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The diffusional transport of water and small solutes in isolated endothelial cells and erythrocytes

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The diffusional permeability coefficients, P_D, for tritiated water (^{3}HHO) ^{14}C-antipyrine (AP) and ^{14}C-iodoantipyrine (IAP) in isolated calf pulmonary artery endothelial cells and dog erythrocytes are measured with the linear diffusion technique at 11.5, 15, 20 and 37°C. The P_D values for both cell populations follow the sequence ^{3}HHO > IAP > AP at each of the temperatures. P_D for water is higher in the erythrocyte compared to the endothelial cells. The differences in P_D for AP and IAP in the erythrocytes and endothelial cells are not dramatic and are similar to the differences seen in comparing permeation of the same solute through bilayers of different composition. A comparison of the values of P_D calculated for the endothelial cells with those for isolated capillaries and the structured endothelium in whole lungs validates the use of the isolated cells as models for the endothelial cells in situ. Incubation of the endothelial cells with *cis*-vaccenic acid or cholesterol produces a reduction in P_D for water and antipyrine. These data are analyzed in terms of Stokesian and non-Stokesian diffusion. The interpretation which best accommodates the data is that the phospholipid area of the membrane, rather than the hydrocarbon core, provides the greatest resistance to permeation for these solutes.

Introduction

Regulation of the movement of solutes in biological systems has been studied extensively on both the cellular and organ level. However, there are few transport studies in which data are available for the same type of cell when it is part of an intact tissue and when this cell type is maintained in vitro. Since the interpretation of these data can yield different conclusions as to the factors which regulate water or solute movement across the endothelium the differences in interpretation can be significant. This report is a continuation of our studies of the permeability characteristics of isolated endothelial cells [1–3] and erythrocytes [4–6] which can be compared to data from studies in lung endothelium [7–9] for the same solutes.

Alterations in membrane lipids or proteins by temperature variation and membrane active agents have been used to investigate diffusional transport processes in cells (e.g., Refs. 10 and 11). We have explored these questions by expanding the studies with the cultured calf pulmonary artery endothelial cells over the temperature range of 11.5°C to 37°C for antipyrine and iodoantipyrine. We have also examined the effect of *cis*-vaccenic acid (*trans*-11-octadecanoic acid) and cholesterol (cholesteryl-hemisuccinate) on the diffusional movement of water and antipyrine in the endothelial cells. When these agents are incorporated into the membrane phospholipids in endothelial cells, cholesterol decreases protein mediated serotonin transport and *cis*-vaccenic acid increases the transport [11]. In these studies we ask questions on three levels, the cellular, tissue and organ levels. (1) Are the P_D values for the solutes different in each cell type and between cell types? (2) What is the significance of the similarities or differences in determining the factors that regulate diffusional transport? (3) Do the data from the cells verify the interpretation of studies in isolated capillaries? (4) Are the data obtained with the cells a good model when compared to data collected in intact organs?

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Materials and Methods

Cell preparations

Endothelial cells are isolated from calf pulmonary artery and maintained and passed in culture without the use of enzymes [12,13]. The glycocalyx and the membrane proteins are, thus, left intact. The cells are characterized as being of endothelial origin by testing for Factor VIII antigen and for angiotensin converting enzyme activity and by morphological criteria under light and electron microscopy [12]. For the diffusional studies the medium is removed from the cells which are grown in T-75 flasks; 5 ml of Puck's A saline (KCl 0.40; NaCl 8.00; $NaHCO_3$ 0.35; D-glucose 1.00; Phenol red 0.005 g/l; GIBCO) with 20 mM Hepes and 0.05% EDTA (pH 7.4) at 4°C are then added to the flasks. After 5 min the cells are removed with a cell scraper and transferred to a centrifuge tube. The cells are centrifuged at $270 \times g$ for 5 min at 4°C. The supernatant fluid is removed and the cells are resuspended in 1.0 ml of Puck's A with 20 mM Hepes by trituration with a 22 gauge needle. The cells are centrifuged in a Microfuge B (Beckman) ($8730 \times g$) for 5 s. The cells are resuspended in medium M199 (GIBCO) with 20 mM Hepes and triturated with a 22 and 26 gauge needle. Then the cells are centrifuged in a Microfuge ($8730 \times g$) for 5 s, the supernatant fluid is removed and saved and the cells and supernatant used in the linear diffusion method [2,14]. Samples of cells are stained with erythrosin B, counted and tested for viability at different stages in the preparation and use of the cells.

