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Endothelial cell permeability to water

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We have calculated diffusional permeability coefficients for tracer water and for [^{14}C]antipyrine in endothelial cells. With these values and those from studies in whole lungs we set a range for diffusional water permeability coefficients of the intact endothelium.

Endothelial cells line the blood vessels in all parts of the body. In the capillaries the endothelium provides a structural barrier as well as a pathway for exchange of various substances in the blood and the tissues. The diffusional permeability of the endothelium is determined by the endothelial cells which regulate the transcellular movement of materials and by other factors that regulate extracellular movement through the paracellular pathway. Any approach to defining the characteristics of the capillary barrier must separate movement through these two pathways. Models of greater or lesser complexity have been developed in recent years to achieve this separation based on data from indicator dilution experiments [1–3]. It has become increasingly evident that for rapidly penetrating substances, such as tracer water, a value for the permeability of the endothelial cells cannot be reliably deduced from this type of study, although a lower bound can be set [4].

We have measured the permeability of isolated endothelial cells directly by the linear diffusion method. We have chosen as test solutes tracer water and antipyrine, a lipid-soluble solute, since both have been used extensively in studies of organ or tissue permeability [5] and data are available to permit comparison of the results of these studies with those in isolated cells. We link here the studies in cells and in organs.

Endothelial cells are isolated from calf pulmonary artery without the use of proteolytic enzymes as described by Ryan et al. [6]. The non-enzymatic separation leaves intact the cell surface layer of the glycocalyx. The cells are characterized as being of endothelial origin by testing for Factor VIII antigen and angiotensin converting enzyme activity and by morphological criteria under light and electron microscopy [7]. For our diffusion studies the endothelial cells are removed from the T-75 flasks with a rubber policeman, titurated with a 26 gauge needle and suspended in 10 ml of Medium 199 or Puck's A saline adjusted to pH 7.4 with 25 mM Hepes. The cells are centrifuged at $400 \times g$ for 5 min at 4 C. The supernatant fluid is removed and the cells are resuspended in Medium 199 and transferred to a 1.5 ml centrifuge tube and centrifuged for 15 s in a Microfuge B (Beckman). A sample is removed to serve as a blank and the remainder of the cells are mixed with 10 μl of ^{125}I-labeled human serum

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albumin (HSA 1 μCi/μl Mallinckrodt Chemical with carrier albumin at 4 g/dl) per 0.1 ml of cells as an extracellular marker. The cells are tested for viability after separation and after the diffusion experiments. Cells treated as a control are resuspended in culture medium and plated into T-25 flasks to ascertain that they can spread and form a monolayer.

The procedures used in the linear diffusion technique have been described in detail [8–10] and are given only briefly here. The cells are drawn into segments of polyethylene tubing (PE 90), one end is sealed and the cells centrifuged in a microhematocrit centrifuge for 30 min. Subsequently, the tubing is sliced below the cell-supernatant fluid interface and taped to a plexiglass support. The open end of the tube is pulsed with a radiolabeled solute (tritiated water, ^{3}HHO, 25 μCi/μl or [^{14}C]antipyrine, 1 μCi/μl, New England Nuclear, in buffer pH 7.4, 300 mosM) and the cells incubated at 37 C. After incubation the cell column is frozen, sliced and each slice is counted in a liquid scintillation counter. From the semilogarithmic plot of the log of the radioactivity against the diffusion distance the diffusion coefficient can be calculated [10]. Diffusion coefficients are obtained for the packed cell preparations (D), the intracellular material (D_2), and the extracellular fluid (D_1) [8,9]. Measurements and calculations are made to obtain the tortuosity of the extracellular path, the relative extracellular volume, cell length in the diffusion direction, the distance between cells in the diffusion direction, the cell surface exchange area and the cross-sectional area for diffusion as described previously [9,10]. The permeability coefficient (P_0) is calculated using the series-parallel pathway model of Perl [10].

