permeability coefficient for 1,3-propanediol relative to propionamide may be attributed to its higher N_H. If one assumes that the measured values of the reflection coefficient, σ, do reflect rates of penetration across cell membranes, then the same comparison could be made and similar conclusions could be drawn for these two molecules in rabbit gallbladder (3). However, extreme care must be exercised in the interpretation of σ measurements in terms of permeabilities, especially in connection with hydrogen-bonding effects. Dipolo et al. (25) have shown that hydrogen bonds exercise more effect on the permeability coefficient, ω, than on σ in cellulose acetate membranes. Accordingly, it is not surprising to find two molecules with the same σ and entirely different ω. For example, in cellulose acetate membrane, ethylene glycol ($K_s = 0.19$, $N_H = 4$) has about the same value of σ as malonamide ($K_s = 0.2$, $N_H = 6$), yet it permeates three times faster. The values for K_s represent the partition between water and the cellulose acetate membrane.

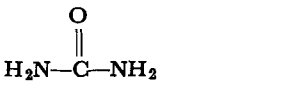
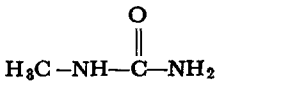
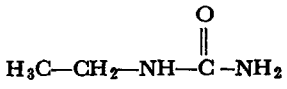
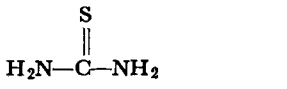
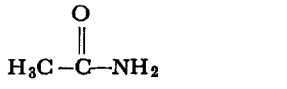
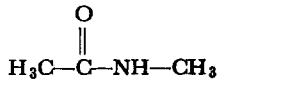
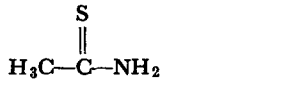
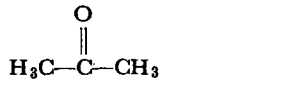
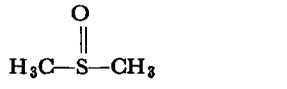
Another example in which the effect of hydrogen bonding on solute permeation is clearly evident is in the butanediol series. As the hydroxyl groups on the molecules are brought closer together from the 1,4- to the 1,3- and finally to the 2,3-position, the permeability coefficient increases. In spite of the increase in size and decrease in k_{ether}, the molecule 2,3-butanediol is more permeant than its isomer 1,3-butanediol because as the hydroxyl groups are increasingly opposed, intramolecular hydrogen bonding increases at the expense of the ability to form hydrogen bonds with external acceptors. Another example in which the effect of hydrogen bonding of a solute on its rate of penetration is evident is in the case of 2-methyl-2,4-pentanediol (mol wt = 118.2, $k_{ether} = 0.51$, $P = 4.5 \times 10^{-5}$ cm/s,) and pinacol (mol wt = 118.2, $k_{ether} = 0.43$, $P = 4.9 \times 10^{-5}$ cm/s). Consequently, for solutes that permeate by dissolving in the membrane fabric, hydrogen bonding is a more important determinant of membrane permeability. Diamond and Wright (21) have also pointed out that the possibility of formation of intramolecular hydrogen bonds increases as the hydroxyl groups move closer together, an effect which is reflected in a concomitant decrease of reflection coefficients in the gallbladder. In terms of membrane structure, this indicates that lipid-soluble solutes traverse a path which brings them into contact with polar moieties such as provided by phosphatides or proteins. An alternative explanation is to postulate that as N_H increases more energy is required to tear the solute loose from water. This will result in a decrease in rate of penetration since Diamond and

Wright have pointed out that nonelectrolyte selection is largely determined by the difference between solute:water and solute:lipid intramolecular forces (26). The partition coefficient most likely does not reflect all these forces since there are many cases in which a change in k_{ether} does not produce a change in N_H and vice versa. According to this explanation, one would expect that hydrogen bonding should exercise more control on the permeability coefficients for large molecules relative to those for small ones since the former must make a transition from polar to nonpolar environment to be able to permeate. Furthermore, the rates of penetration of small hydrophilic solutes should be the least affected by variation in N_H. This is indeed the case as will be shown later. In general, the present study suggests that the permeability coefficient for a large solute is determined by its lipid solubility, its molecular size, and its hydrogen-bonding ability. The permeability coefficient increases with increasing k_{ether} and a decreasing N_H whereas it decreases with increasing molecular size.