Erythrocytes are obtained from mongrel dogs of either sex by venipuncture. The plasma is removed by centrifugation and saved and the erythrocyes are used in the linear diffusion technique as described previously [5,14,15]. There is one change from previous reports in the preparation and use of the erythrocytes, hemoglobin and plasma for diffusion experiments. The hemoglobin is released from the cells and prepared for concentration as described previously but the concentration step is performed with Centriprep (Amicon) tubes. After cell lysis, centrifugation and filtration (0.45 μm) the filtrate is added to a Centriprep tube (88103-30), spun for 50 min at $3000 \times g$, decanted and the procedure repeated two more times. The retentate is then decanted and the hemoglobin concentration determined [15]. This procedure produces a more consistent hemoglobin preparation than the earlier technique [5,15].

Membrane alteration

Cholesterol (cholesteryl-hemisuccinate) (0.1 mM) or *cis*-vaccenic acid (0.1 mM) are added to the endothelial cell medium in the T-75 flask and incubated at 37°C for 3 h. Ethyl alcohol (0.1%) is the vehicle for both substances and is added to the control flasks. Under these conditions these agents are incorporated into the endothelial cell membrane [11]. After three hours the medium is removed and the cells are isolated for the diffusion studies as described above.

Measurement of cell permeability

We have obtained the values for the permeability coefficients for tracer water, antipyrine and iodoantipyrine in the cells by measuring the diffusion coefficients with the linear diffusion technique which has been described in detail elsewhere [3,4,14,16]. The principle aspects of the method are as follows. The cells are mixed with 10 μl of ^{125}I-labeled human serum albumin (HSA 1 Ci/l (Mallindcrodt Chemical) with carrier albumin at 4 g/dl) per 0.1 ml of cells as an extracelllular marker. The cells are drawn into segments of polyethylene tubing (PE 60 or 90), one end of which is sealed, and centrifuged in a microhematocrit centrifuge for 15 min at 4°C. The supernatant is removed and saved for the detemination of extracellular volume, the tube is sliced below the cell-supernatant fluid interface and taped to a plexiglass tray. The entire tray and cells in the tubing are preincubated at the temperature of the diffusion study.

Subsequently, the open end of the tube is pulsed with 1 μl of either [^{14}C]antipyrine (1 Ci/l), [^{14}C]iodoantipyrine (1 Ci/l) or tritiated water (25 Ci/l) and the cells incubated at the desired temperature for a set period of time. After incubation the cell column is frozen with solid CO_2, sliced and the radioactivity in each slice is determined in a liquid scintillation counter. The diffusion coefficient for the column of packed cells, D, can be calculated from the plot of the log of the radioactivity against the diffusion distance [2,14]. Diffusion coefficients are obtained for the intracellular material, D_2, and the extracellular (supernatant) fluid, D_1, by similar procedures as described previously [14,16]. Endothelial cell viability is monitored throughout the procedure.

The permeability coefficient, P_D, is calculated using the series-parallel pathway model of Perl [15]. D represents a bulk diffusion coefficient through the cells packed in the tubing and includes resistances due to the extracellular fluid, the plasma membrane and the cytoplasmic contents. In the model P_D is obtained by separating the resistance provided by the plasma membrane from the resistances provided by the extracellular fluid (an unstirred layer in which the diffusion coefficient is D_1) and the intracellular material (in which the diffusion coefficient is D_2). This allows calculation of the mean membrane permeability coefficient for the endothelial cells. This requires measurement or calculation to obtain the values for: the tortuosity of the extracellular path (from relative diffusion coefficients); the relative extracellular volume (from ^{125}I-HSA measurements); the cell length in the diffu-

sion direction (from microscopy); the distance between the cells in the diffusion direction; the cell surface exchange area; and the cross sectional area for diffusion calculated from the extracellular volume and cell size [14–16]. A computer program for these calculations has been published [14].

We have used the Arrhenius equation to relate changes in the coefficients to changes in temperature and have calculated the activation energy (E_a) to represent this relationship. We do not assume that the E_a values for P represent the temperature dependence of a single molecular process since both partitioning and diffusion are affected by temperature changes [17]. Comparison of P_D values is performed with two factor analysis of variance for unbalanced data.

Results

Diffusion coefficients

Diffusion coefficients in the extracellular fluid, D_1, in the intracellular material, D_2, and packed endothelial cells, D, for tritiated water, [^{14}C]antipyrine and [^{14}C]iodoantipyrine at different temperatures are listed in Table I. The diffusion coefficients for all solutes increase with an increase in temperature although the relative increase is not the same for D, D_1 and D_2. The value for D_1 is lower for the larger iodoantipyrine (mol. wt. 314) than for antipyrine (mol. wt. 188) at each temperature. If D_1 for antipyrine and iodoantipyrine are evaluated for temperature dependence the E_a values are 16.1 and 14.0 kJ/mol, for AP and IAP, respectively. These values are similar to E_a for diffusion in water where the change in diffusion rate is due to the changes of the viscosity of water between approximately 5 and 40°C [18]. For each solute, at each temperature $D_1 > D_2 > D$. This relation is the basis for calculation of the permeability coefficient by the series-parallel pathway model [15].

