Water transport and the distribution of aquaporin-1 in pulmonary air spaces

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Effros, R. M., C. Darin, E. R. Jacobs, R. A. Rogers, G. Krenz, and E. E. Schneeberger. Water transport and the distribution of aquaporin-1 in pulmonary air spaces. J. Appl. Physiol. 83(3): 1002–1016, 1997.—Recent evidence suggests that water transport between the pulmonary vasculature and air spaces can be inhibited by HgCl2, an agent that inhibits water channels (aquaporin-1 and -5) of cell membranes. In the present study of isolated rat lungs, clearances of labeled (3HOH) and unlabeled water were compared after instillation of hypotonic or hypertonic solutions into the air spaces or injection of a hypotonic bolus into the pulmonary artery. The clearance of H2O between the air spaces and perfusate after intratracheal instillation and from the vasculature to the tissues after pulmonary arterial injections was invariably greater than that of unlabeled water, indicating that osmotically driven transport of water is limited by permeability of the tissue barriers rather than the rate of perfusion. Exposure to 0.5 mM HgCl2 in the perfusate and air-space solution reduced the product of the filtration coefficient and surface area (PfS) of water from the air spaces to the perfusate by 28% after instillation of water into the trachea. In contrast, perfusion of 0.5 mM HgCl2 in air-filled lungs reduced PfS of the endothelium by 86% after injections into the pulmonary artery, suggesting that much of the action of this inhibitor is on the endothelial surfaces. Confocal laser scanning microscopy demonstrated that aquaporin-1 is on mouse pulmonary endothelium. No aquaporin-1 was found on alveolar type I cells with immunogold transmission electron microscopy, but small amounts were present on some type II cells.

28-kDa channel-forming integral membrane protein; mercuric chloride; epithelium; endothelium; immunogold

FUNCTIONAL EVIDENCE for water channels in cell membranes was first reported by Paganeli and Solomon (24) and Sidel and Solomon (32) nearly 40 years ago. They found that osmotic water permeability exceeds diffusional water permeability in red blood cells and attributed this difference to the movement of water through pores in the red blood cell membranes. Macey (19) subsequently showed that water transport across red blood cell membranes is inhibited by organic mercurials and that the activation energy of water movement through red blood cell membranes is low in the absence of mercurials but high when they are present. This suggests that in the presence of osmotic gradients, most of the water moves through aqueous channels that can be inhibited by mercurials. The structure of the red blood cell water channels has been recently elucidated by Agre et al. (1), who cloned a protein of 28,000-Da molecular mass, designated as the channel-forming integral membrane protein (CHIP28). This represented the first member of a growing family of membrane proteins referred to as “aquaporins,” many of which transport water, and CHIP28 is now referred to as aquaporin-1.

When lungs are perfused with hypertonic solutions of NaCl, urea, or sucrose, the fluid that is extracted from the pulmonary parenchyma is essentially free of any solutes (7). This observation was consistent with the hypothesis that most of the water removed from the lungs with these perfusion solutions crosses cell membranes, which are impermeable to extracellular electrolytes and other small hydrophilic solutes. In a subsequent study (11), our laboratory found that perfusion of fluid-filled lungs with hypertonic solutions of glucose or sucrose resulted in the loss of virtually solute-free water from the air spaces, suggesting that most of the water had crossed cellular membranes rather than through the intercellular junctions.