Behavior of Small Molecules

In the case of small solutes N_H and k_{ether} seem to exercise much smaller effects on the permeability coefficient as compared to larger molecules. The permeability coefficients, k_{ether}, molecular weight, and N_H of various small solutes are summarized in Table IV. As illustrated in the table, there does not seem to be any correlation between permeability coefficient on one hand and N_H and k_{ether} on the other. For example, urea is much more permeant than its other derivatives in spite of its low value of k_{ether} and high value of N_H. This also applies to the remaining groups in the table. This is true not only for hydrophilic solutes but also for lipophilic solutes. For example, in the case of one alcohol series, methanol is more permeant than butanol in spite of higher lipid solubility for the latter solutes. Replacing an oxygen atom by a sulfur atom increases lipid solubility and decreases bond strength since sulfur, being bigger and less electronegative than oxygen, forms a weaker hydrogen bond. In spite of this, the permeability coefficient decreases rather than increases. In the case of large molecules such substitution invariably increases permeability coefficient (thiodiglycol, $P = 1.7 \times 10^{-5}$ cm/s; diethylene glycol, $P = 0.63 \times 10^{-5}$ cm/s). For these small solutes, the predominant diffusion factor is steric. The 300-fold decrease in the permeability coefficient as one moves from urea to thiourea cannot be explained entirely on the basis of size, particularly since no such difference can be found in the permeability coefficients for acetamide and thiocetamide. This may give some support to the idea that urea is transported across human red cell membranes by facilitated transport mechanism (18). On the other hand, the sharp decrease in the permeability coefficient when one goes from acetone to dimethyl sulphoxide probably does not reflect just the increase in molecular size. The molecule dimethyl sulphoxide has a number of peculiar physical properties including its ability to form extremely strong hydrogen bonds, much stronger than those of ketones (21).

TABLE IV
BEHAVIOR OF SMALL MOLECULES

Name	Formula	M	k_{ether}	N_H*	$P \times 10^{-5}$ cm/s
Urea		60	0.00047	5	23.87
Methyl urea		74.1	0.0012	4	1.83
Ethyl urea		88.1	0.0041	4	0.25
Thiourea		76.1	0.0063	5	0.07
Acetamide		59.1	0.0025	3	4.20
Methyl acetamide		73.1	—	2	3.18
Thioacetamide		75.1	—	3	3.39
Methanol	$H_3C—OH$	32.0	0.14	2	11.35
Ethanol	$H_3C—CH_2—OH$	46.1	0.26	2	8.76
n-Propanol	$H_3C—CH_2—CH_2—OH$	60.1	1.9	2	6.35
n-Butanol	$H_3C—CH_2—CH_2—CH_2—OH$	74.1	7.7	2	4.12
Acetone		58.1	0.62	—	9.75
Dimethyl sulphoxide		78.1	—	—	1.30

* Values are taken from reference 6 except for methyl acetamide and thioacetamide which were estimated.

Having discussed the three important variables which need to be considered separately in understanding the permeation process for solutes, we shall proceed now to discuss the effect of various chemical modifications of solute on these three variables.

HYDROCARBON CHAIN LENGTH Increasing hydrocarbon chain length by introduction of CH_2 units invariably increases both lipid solubility and molecular size of the molecule. The net effect on its rate of transfer will be governed by these two variables (k_{ether} and molecular weight). In general, the permeability coefficient for small hydrophilic or lipophilic molecules is decreased by increasing hydrocarbon chain length. This decrease is due to increase in molecular size. On the other hand, permeation by large solutes is increased. This is due to increase in k_{ether}. In the case of the latter molecules, the addition of one CH_2 group leads to an increase in permeability of 1.5 ± 0.3 times and in k_{ether} of 2.8 ± 1.1 times. The fact that the increase in permeability is less than that in k_{ether} should not be surprising since addition of CH_2 increases molecular size which invariably decreases permeability.

OXYGEN ATOM As has been discussed earlier, the number of hydrogen bonds a solute is able to form with external acceptor and the strength of these bonds are important parameters in determining its rate of permeation across cell membranes. There are two factors which determine the hydrogen-bonding ability of a molecule: (*a*) the number of proton acceptors (oxygen and nitrogen atoms) and (*b*) the number of protons (hydrogen atoms) which can be donated bound to the proton acceptors. Accordingly, increasing the number of OH groups on a molecule leads in general to a decrease in its rate of permeation as it is evident in Table V. It is extremely difficult to quantify the decrease in permeability as a result of increasing the number of OH groups. The situation is complicated by the fact that intramolecular hydrogen bonds can be formed when hydroxyl groups are on adjacent carbons.

Diamond and Wright (21) have shown that introduction of an ether linkage (R-O-R′) decreases the permeability coefficient (increases the reflection coefficient) for nonelectrolytes in gallbladder. They have also shown that the effect of one hydroxyl group in decreasing the rate of permeation is significantly greater than the effect of one ether link. In general, the results of the present studies which are summarized in Table VI confirm these two conclusions. There are, however, some peculiarities. For example, two sets of solutes (*n*-propanol against ethylene glycol monomethyl ether and 3-pentanol against diethylene glycol methyl ether) deviate from the general pattern. In the studies of Diamond and Wright, the two sets of molecules did not deviate from the general pattern. This must reflect differences in the two systems with respect to the ethylene glycol series. It is more so since the permeability coefficients of some members of this series seem to deviate from expected behavior. Also the molecule dioxane is more permeant than tetrahydrofuran in spite of a lower number of ether links in the latter.