Ideally, D_2 is measured through a column of intracellular material from which only the plasma membrane has been removed. We have found that the most satisfactory method to simulate this is to freeze and thaw the packed cells. This procedure ruptures the plasma membrane but appears to leave intact the remainder of the cell [4]. D is lower than D_2 for each solute at each temperature. We interpret this as an indication of the resistance due to the intact plasma membrane when D is measured, compared to its absence when D_2 is measured.

The diffusion coefficients for D, D_1 and D_2 for antipyrine and iodoantipyrine with erythrocytes, plasma and hemoglobin are listed in Table II. D_1 and D_2 for iodoantipyrine are consistently lower than those for antipyrine at each temperature. D is consistently higher for iodoantipyrine than for antipyrine at each temperature. This is contrary to what is expected in an aqueous solution.

Permeability coefficients

The permeability coefficients, P_D, in the endothelial cells for [^{14}C]AP, [^{14}C]IAP and ^{3}HHO at different temperatures are listed in Table III and plotted in Fig. 1. P_D is calculated from the diffusion coefficients with the series parallel pathway model [14,15]. The permeability coefficients for antipyrine are lower than those for iodoantipyrine and both are lower than that for ^{3}HHO. P_D increases with an increase in temperature. The change in P_D with a change in temperature is calculated, as E_a, to be 35.2, 46.0 and 42.2 kJ/mol for ^{3}HHO, AP and IAP, respectively. The E_a values for

TABLE I

Diffusion coefficients in endothelial cells for [^{14}C]antipyrine (AP), [^{14}C]iodoantipyrine (IAP) and tritiated water (^{3}HHO)

Diffusion coefficients for packed cells (D), extracellular fluid (D_1) and intracellular material (D_2) are the means ± S.E.; the number of determinations is in parentheses. Values at 20 and 37°C for ^{3}HHO and at 37°C for AP are from Refs. 2 and 3.

	Temp. (°C)	Diffusion coefficients (10^{-5} cm^2/s)		
		D	D_1	D_2
AP	11.5	0.084 ± 0.002 (3)	0.633 ± 0.024 (4)	0.145 ± 0.004 (3)
	15	0.120 ± 0.007 (5)	0.723 ± 0.035 (8)	0.216 ± 0.022 (4)
	20	0.202 ± 0.012 (7)	0.810 ± 0.068 (6)	0.314 ± 0.030 (5)
	37	0.273 ± 0.006 (6)	1.13 ± 0.029 (16)	0.355 ± 0.018 (6)
IAP	11.5	0.099 ± 0.005 (7)	0.569 ± 0.029 (3)	0.168 ± 0.002 (3)
	20	0.212 ± 0.010 (4)	0.610 ± 0.038 (7)	0.286 ± 0.016 (6)
	37	0.277 ± 0.024 (8)	0.767 ± 0.040 (9)	0.444 ± 0.032 (10)
^{3}HHO	11.5	0.227 ± 0.006 (7)	1.46 ± 0.074 (4)	0.269 ± 0.029 (3)
	15	0.282 ± 0.020 (5)	1.54 ± 0.057 (8)	0.452 ± 0.026 (5)
	20	0.411 ± 0.019 (10)	1.81 ± 0.056 (14)	0.767 ± 0.043 (7)
	37	0.682 ± 0.029 (7)	2.45 ± 0.042 (20)	0.932 ± 0.057 (5)

TABLE II

Diffusion coefficients in erythrocytes for [^{14}C]antipyrine (AP) and [^{14}C]iodoantipyrine (IAP)

Diffusion coefficients for packed cells (D), extracellular fluid (D_1) and intracellular material (D_2) are the means ± S.E.; the number of determinations is in parentheses.