Nielsen et al. (23) recently reported that the mRNA for aquaporin-1 is present in the endothelium of lungs and a variety of other organs with continuous endothelial membranes. Hasegawa et al. (15) found that mRNA encoding aquaporin-1 is present in a subpopulation of pulmonary alveolar epithelial cells, tracheal epithelium, and the tracheal adventitia. Immunohistochemically stained lung tissues examined by light microscopy revealed what was interpreted as strong staining of the pulmonary epithelium. Folkesson et al. (12) showed that the osmotic water permeability of freshly isolated rat alveolar type II cells is weakly temperature dependent and inhibited by 0.5 mM HgCl2. Because it has not been possible to isolate type I alveolar cells, which cover 97% of the alveolar surface, these investigators conducted additional experiments in perfused sheep lungs. Instillation of hyperosmolar saline (900 mM NaCl) into these lungs resulted in the flow of fluid from the vasculature into the air spaces. This movement of water was inhibited by HgCl2 in a manner suggesting that aquaporin-1 molecules play an important role in the movement of water between the air spaces and vasculature of the lungs, and they suggested that much of the aquaporin-1 is located on the pulmonary epithelium. However, it was not determined whether the osmotic shift of water across the alveolar-capillary barrier was limited by permeability of the membranes rather than the rate at which the lungs were perfused. In view of the observation that HgCl2 can increase pulmonary vascular resistance (2), the effects of HgCl2 might reflect a decrease in the alveolar-capillary sur-
face area of the lungs rather than inhibition of aquaporins in the alveolar membranes. Furthermore, they did not determine whether endothelial rather than epithelial aquaporin-1 activity might be responsible for the effect of HgCl₂ on water transport across the alveolar-capillary barrier.

In the present study, the movement of water in response to osmotic gradients was monitored after intratracheal instillation and pulmonary arterial injections by using both labeled (³HOH) and unlabeled water (HOH). New indicator dilution approaches were used to estimate the osmolality of the pulmonary vasculature after instillation of hypotonic and hypertonic solutions into the air spaces and to determine the effect of HgCl₂ on epithelial and endothelial permeability to water. In addition, light- and electron-microscopic immunochemical studies were used to localize more precisely the distribution of aquaporin-1 in the lungs.

METHODS

Water and Solute Exchange

Experimental procedures. The physiological studies were divided into two sets of experiments (Table 1). In the first set (groups 1–7), 5 ml of hypotonic or hypertonic fluids containing ³HOH were instilled into the air spaces of isolated, perfused rat lungs, and measurements were made of the movements of labeled and unlabeled water between the air-space and vascular compartments. In the second set (groups 8 and 9), 0.7 ml of solute-free water with ³HOH were injected into the pulmonary artery of air-filled lungs, and samples of the pulmonary venous outflow were collected to determine how much of the ³HOH and HOH had exchanged between the tissues and vasculature. No attempt was made to subdivide the cellular and interstitial compartments of the lungs in these studies.

In all studies, Sprague-Dawley rats [average wt 343 ± 67 (SD) g, n = 46] were anesthetized with 0.7 ml of a 64.8 mg/ml pentobarbital sodium solution. The chest was opened, catheters were placed in the trachea, pulmonary artery, and left atrium, and blood was flushed from the vasculature at constant temperature (see Table 1) with 10 ml of perfusion fluid that contained (in mM) 25 NaHCO₃, 110 NaCl, 4 KCl, 2.5 CaCl₂, and 0.8 MgSO₄, as well as 150 mg/dl glucose, 10 mg/dl urea, and 5 g/dl bovine serum albumin (BSA; Cohn Fraction V, 98–99% albumin, Sigma Chemical, St. Louis, MO) adjusted to pH 7.4 with 1N NaOH when exposed to 5% CO₂. The albumin was labeled with Evans blue (EB; 10 mg/l). The lungs were ventilated with a 5% CO₂/95% O₂ mixture at a tidal volume of 4.5 ml and at a rate of 30 breaths/min. Ventilation was discontinued just before the beginning of the experiments. Pulmonary arterial pressures were measured just proximal to the pulmonary arterial catheter.

AIRWAY INSTILLATION. In the first set of experiments (groups 1–7, Table 1), perfusate was pumped through the lungs at either 6.45 or 22.1 ml/min with a nonpulsatile pump from a reservoir that was heated to 37°C, cooled to 11°C, or left at room temperature (25°C) to keep the pulmonary arterial temperature constant. Temperatures were measured with a thermocouple inserted into the arterial line just proximal to the point at which the catheter entered the pulmonary artery. Left atrial pressures were kept at zero. After 70 s of perfusion, an experimental solution was instilled into the air spaces with a no. 50 polyethylene catheter that was inserted through the endotracheal tube to the carina. The outflow from the left atrium was collected in serial beakers over a 120- to 240-s interval, and, in some experiments, samples were collected from the air spaces at the end of the experiments.