Replacement of a hydroxyl group by a carbonyl group as one moves from isopropanol [$(CH_3)_2-C-OH$, $P = 4.4 \times 10^{-5}$ cm/s] to acetone [$(CH_3)_2-C=O$, $P = 9.8 \times 10^{-5}$ cm/s] tends to increase the permeability coefficient. The

TABLE V

EFFECT OF NUMBER AND POSITION OF HYDROXYL GROUPS ON PERMEABILITY COEFFICIENT IN HUMAN RED CELLS

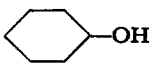
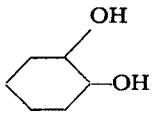
Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
Ethanol	$H_3C—CH_2—OH$	0.26	8.76
Ethylene glycol	$H_2C(OH)—CH_2(OH)$	0.0053	3.38
n-Propanol	$H_3C—CH_2—CH_2—OH$	1.9	6.35
1,2-Propanediol	$H_2C(OH)—CH(OH)—CH_3$	0.018	1.79
1,3-Propanediol	$H_2C(OH)—CH_2—CH_2(OH)$	0.012	0.91
Glycerol	$H_2C(OH)—CH(OH)—CH_2(OH)$	0.00066	0.58
n-Butanol	$H_3C—CH_2—CH_2—CH_2—OH$	7.7	4.12
1,3-Butanediol	$H_2C(OH)—CH_2—CH(OH)—CH_3$	0.042	2.17
1,4-Butanediol	$H_2C(OH)—CH_2—CH_2—CH_2(OH)$	0.029	1.15
1,2,4-Butanetriol	$H_2C(OH)—CH(OH)—CH_2—CH_2(OH)$	—	0.24
Ethylene glycol monoethyl ether	$HO—CH_2—CH_2—O—CH_2—CH_3$	0.20	12.82
Diethylene glycol	$HO—CH_2—CH_2—O—CH_2—CH_2—OH$	0.004	0.63
3-Pentanol	$H_3C—CH_2—CH(OH)—CH_2—CH_3$	—	1.75
1,5-Pentanediol	$H_2C(OH)—CH_2—CH_2—CH_2—CH_2(OH)$	0.055	1.64
Cyclohexanol		—	4.44

TABLE V—*Concluded*

Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
Catechol		—	0.01
1,6-Hexanediol	CH_2—CH_2 –CH_2—CH_2—CH_2—CH_2 (OH, OH)	0.12	2.26
2,5-Hexanediol	CH_3—CH—CH_2—CH_2 –CH—CH_3 (OH, OH)	–	3.25

situation seems to be different when one moves from 2,5-hexanediol [CH_3—CH(OH)—$(CH_2)_2$—CH(OH)—CH_3, $P = 3.3 \times 10^{-5}$ cm/s] to 2,5-hexanedione [CH_3—C(O)—$(CH_2)_2$—C(O)—CH_3, $P = 2.5 \times 10^{-5}$ cm/s]. It must be pointed out, however, that extreme care should be exercised in the interpretation of the permeability coefficients for carbonyl compounds because of their instability in aqueous solution. As it has been suggested by Diamond and Wright (21) these compounds undergo a transformation to hydroxyl compounds either by an intramolecular hydrogen migration (O=C—CH_2 → OH—C=CH—) or a diol formation [—C=O $\xrightarrow{H_2O}$ —$C(OH)_2$]. Acetone is one of a few carbonyl compounds which is neither hydrated (27) nor appreciably enolized (28). Although the solute 2,5-hexanedione should not be significantly enolized or hydrated on chemical grounds, no published data are available. Also Diamond and Wright (21) have found that the molecule 1,4-cyclohexanedione [O=C—$(CH_2)_4$—C=O] is much less permeant (high σ) than 2,5-hexanedione in gallbladder. The solute 1,4-cyclohexanedione is not likely to be significantly enolized or hydrated. They have pointed out that the reason for this is unclear. In the case of red cell membranes, we find that the two molecules show about equal permeabilities. This reflects probable differences in the two systems.