	Temp. (°C)	Diffusion coefficients (10^{-5} cm^2/s)		
		D	D_1	D_2
AP	11.5	0.017 ± 0.001 (16)	0.396 ± 0.010 (8)	0.178 ± 0.008 (8)
	20	0.034 ± 0.001 (7)	0.600 ± 0.020 (14)	0.191 ± 0.008 (5)
	29	0.052 ± 0.001 (8)	0.622 ± 0.020 (7)	0.294 ± 0.008 (18)
	37	0.101 ± 0.004 (8)	0.736 ± 0.032 (7)	0.412 ± 0.013 (8)
IAP	11.5	0.027 ± 0.001 (8)	0.334 ± 0.016 (7)	0.147 ± 0.003 (7)
	15	0.037 ± 0.001 (8)	0.467 ± 0.021 (8)	0.140 ± 0.059 (7)
	20	0.058 ± 0.001 (15)	0.493 ± 0.015 (6)	0.153 ± 0.007 (8)
	37	0.126 ± 0.005 (15)	0.680 ± 0.030 (7)	0.266 ± 0.016 (16)

^{3}HHO, AP and IAP are not significantly different ($p > 0.05$) by two factor analysis of variance.

The permeability coefficients for ^{3}HHO [4,5], [^{14}C]IAP and [^{14}C]AP in the erythrocytes at different temperatures are listed in Table IV and ploted in Fig. 2. There is a consistent relationship of ^{3}HHO > IAP > AP. The E_a for the change in P_D with a change in temperature is 69.9, 64.9 and 25.0 kJ/mol for AP, IAP and ^{3}HHO, respectively. 2. The E_a values for AP and IAP are not significantly different ($p > 0.05$) but that for ^{3}HHO compared to both IAP and AP is significantly different ($p < 0.05$). The permeability coefficients in the erythrocytes are significantly different from those in the endothelial cells for water ($p < 0.0001$) and for iodoantipyrine ($p < 0.0001$) but not for antipyrine ($p = 0.483$).

The permeability coefficients for the endothelial cells after exposure to cholesterol and *cis*-vaccenic acid are listed in Table V. Cholesterol and *cis*-vaccenic acid in the endothelial cell membrane do affect the rotation of fluorescent probe molecules differently [11]. Increased motion, interpreted to reflect increased fluidity

TABLE III

Permeability coefficients (P_D) and activation energies E_a) for AP, IAP and ^{3}HHO in isolated calf pulmonary artery endothelial cells over the temperature range of 11.5 to 37°C

Values of P_D are means ± S.E.; number of determinations is in parentheses. Values at 20 and 37°C for ^{3}HHO are from Ref. 2. For calculating P_D [14] the tortuosity, L_1/L_2, is 1.14; cell length in the diffusion direction is 22 μm; L_o, the intercellular distance, differs with the extracellular volume but is in the range of 0.9 to 1.5 μm; the side face to end face exchange area, P/S_A, is 4 and is used to calculate the total cross-sectional exchange area. The extracellular volume varies with each preparation in the range 0.15 to 0.20.

	P_D (10^{-5} cm/s)				E_a (kJ/mol)
	11.5°C	15°C	20°C	37°C	
AP	33 ± 3 (3)	56 ± 5 (5)	65 ± 15 (7)	181 ± 35 (6)	41.8 ± 3.76
IAP	42 ± 9 (7)	–	84 ± 13 (4)	249 ± 40 (8)	48.5 ± 4.31
^{3}HHO	91 ± 6 (7)	125 ± 27 (5)	160 ± 23 (10)	304 ± 42 (7)	35.1 ± 2.68

TABLE IV

Permeability coefficients (P_D) and activation energies (E_a) for AP, IAP and ^{3}HHO in dog erythrocytes over the temperature range of 10 or 11.5 to 37°C

Values of P_D are means ± S.E.; number of determinations is in parentheses. Values for ^{3}HHO are from Refs. 5 and 10, the lowest value is at 10°C.

	P_D (10^{-5} cm/s)					A_a (kJ/mol)
	11.5°C	15°C	20°C	29°C	37°C	
AP	20 ± 2 (8)	–	43 ± 2 (7)	85 ± 3 (8)	230 ± 15 (12)	69.8 ± 1.30
IAP	42 ± 3 (8)	65 ± 33 (8)	147 ± 6 (15)	–	449 ± 32 (14)	54.8 ± 1.59
^{3}HHO	295 ± 10 (8)	438 ± 20 (11)	554 ± 21 (17)	–	841 ± 27 (43)	25.5 ± 0.67