The diffusion coefficients obtained for labeled water and antipyrine are listed in Table I. The extracellular diffusion coefficients (D_1), which are through the supernatant fluid from the last cell washing, are lower than those in water as is usually found in physiological buffer solutions [9]. The intracellular diffusion coefficients (D_2) are measured through packed cells that have been frozen and thawed three times to disrupt the plasma membranes [8]. Diffusion coefficients in the intracellular material are significantly smaller than the coefficients in the supernatant fluid, D_1. The ratio $(D_2 - D)/D_2$ allows comparison of diffusion coefficients in the intracellular material with overall diffusion coefficients when the plasma membrane is intact [8]. The closer this ratio is to one, the larger is the proportion of the total resistance due to the membrane. This ratio is 0.27 and 0.23 for water and antipyrine, respectively, for the endothelial cells. We take this as an indication that cytoplasmic resistance may be an important component of total cellular resistance to diffusional permeability for these substances.

The mean membrane permeability coefficient for water is 304 ($\pm$42, $n = 7$) $\cdot 10^{-5}$ cm $\cdot$ s^{-1} at 37°C. This is considerably lower than that reported for erythrocytes or mixed lung cells but higher than that found for alveolar macrophages (Table II). The permeability coefficients in Table II have been determined with the linear diffusion technique except for the last two values for the erythrocytes which have been determined in a rapid reaction continuous flow system and from the efflux of tritiated water from the erythrocytes, respectively. Interpretation of data from the cell studies has resulted in proposals for protein-associated (e.g., erythrocytes, [11]) and/or lipid (e.g., lung cells [9]) pathways for diffusional movement

TABLE I

DIFFUSION COEFFICIENTS FOR PACKED CELLS (D), EXTRACELLULAR MATERIAL (D_1) AND INTRACELLULAR MATERIAL (D_2)

All were measured at 37°C. The values given are mean $\pm$ S.E. with the number of samples in parenthesis.

	Diffusion coefficient (10^{-5} cm$^2 \cdot$ s^{-1})		
	D	D_1	D_2
^{3}HHO	0.682 $\pm$ 0.0294 (7)	2.45 $\pm$ 0.042 (20)	0.932 $\pm$ 0.0570 (5)
[^{14}C]Antipyrine	0.273 $\pm$ 0.0058 (6)	1.13 $\pm$ 0.029 (16)	0.355 $\pm$ 0.0180 (6)

TABLE II

PERMEABILITY COEFFICIENTS FOR DIFFERENT CELL PREPARATIONS ALL MEASURED AT 37°C

The permeability coefficients are calculated using the relative extracellular volume (V_1/V) calculated for each tube of cells this was approx. 0.15; the tortuosity (L_1/L_2) = 1.14; the cell diameter = 20.25 μm; the cell to cell distance (L_0) = 1.07 μm.

	P (10^{-5} cm·s^{-1})	
	^{3}HHO	[^{14}C]antipyrine
Endothelial cells	304	181
Alveolar macrophages [9]	110	232
Lung cells [9]	755	444
Erythrocytes		
dog [16]	908	318
human	520	
human [21]	562	
human [22]	340	

through the plasma membrane. The presence of protein associated pathways does not exclude water movement through lipid areas but the permeability coefficient through the lipid areas can be measured only when the protein pathways are blocked [12]. It is possible that the estimate of P for water should include a contribution from the glycocalyx but we have no evidence for this at present. From the data presented here, we cannot determine yet the particular process by which water permeates the endothelial cell.

Some years ago Perl et al. [13] obtained, from multiple indicator dilution experiments, a value of $300 \cdot 10^{-5}$ cm · s^{-1} for the permeability coefficient to water of the endothelium in the pulmonary vasculature in anesthetized dogs. This value was based on the Crone relation [2], $PS = F \ln(1/(1 - E)$ where S is the surface area, F is flow and E is extraction of tracer water and included important corrections for back-diffusion. The value of S used was 500 cm^2 · (g lung)$^{-1}$, a value considerably less than that of 3500 cm^2 · (g lung)$^{-1}$, now more commonly accepted. If the latter value is used, P, calculated from the indicator dilution studies, drops to about $42 \cdot 10^{-5}$ cm · s^{-1}, an order of magnitude less than the values obtained for the cells.