PULMONARY ARTERIAL INJECTIONS. In the second set of experiments (groups 8 and 9, Table 1), 0.7 ml of water with ³HOH, [¹⁴C]dextran, and 1 g/l albumin labeled with 10 mg/l EB were introduced into the pulmonary arterial line (see description of perfusate above), and the outflow from the left atrium was collected at 2.66-s intervals with an automated collection rack over an 80-s interval. These injections were made with a chromatography valve, which enabled us to rapidly switch the inflow from tubing that was filled with the control perfusion solution to tubing that contained the injection bolus.

MEASUREMENTS. Indicator and solute concentrations were measured in 0.4-ml samples of the injected solutions, the perfusate, and, in group 3 and 4 experiments, the fluid remaining in the air spaces at the end of the experiments. EB-labeled albumin concentrations were measured in 60-µl samples that were diluted 10-fold in isotonic saline and then centrifuged at 1,000 × g for 10 min. The supernatant was removed, and the absorbance was determined spectrophotometrically at 620 nm. Concentrations of ³HOH and in the group 2 of experiments, [¹⁴C]dextran, molecular weight 45,000, were determined with samples of perfusate and the instilled solutions by diluting 50 µl in 2 ml of Hi-Safe III scintillation fluid and counting these samples with an automated beta counter. Corrections were made for background radiation and crossover between the ³⁴C and ³H labels. Na⁺ ([Na⁺]) and K⁺ concentrations ([K⁺]) were determined with ion-specific electrodes. In most of the group 1–7 studies osmolality was measured with a vapor pressure osmometer. In the group 8 and 9 studies (see Table 1), osmolality was estimated from EB-labeled albumin concentrations. Previous studies from our laboratory (7) indicated that solvent drag reflection coefficients (αᵣ) of small hydrophilic solutes and electrolytes across the endothelium are close to 1.0. After introduction of water into the arterial inflow, the loss of water from the capillaries should result in increases in osmolality that are proportionate to those of EB-labeled albumin

\[
C_{\text{v}} = \frac{[\text{EB}]_{\text{v}}}{[\text{EB}]_{\text{a}}} C_{\text{a}}
\]

where \( C_{\text{v}} \) and \( C_{\text{a}} \) are the venous and arterial osmolalities, and \([\text{EB}]_{\text{a}}\) and \([\text{EB}]_{\text{v}}\) are the concentrations of EB-labeled albumin in the venous and arterial perfusate. When air-space samples

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Flow, ml/min</th>
<th>Air Space Osmolality, mosmol/kgH₂O</th>
<th>Temperature, °C</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>6.45</td>
<td>0</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>2 (High flow)</td>
<td>22.1</td>
<td>0</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>3 (Hypotonic)</td>
<td>6.45</td>
<td>154</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>4 (Hypertonic)</td>
<td>6.45</td>
<td>508</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>5 (Cold)</td>
<td>6.45</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>6 (No HgCl₂)</td>
<td>6.45</td>
<td>154</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>7 (HgCl₂)</td>
<td>6.45</td>
<td>154</td>
<td>25</td>
<td>0.5 mM HgCl₂</td>
</tr>
<tr>
<td>Pulmonary arterial injections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (No HgCl₂)</td>
<td>6.45</td>
<td>0</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>9 (HgCl₂)</td>
<td>6.45</td>
<td>0</td>
<td>37</td>
<td>0.5 mM HgCl₂</td>
</tr>
</tbody>
</table>

Cold, hypothermia (11°C); hypotonic, 77 mM NaCl; hypertonic, 308 mM NaCl.
were collected, [Na\(^+\)], [K\(^+\)], and osmolality were measured (none of the EB entered the air space). The conditions of each set of experiments are indicated in Table 1. Binding of EB to albumin in the experimental solutions was checked by filtering them through ultrafilters (Centricon) with a molecular cutoff of 30,000. No unbound albumin was detected in these experiments. The binding of EB to albumin and its color remained uninfluenced between osmolalities of 10 and 500 mosmol/kg H\(_2\)O. Measurements of lactic dehydrogenase (kit, Sigma Chemical) were made in the air-space and perfusate solutions at the end of some of the experiments, but none was detected.