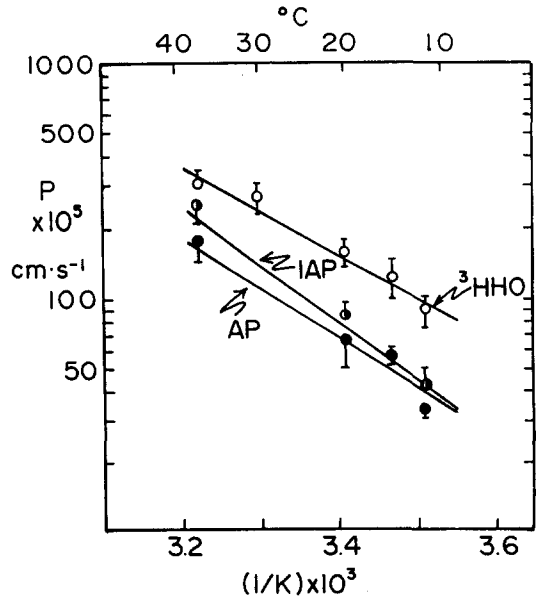

Fig. 1. Endothelial cells. Permeability coefficients, P (mean ± S.E.) are plotted semilogarithmically against the reciprocal of temperature, K, for tritiated water (^{3}HHO, open circles), [^{14}C]iodoantipyrine (IAP, half-filled circles) and [^{14}C]antipyrine (AP, filled circles). The lines are drawn by analysis of variance for linear regression of all data points.

(i.e., decreased viscosity), is expected to result in more rapid diffusion in a Stokesian system and decreased motion, due to increased viscosity, should result in slower diffusion [17]. The P_D for ^{3}HHO is reduced about 30% after incubation with cholesterol and with *cis*-vaccenic acid. The permeability coefficient to antipyrine is also reduced after both treatments but by 70–80%, considerably more than for water.

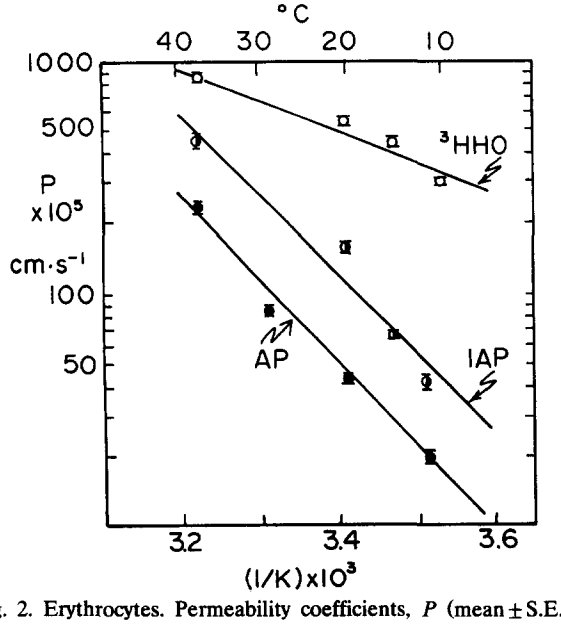

Fig. 2. Erythrocytes. Permeability coefficients, P (mean ± S.E.) are plotted semilogarithmically against the reciprocal of temperature, K. Notations are the same as for Fig. 1.

TABLE V

Permeability coefficients for endothelial cells after incubation (as in Methods) with cis-vaccenic acid (0.1 mM) or cholesterol (0.1 mM) at 37°C

P_D for ^{3}HHO determined with 0.1% ethanol present is $287 \cdot 10^{-5}$ cm/s.

Addition	P_D (10^{-5} cm/s)	
	^{3}HHO	AP
cis-Vaccenic acid	207 ± 31 (6)	33 ± 3 (3)
Cholesterol	212 ± 31 (4)	48 ± 7 (4)

Discussion

The permeability coefficients in calf pulmonary artery endothelial cells and in erythrocytes follow the sequence ^{3}HHO > IAP > AP at each temperature from 10–11.5 to 37°C. The permeability coefficients for AP and IAP are higher in the erythrocytes than in the endothelial cells at most temperatures. The P_D for water diffusional transport is significantly higher ($p < 0.0001$) in the erythrocyte, where it is primarily a protein associated process, than in the endothelial cells where water moves through the lipid areas of the membrane [2,5,10]. Evidence that antipyrine and iodoantipyrine permeate the lipid areas of the plasma membrane in both cell types is found: (1) in the more rapid permeation of the larger but more lipophilic iodoantipyrine, (2) the E_a values for permeation of 46.0 and 42.2 kJ/mol in the endothelial cells and 69.9 and 64.9 kJ/mol in the erythrocyte and (3) the lack of an effect on their transport by sulfhydryl or disulfide agents [10]. Both the plasma membrane and the cytoplasm contribute to the diffusional resistance in these cells for water, antipyrine and iodoantipyrine (i.e., D is always smaller than D_2) [16].