In indicator dilution experiments from one of our laboratories, we found no change in tracer water extraction with temperature in dog [4] or rat [18] lungs between 27 and 15°C [4] or 27 and 8°C [18], respectively. If the permeability coefficient to tracer water was in fact measured in these experiments we would have expected to see a change in P calculated over this temperature range. Both in vivo and in isolated perfused dog lungs, tracer water extraction and calculated PS products increase in the presence of alveolar flooding [19]. However, even with the highest value obtained for E, i.e., 0.994, with $S = 3.5 \cdot 10^3$ cm^2 · g^{-1}, the P calculated is only $44 \cdot 10^{-5}$ cm · s^{-1}. It is only if S is taken as 500 cm^2 · g^{-1} that P reaches $308 \cdot 10^{-5}$ cm · s^{-1}. These findings can be interpreted as indicating that the distribution of tracer water is not demonstrably barrier-limited in the time domain of indicator dilution experiments. Therefore, we cannot use data from these indicator dilution experiments to obtain actual values for the permeability coefficient to water, but we can, nevertheless, use the values obtained as lower bounds for this coefficient. Thus, we have established a range of measured permeability coefficients for endothelium between $44 \cdot 10^{-5}$ cm · s^{-1} in the lung and $304 \cdot 10^{-5}$ cm · s^{-1} in the isolated cells.

We note that these values for the permeability coefficient of the pulmonary endothelium to water fall within the range of the values of $2.5 \cdot 10^{-5}$ to $3.6 \cdot 10^{-2}$ cm · s^{-1} for the filtration permeabilities of the endothelium that can be calculated from filtration coefficients of $5 \cdot 10^{-3}$ to $7 \cdot 10^{-1}$ cm^3 · min^{-1} · mmHg^{-1} · (100 g)$^{-1}$ obtained from lymph flow and pressure increment experiments [20]. A comparison of the diffusional permeabilities and filtration permeabilities provides the basis for considering that the passage of tracer water across the pulmonary endothelium could be either diffusive or convective.

The value of P calculated for antipyrine, 181 (± 35, $n = 6$) $\cdot 10^{-5}$ cm · s^{-1} is lower than that for water in endothelial cells. It is also lower than the value of P for this solute reported for mixed lung cells, erythrocytes or alveolar macrophages (Table II). A permeability coefficient for antipyrine that is lower than that for water is usual in our cell studies and in epithelium [9,14], although the alveolar macrophages seem to be an exception at 37°C [9]. In all of these studies it is assumed that antipyrine permeates the lipid bilayer of the plasma membrane: the differences in P could be a

reflection of differences in the composition of the bilayers in these cells [9]. The same assumption applies to the endothelial cell. In the structured pulmonary endothelium, the interpretation of data obtained with antipyrine is that it has a lower permeation rate than for water [15]. This concords with the results obtained with the endothelial cells.

The permeability coefficients for the isolated endothelial cells reported here are the first values obtained with these cells. These values provide a basis for comparison with other cell types, both in the values obtained and in the pattern seen in the relative permeabilities of water and antipyrine. The permeability coefficients for the endothelial cells also provide a basis of reference that can be used in modeling indicator dilution experiments. As we accumulate data with the endothelial cells it will be possible to test the hypothesis that data obtained with isolated cells are comparable to data obtained with endothelial cells in a structured tissue or organ system when the limitations of other techniques are taken into account. Finally, the data may make it possible to determine whether the transendothelial passage of tracer water and, inferentially, of ordinary water, is diffusive or convective, transcellular or paracellular.

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