Calculation of water transport. AIRWAY INSTILLATION EXPERI-
MENTS. After instillation of hypotonic solutions into the air
spaces (groups 1–3 and 5–7, Table 1), entry of water into the pulmonary exchange vessels increased the flow leaving these vessels and decreased EB-labeled albumin concentrations. Assuming no loss of EB and steady-state conditions, the venous outflow (F\(_v\)) was calculated from the arterial inflow (F\(_a\)), [EB]\(_a\), and [EB]\(_v\), with the equation

\[
F_v = \frac{[EB]_a}{[EB]_v}
\]

(2)

The clearance of water (C\(_{\text{CHOH}}\)) from the air spaces to the perfusate was calculated from the difference between the venous and arterial flows

\[
C_{\text{CHOH}} = F_v - F_a = \frac{[EB]_a - [EB]_v}{[EB]_v}
\]

(3)

The volume of water remaining in the air spaces at time \(i\) (V\(_{\text{as},i}\)) was assumed to be equal to the amount originally instilled into the lungs (5 ml) minus the volume of water that was transferred from the air spaces to the perfusate before that time

\[
V_{\text{as},i} = 5 - \sum_{t=0}^{n} C_{\text{CHOH}} \Delta t_i
\]

(4)

where \(\Delta t_i\) represents the duration of the time interval during which the \(i\)th sample was collected.

The rate of loss (I\(_{\text{as},i}\)) of \(^3\)HOH from the air spaces during the \(i\)th interval was calculated with the equation

\[
I_{\text{as},i} = F_v[3\text{HOH}]_{\text{as},i} - F_a[3\text{HOH}]_{\text{ki},i}
\]

(5)

where [3\text{HOH}]\(_{\text{ki},i}\) is the venous concentration of \(^3\)HOH in blood collected from the left atrium during the \(i\)th interval.

The amount of \(^3\)HOH remaining in the air spaces during the collection of the \(i\)th sample ([3\text{HOH}]\(_{\text{as},i}\)) was assumed equal to the original amount instilled into the air spaces ([3\text{HOH}]\(_{\text{as},0}\)) minus the quantity that had left in the venous outflow

\[
[3\text{HOH}]_{\text{as},i} = [3\text{HOH}]_{\text{as},0} - \sum_{t=0}^{n} L_{\text{as},i} \Delta t_i
\]

(6)

The concentration of \(^3\)HOH in the air-space fluid at the time that the \(i\)th sample of perfusate was collected ([3\text{HOH}]\(_{\text{ki},i}\)) was calculated from Eqs. 3 and 5

\[
[3\text{HOH}]_{\text{ki},i} = \frac{[3\text{HOH}]_{\text{as},i}}{V_{\text{as},i}}
\]

(7)

The clearance of \(^3\)HOH (C\(_{3\text{HOH}}\)) from the air spaces during each collection interval was calculated with the equation

\[
C_{3\text{HOH}} = \frac{I_{\text{as},i}}{[3\text{HOH}]_{\text{as},i}}
\]

(8)

In the group 4 experiments, instillation of hypertonic saline into the air space resulted in the movement of fluid from the vasculature to the air spaces. This caused \(F_v\) to fall relative to \(F_a\) and increased the concentrations of EB-labeled albumin in the perfusate leaving the pulmonary exchange vessels. The clearance of water into the air spaces was calculated from Eq. 2. This value was multiplied by -1 to yield a positive value for this parameter. In this group of experiments, \(^3\)HOH was incorporated in the perfusate rather than the air-space solution, and it moved into the hypertonic fluid that had been instilled into the air spaces. The influx of \(^3\)HOH into the air space (I\(_{\text{as},i}\)) was calculated with the equation

\[
I_{\text{as},i} = F_a[3\text{HOH}]_{\text{as},i} - F_v[3\text{HOH}]_{\text{ki},i}
\]