The E_a values for permeation of antipyrine and iodoantipyrine in each cell type (and for water in endothelial cells) are not significantly different ($p > 0.05$). The differences in P_D and E_a in the endothelial cells compared to the erythrocytes for AP and IAP are well within the range that could be expected due to the differences in lipid composition of endothelial cell and erythrocyte membrane lipids (based on differences for D and E_a in bilayers) [19,20]. The lower P_D for water in the endothelial cells after the incorporation of *cis*-vaccenic acid and cholesterol is unlike the results reported with these two agents when a protein-mediated process, serotonin transport, was tested [11]. We can use these results to: (1) compare to the results in isolated capillaries or intact organs or (2) explore hypotheses of the processes and regulation for diffusional transport in cells.

Cellular, capillary and whole lung studies

Curry [21] reported a rapid permeation of aminopyrine and antipyrine and a very high E_a for both solutes in the endothelium of isolated mesenteric capillaries of the frog at 25°C. Curry proposed that AP and ^{3}HHO both move through extracellular and transcellular pathways and that aminopyrine moves through only the transcellular pathway across the endothelium. The 'cellular' permeability coefficient measured ($67.5 \cdot 10^{-5}$ cm/s) or calculated ($34.8 \cdot 10^{-5}$ cm/s) for antipyrine by Curry and the E_a of 46 kJ/mol he calculates are similar to those we report here for isolated endothelial cells. Our data indicate it is not necessary to invoke movement through an extracellular pathway to explain the relations among the data for the isolated capillaries since a similar relationship for water, AP and IAP is seen in isolated endothelial cells.

Chinard et al. [7] and Cua et al. [9] calculated $P \cdot S$ (permeability-surface area) products from indicator dilution experiments for water, antipyrine and iodoantipyrine in isolated dog and rat lungs over the temperature range from 8 to 37°C. In these studies solute movement across the pulmonary endothelium is assessed. The pattern of $P \cdot S$ products is IAP > ^{3}HHO > AP at 37°C which changes to ^{3}HHO > IAP > AP below 21°C. They have shown that an accurate value for P_{AP} can be calculated only below 21°C due to the rapid movement of antipyrine across the endothelium compared to the velocity of blood flow [9]. Below 21°C a value of P can be calculated. At 15°C the P_D values calculated for AP in the isolated endothelial cells is $56 \cdot 10^{-5}$ cm/s (Table III) and close to the value of $38 \cdot 10^{-5}$ cm/s obtained for the intact endothelium of the lung in these studies [2,3,8,9]. This finding supports our contention that the two experimental approaches, with isolated cells and isolated organs, complement each other.

Since an accurate value of P_D for water and small lipophilic solutes cannot be calculated at mammalian body temperatures from whole organ studies, the isolated endothelial cells are a reliable alternate source for these values for measuring transcellular movement. We recognize that endothelial (compared to cellular) permeability can include extracellular movement, we provide a measure of transcellular movement so that the contribution of the extracellular pathway can be estimated more accurately by other means.

Regulation of membrane transport

Analysis of cellular diffusional transport and the factors which regulate it has long been based on Overton's conclusion that diffusional transport in the plasma membrane occurs through membrane lipid for most molecules. Earlier data have been analyzed and interpreted in terms of the Stokes-Einstein equation for diffusion and the predictions which are based on this equation. Lieb and Stein [17] point out that two features of the cellular data accumulated over the years (primarily in erythrocytes) are especially noteworthy, (1) the steep relationship between solute size (MV) and ln $P_D/K_{o/w}$ (equated to ln $D_{membrane}$), where $K_{o/w}$ is the oil/water partition coefficient, and (2) the variation in E_a with different solutes [17]. In a system where Stokesian diffusion is occurring the relation between solute size (MV) and the diffusion coefficient (D_m) should be relatively constant ($D(MV)^{1/3}$ = constant) and E_a should depend upon the change in viscosity of the diffusing medium and be more or less the same for all small solutes (e.g., similar to E_a for D_1 in this study).

Lieb and Stein [17] propose that non-Stokesian diffusion in the membrane lipid would explain the difference in the expected relationships of D_m and MV and those actually seen in the erythrocytes and propose a method for analysis of data to test this. The particular model for non-Stokesian diffusion which they propose for erythrocytes is similar to that proposed for polymers, in which the solutes move through the membrane in the areas of free volume (or 'holes') which comprise 35% of the hydrocarbon area [17]. Within this area the number of 'holes', the rate of appearance and disappearance of them and the volume of particular holes can change. The rate at which these changes occur will determine the diffusion rate within the membrane and will change with temperature changes [17]. Within biological membranes both Stokesian, the interior hydrocarbon core, and non-Stokesian media, the phospholipid areas, are present.