The effect of these molecular alterations on the permeability coefficient can be accounted for on the basis of changes in the molecule's ability to form hydrogen bonds with an external acceptor. For example, the addition of a hydroxyl group to a solute reduces its permeance more than the addition of a carbonyl, because the former can form two hydrogen bonds whereas the latter

TABLE VI

EFFECT OF ETHER LINKAGE ON PERMEABILITY COEFFICIENTS IN HUMAN RED CELLS

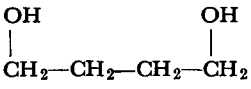
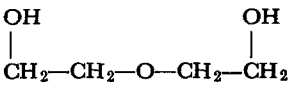
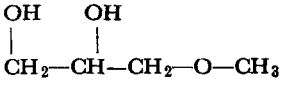
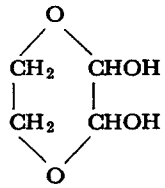
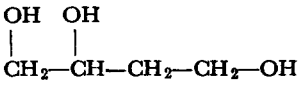
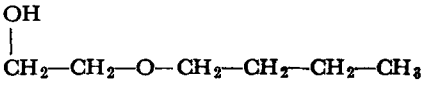
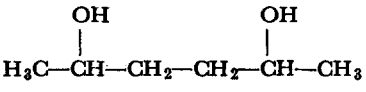
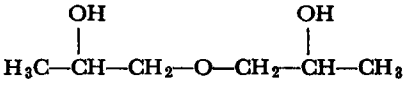
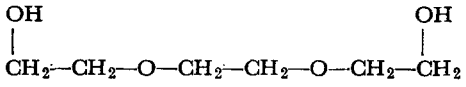
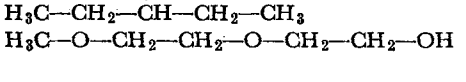
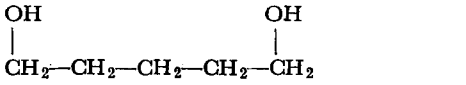
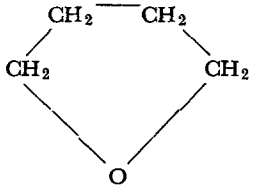
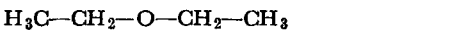
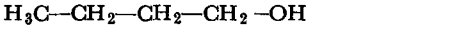
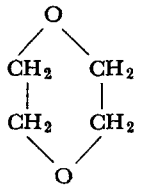
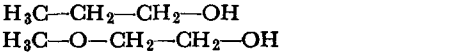
Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
Ethylene glycol ethyl ether	$H_3C-CH_2-O-CH_2-CH_2-OH$	0.20	12.82
1,4-Butane diol		0.029	1.15
Diethylene glycol		0.004	0.63
3-Methoxy-1,2-propane-diol		0.019	1.00
2,3-Dioxanediol		—	0.01
1,2,4-Butanetriol		—	0.24
Ethylene glycol butyl ether		—	4.10
2,5-Hexanediol		—	3.25
Dipropylene glycol		0.035	1.54

TABLE VI—*Concluded*

Name	Formula	k_{ether}	$P \times 10^{-5}$ cm/s
Triethylene glycol		0.0031	0.10
3-Pentanol		—	1.73
Diethylene glycol methyl ether		0.037	4.94
1,5-Pentanediol		0.055	1.64
Tetrahydrofuran		—	6.99
Diethyl ether		10	11.17
n-Butanol		7.7	4.12
Dioxane		—	11.94
n-Propanol		1.9	6.35
Ethylene glycol monomethyl ether		0.061	12.15

can form only one. Similar arguments can be advanced for the difference between a hydroxyl group and an ether link.

AMIDE GROUP The effect of replacing a hydroxyl group by an amide group (—C—NH_2) on the permeability coefficient and molecular weight is best illustrated by comparing the two sets of molecules *n*-propanol (mol wt = 60.1, $P = 6.35 \times 10^{-5}$ cm/s) to propionamide (mol wt = 73.1, $P = 3.8 \times 10^{-5}$ cm/s) and isovaleramide (mol wt = 101.2, $P = 4.14 \times 10^{-5}$ cm/s)to isoamyl alcohol (mol wt = 88.1, $P = 7.06 \times 10^{-5}$ cm/s). In agreement with

previously reported data (21), an amide group in general causes a greater decrease in permeability coefficient than a hydroxyl group. Also, an amide group, in general, causes a greater decrease in permeability coefficient than an ether linkage. This is evident in comparing the two molecules *n*-butyramide ($P = 4.88 \times 10^{-5}$ cm/s) and diethyl ether ($P = 11.17 \times 10^{-5}$ cm/s). There are two major differences between hydroxyl and amide groups: (*a*) difference in size and (*b*) difference in hydrogen-bonding ability. As discussed earlier, hydrogen-bonding ability is somewhat greater for amides than for alcohols as illustrated by differences in N_H. Franks and Ives (22) give this number as 2 for the alcohol group, whereas the most likely value for the amide group is 3 (23). These two factors, increase in molecular size and N_H, can account for the observation that an amide group causes greater decrease in permeability than does a hydroxyl group.

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