(9)

C\(_{3\text{HOH}}\) into the air spaces was calculated on the basis of the concentration of \(^3\)HOH entering the arterial end of the exchange vessels ([3\text{HOH}]\(_{\text{a}}\))

\[
C_{3\text{HOH}} = \frac{I_{\text{as},i}}{[3\text{HOH}]_{\text{a}}}
\]

(10)

For purposes of comparison, values were also calculated for the peak values of C\(_{\text{CHOH}}\) divided by peak values of C\(_{3\text{HOH}}\) (see Table 2).

PULMONARY ARTERIAL INJECTIONS. In the second group of experiments, the lungs were perfused with the EB-labeled albumin solution described above for 2 min, and a 0.7-ml bolus of water was injected into the arterial inflow. The injectate bolus contained \(^3\)HOH and [14C]dextran that were dissolved in water containing the same concentration of EB as that present in the perfusion solution and 0.1 g/dl albumin. As the hypertonic injection bolus traverses the pulmonary capillaries, HOH is lost from the vessels, resulting in an increase in the concentration of EB-labeled albumin. The rate at which HOH was lost from the vascular compartment to the tissues (which is equivalent to its clearance, C) was calculated by using the equation

\[
C_{\text{CHOH}} = F_a \frac{[EB]_a - [EB]_v}{[EB]_v}
\]

(11)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>C(_{\text{CHOH}}) peak, ml/s</th>
<th>C(_{\text{CHOH}}) peak, ml/s</th>
<th>C(<em>{\text{CHOH}}) peak/ C(</em>{\text{CHOH}}) peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>0.0147 ± 0.0010</td>
<td>0.0311 ± 0.0067</td>
<td>0.526 ± 0.064</td>
</tr>
<tr>
<td>2 (High flow)</td>
<td>0.0390 ± 0.0056*</td>
<td>0.0854 ± 0.0115*</td>
<td>0.455 ± 0.008</td>
</tr>
<tr>
<td>3 (Hypotonic)</td>
<td>0.0151 ± 0.0006</td>
<td>0.0472 ± 0.0032</td>
<td>0.325 ± 0.026*</td>
</tr>
<tr>
<td>4 (Hypertonic)</td>
<td>0.0240 ± 0.0014*</td>
<td>0.0493 ± 0.0023</td>
<td>0.489 ± 0.029</td>
</tr>
<tr>
<td>5 (Cold)</td>
<td>0.0152 ± 0.0009</td>
<td>0.0401 ± 0.0044</td>
<td>0.392 ± 0.034*</td>
</tr>
<tr>
<td>6 (No HgCl(_2))</td>
<td>0.0132 ± 0.0009</td>
<td>0.0468 ± 0.0020</td>
<td>0.285 ± 0.022*</td>
</tr>
<tr>
<td>7 (HgCl(_2))</td>
<td>0.0111 ± 0.0005</td>
<td>0.0493 ± 0.0034</td>
<td>0.227 ± 0.015*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 5\) subjects in each group. C\(_{\text{CHOH}}\) peak, peak clearance of unlabeled water; C\(_{\text{CHOH}}\) peak, peak clearance of labeled water. * Significantly different from group 1, \(P < 0.05\).
The fraction (DHOH) of the injected volume of water (0.7 ml) that was lost each second from the pulmonary venous outflow was calculated by dividing CHOH by 0.7 ml (see Fig. 6). The fraction of the injected H2O (D1H2O) that was lost each second from the perfusate samples was calculated in the following fashion. It was assumed that the labeled dextran (which was injected into the pulmonary artery with 0.7 ml of water containing 3H2O) does not leave the vasculature during transit through the lungs. The concentration of 3H2O (designated as the calculated value, [3H2O]a) that would have been present in each of the venous samples had there been no diffusion of 3H2O out of that sample in transit through the lungs was determined with the equation