An analysis of some of the preliminary data from this study, primarily from erythrocytes but also from endothelial cells, using the quantitative approach of Lieb and Stein [17] indicated that permeation is non-Stokesian (Garrick, R.A. (1989) FASEB J. 3, A1386). The data reported here allow us to expand this analysis.

The analysis described by Lieb and Stein is straightforward and the steps in the analysis consist of: (1) choosing the organic solvent in which $K_{o/w}$ for each solute is measured to use as a model for partitioning; (2) calculating the van der Waals radius for the test solutes; (3) calculating a ' size corrected P_D', $P^{V=0}$, with the van der Waals radius to use in estimating D_m (ln D_m = ln $P_D/K_{o/w}$; (4) determining whether the dependence of D_m on molecular size is a constant and how steep a change there is in D_m in relation to MV of different test solutes; (5) calculating E_a for P_D, $K_{o/w}$ and, from these, estimating E_a for D_m; and (6) evaluating E_as in terms of Stokesian or non-Stokesian processes. The details for doing these calculations are given in Lieb and Stein [17].

Hexadecane and octanol are identified as potentially good model solvents for estimating the plasma

TABLE VI

Oil / water partition coefficients ($K_{o/w}$) and activation energies for partitioning (E_a) over the temperature range 8.5–37 and 20–42°C for antipyrine and iodoantipyrine

Method in Appendix. $K_{o/w}$ and E_a are means ± S.E.; number of determinations is in parentheses. E_a is calculated from the average at each temperature.

Temp. (°C)	Partition coefficient, $K_{o/w}$			
Solute:	AP		IAP	
Solvent:	octanol	hexadecane	octanol	hexadecane
42.0	–	0.0069 ± 0.0001 (27)	–	0.150 ± 0.015 (27)
37.0	2.23 ± 0.019 (27)	–	11.91 ± 1.076 (33)	–
30.0	–	0.0040 ± 0.0001 (27)	–	0.108 ± 0.0011 (27)
22.0	1.49 ± 0.072 (27)	–	9.56 ± 0.087 (18)	–
20.0	–	0.0035 ± 0.0002 (27)	–	0.070 ± 0.0008 (27)
8.5	1.09 ± 0.009 (15)	–	7.93 ± 0.256 (19)	–
	E_a (kJ/mol)			
	AP		IAP	
	octanol	hexadecane	octanol	hexadecane
	18.2 ± 0.001 (3)	24.0 ± 0.007 (3)	10.4 ± 0.0004 (3)	26.5 ± 0.003 (3)

membrane partition coefficient [17], however, partition coefficients for antipyrine and iodoantipyrine in these solvents have not been published. Therefore, for this analysis we measured partition coefficients for AP and IAP in hexadecane (20–37°C) and in octanol (10–37°C) as described in the Appendix. The coefficients obtained are listed in Table VI. With these values of $K_{o/w}$ we determined that the best model for the erythrocytes is hexadecane while for the endothelial cells either hexadecane or octanol can be used since both yield a slope of one in a plot of log $P^{V=0}$ vs. log $K_{o/w}$.

If we assume that the temperature dependence, E_a of $K_{o/w}$ for each solute is an estimate of E_a for partitioning of that solute into the cell membrane then we can estimate E_a for membrane diffusion (E_a for D_m) from the relation E_a for $P_D = E_a$ for $D_m + E_a$ for $K_{o/w}$ [17]. The results of these calculations are given in Table VII. The E_a values for diffusion of IAP, AP and water in aqueous solutions (D_1) are given for comparison.

If we compare E_a for the diffusion coefficients in D_1 to the values for D_m in the cells then the E_a values for diffusion in the erythrocyte membrane are clearly higher than E_a for diffusion in solvents which indicates non-Stokesian diffusion for antipyrine and iodoantipyrine in the erythrocyte. Lieb and Stein gave the

TABLE VII

The estimated activation energy (E_a) for membrane diffusion (D_m) calculated from E_a for partitioning ($K_{o/w}$) in model solvents and E_a for the permeability coefficient (P_D) for AP, IAP and water in endothelial cells and erythrocytes

E_a values for diffusion in plasma and supernatant fluid (D_1) are given for comparison. The activation energy for 'D_m' is calculated accordig to Lieb and Stein [17].