\[ [3H2O]_b = \frac{[dex]_b}{[dex]_n} [3H2O]_{inj} \]  

where [dex]b represents the observed concentration of 14C-dextran in the venous sample, and [dex]n designates the corresponding concentration in the injected bolus. D3H2O of the injected 3H2O that was lost from each sample because of diffusion into the tissues is therefore

\[ D3H2O = \frac{([3H2O]_a - [3H2O]_b)F_v\Delta t}{[3H2O]_{inj}V_{inj}} \]  

\[ = \frac{[dex]_b [3H2O]_{inj} - [3H2O]_nV_{inj}}{[3H2O]_{inj}V_{inj}} \]  

where \( \Delta t \) is the collection interval. The hypotonic bolus was followed by isotonic perfusate containing no 3H2O, and there was consequently return of both 3H2O and H2O to the vasculature at later times, shown as negative values (see Fig. 6). Presumably because of differences in the perfused volumes of different lungs, arrival of indicators in the outflow varied between experiments. To facilitate graphic presentation of these data, the samples with peak 3H2O are shown at “zero time” and other samples at the number of seconds before and after the peak 3H2O concentrations had been attained.

Calculation of the product of the filtration coefficient (P,<sub>c</sub>) and surface area (S) of the alveolar-capillary wall ([P,<sub>c</sub>S]<sub>a</sub>) was made with the following equation, which is derived in the APPENDIX

\[ (P,<sub>c</sub>S)_a = \frac{CHOH}{(c_i - c_o)V_w} \]  

where \( V_w \) is the molar volume of water (18 cm<sup>3</sup>/mol), c<sub>i</sub> represents the estimated osmolarities of the perfusate in the exchange vessels and \( V_{inj} \) is volume injected into pulmonary artery (0.7 ml) (see APPENDIX). Although the subscript c is used, it must be emphasized that exchange may not be limited to the capillaries but may include other microvessels within the lungs. The osmolarity of the air-space fluid (c<sub>o</sub>) was zero in groups 1, 2, and 5. In the other groups, c<sub>o</sub> was calculated from the amount placed in the air spaces, and subsequent changes in volume were calculated by using Eq. 3. Values of P<sub>c</sub>S are expressed in units of milliliters per second, and mean values shown are based on the values calculated from samples collected near the peak of the clearance curves (60 s after instillation of fluid into the air spaces in groups 1 and 3–7 and 30 s after instillation of fluid in group 2).

Similarly, the P<sub>c</sub>S product of the endothelium [(P,<sub>c</sub>S)<sub>endo</sub>] was estimated by using the equation

\[ (P,<sub>c</sub>S)_{endo} = \frac{CHOH}{(c_{inj} - c)v_w} \]  

where \( c_{inj} \) designates the estimated osmolality of the tissue just before the nth sample had traversed the exchange vessels.

Statistical analysis. Values are means ± SE. The significance of differences between mean peak clearance values were tested with a one-way analysis of variance. A Newman-Keuls test was used to compare individual mean values (SigmaPlot, J andel, San Rafael, CA). A similar approach was used to compare P<sub>c</sub>S values, but a separate Student’s t-test was also used to compare (P,<sub>c</sub>S)<sub>a</sub> in the presence or absence of HgCl<sub>2</sub>. Comparisons between peak losses of HOH and 3H2O in the pulmonary arterial injection experiments were made with a Student’s t-test.

Morphological Studies

Reagents. Rabbit anti-aquaporin-1 and preimmune sera were a generous gift of Dr. Dennis Brown (Renal Unit, Massachusetts General Hospital). Previous studies established that there was no difference in the specificity between the whole anti-serum and the affinity-purified anti-aquaporin-1 antibody, as determined by Western blot analysis and immunogold staining of rat kidneys (29). CY3-conjugated goat anti-rabbit immunoglobulin G (10 nm) was from Ted Pella (Redding, CA). Glutaraldehyde, acrolein, and Epon were obtained from Ladd Research Industries (Burlington, VT); uranyl acetate and propylene oxide were from Polysciences (Warrington, PA).