	Cells:	Model solvent: hexadecane				
		endothelial cells			erythrocytes	
Solute		E_a of $K_{o/w}$ (kJ/mol)	E_a of P (kJ/mol)	E_a of 'D_m' (kJ/mol)	E_a of P (kJ/mol)	E_a of 'D_m' (kJ/mol)
AP		24.0	46.0	22.0	69.9	45.9
IAP		26.6	42.2	15.6	64.9	38.3
^{3}HHO		34.0 [20]	35.2	1.2	–	–
		Model solvent: octanol				
AP		18.3	46.0	27.7	69.9	51.8
IAP		10.4	42.2	31.8	64.9	55.2
^{3}HHO		28.1 [25]	35.1	7.1	–	–
Extracellular fluid		E_a of D_1 (kJ/mol)			E_a of D_1 (kJ/mol)	
AP		–	–	16.1	–	18.5
IAP		–	–	14.0	–	17.7
^{3}HHO		–	–	15.0	–	13.4

same interpretation with another set of solutes for human and dog erythrocytes. Our data indicate that the non-Stokesian medium in the phospholipid of the erythrocyte provides the major resistance to diffusion for the solutes we have tested.

The data for the endothelial cells present a somewhat different picture. The estimated E_as for D_m of antipyrine and iodoantipyrine are close to those in D_1 if hexadecane is the model solvent and somewhat higher if octanol is used. Since water diffuses through the membrane lipid in endothelial cells we can also estimate the E_a for water diffusion. Based on the calculated E_a values the main resistance to water appears to be partitioning into the membrane. For antipyrine and iodoantipyrine if we use the data with hexadecane we would conclude that the diffusion is Stokesian, i.e., the hydrocarbon core provides the main diffusional resistance. If we use octanol as the model solvent we would conclude the diffusion is non-Stokesian, i.e., the phospholipid membrane interface provides the main resistance to diffusion. The choice of the model solvent for membrane partitioning for the endothelial cells is based on far fewer test solutes than for the erythrocytes so we cannot definitely say which solvent is more appropriate. The data for permeation when *cis*-vaccenic acid or cholesterol is present helps in interpreting these data.

Incubation of the cells with cholesterol and *cis*-vaccenic acid produces a decrease in the permeability coefficients for antipyrine and water unlike the results with serotonin transport [11]. In a non-Stokesian system incorporation of substances into the membrane could decrease the free volume available for diffusion and the opening and closing of the volumes necessary for movement. Both of these agents interact with the mid-acyl region of the membrane phospholipids. This is also the interface for partitioning between the extracellular aqueous and membrane lipid areas.

The best interpretation of the data presented here is that the primary resistance to membrane diffusion of small lipid soluble molecules is present in the phospholipid layer – a non-Stokesian medium. The difference in the effect of membrane alteration on water and antipyrine would correspond to the size difference in the free volume necessary for diffusional movement for the two solutes. This also suggests that predictions of permeability characteristics (e.g., for drugs, toxins etc.) and the effects of alterations in the lipid of the plasma membrane (e.g., from oxidants, metabolic products, etc.) need to be explored more fully in terms of alternate explanations to those for Stokesian diffusion.

Summary

Cultured pulmonary artery endothelial cells are a good model for estimating transport across the cells in the intact endothelium. Studies with these cells also provide a basis for distinguishing between transcellular and intercellular movement in the endothelium. Movement of lipophilic solutes through the plasma membrane is restricted by the phospholipid areas rather than the hydrocarbon core.

Appendix

Measurement of oil / water partition coefficients ($K_{o/w}$)

A semi-micro method was developed to measure the partition coefficients between an organic phase (hexadecane or octanol) and water for AP and IAP. To a 25 ml Ehrlenmeyer flask are added 4.0 ml of 150 mM NaCl containing 1 mM $CaCl_2$ and 4 ml of an organic phase (octanol or hexadecane). Approx. 1 μCi of [^{125}I]IAP or of [^{14}C]AP are added in a volume of 10 μl. The mixtures are shaken gently at designated temperatures (37, 22 and 8.5°C for octanol and 42, 30 and 20°C for hexadecane) for 1–30 h, the periods are longer for the lower temperatures to ensure equilibrium between the aqueous and organic phases. The phases are sampled by means of a microhematocrit capillary tube which is flame sealed at one end and spun at 13 500 × g in a micro-capillary centifuge (IEC, model MB) for 10 min in a cold room at 8.5°C. The tubes are scored to provide segments containing 25 μl and broken at the scored points. The segments are placed in 7 ml scintillation vials which contain 5 ml of Ecolume^R. To the vials into which the organic phase segments have been placed 25 μl of aqueous solution are added and 25 μl of the organic phase are added to the vials in which the aqueous phase segments have been placed. The radioactivity of each vial is determined and the ratio of counts in the organic phase to counts in the aqueous phase are calculated for each solute. For each experiment at least four segments from each phase are used for each of the solutes at each temperature. Hexadecane solidifies below 20°C and can not be used below that temperature. The results are listed in Table VI.

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