Procedures. Immunofluorescence localization of aquaporin-1 by using confocal microscopy (see Fig. 7). Female BALB/c mice, 6–8 wk old, were anesthetized with pentobarbital sodium (5 mg/100 g body wt). The thorax was opened, and fixative containing 2% paraformaldehyde, 75 mM lysine, and 10 mM sodium periodate (20, 21) was used to gently inflate the lung. After 5 min, slices of lung were fixed overnight in sodium periodate at 4°C. The next day, small pieces (1 mm<sup>3</sup>) of lung were cut, rinsed in phosphate-buffered saline (PBS), dehydrated in graded ethanol, embedded in Epon, and polymerized overnight at 60°C. Sections, 1 μm thick, were cut and dried onto Superfrost/Plus microscope slides at 60°C overnight. Before immunostaining, the resin was etched by treating the sections for 5 min with a solution consisting of 2 g KOH, 5 ml propylene oxide, and 10 ml methanol (20). After being washed in methanol-PBS (50:50 vol/vol) and PBS for 5 min each, sections were blocked for 15 min with 1% BSA in PBS. They were then incubated with either aquaporin-1 anti-serum or preimmune serum (both at 1:800) for 2 h, followed by goat anti-rabbit immunoglobulin G-CY3 conjugate for 1 h. All incubations were followed by two 5-min washes each of 2.7% NaCl and PBS. Sections were mounted in 50% glycerol in 0.2 M tris(hydroxymethyl)aminomethane-HCl, pH 8.0, containing 2% n-propyl galate to retard quenching of the fluorescence signal. Sections were examined by using an Arasastro 2000 confocal laser scanning microscope (Molecular Dynamics, Sunnyvale, CA) fitted with a 25-mW argon-ion laser and adjusted for simultaneous transmitted light (lung tissue) and >535-nm fluorescent light (aquaporin-1) imaging. Transmitted light and fluorescent images of the lung and aquaporin-1, respectively, were superimposed by image processing.

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IMMUNOGOLD LOCALIZATION (see Fig. 8). Rats were anesthetized as described above, the thorax was opened, and 4% paraformaldehyde was gently instilled into the lungs. The lungs were removed, and sections were placed overnight in 2% paraformaldehyde and 1% acrolein at 4°C. For immunogold staining, ultrathin frozen sections of lung were prepared as described by Tokuyasa (36). Briefly, small blocks of fixed lung were placed in 10% gelatin in PBS, chilled, infiltrated with 2.3 M sucrose overnight and frozen in liquid nitrogen. Ultrathin cryosections, 60 nm thick, were cut on a Reichert FC4E ultracryomicrotome and mounted on Parlodion/carbon-coated Ni grids. They were stored overnight on drops of 2.3 M sucrose on solidified 2% gelatin. After being rinsed in PBS, sections were quenched with 0.02 M glycine-PBS and rinsed in PBS, each for two 5-min periods. Sections were preincubated for 15 min in 0.1% BSA-0.4% gelatin-PBS (alg/bal/gel/PBS) for 15 min and then incubated with aquaporin-1 anti-serum and diluted 1:1,200 with alg/bal/gel/PBS for 2 h at room temperature. After being washed in 0.1% BSA-PBS-0.3% Tween-20 (rinse solution), sections were incubated with protein A-gold (10 nm, diluted 1:100) for 1 h and washed 3 × 5 min in rinse solution and once in PBS. Sections were then fixed for 10 min with 1% glutaraldehyde in PBS, washed in PBS, and rinsed 4 × 1 min in water. They were stained with 2% aqueous uranyl acetate for 4 min and exposed to 0.5% methyl cellulose in water for 16 s. Grids were dried and viewed in a Philips 301 electron microscope.

RESULTS

Water and Solute Exchange

Clearance of 3H2O and HOH from airways. After instillation of 5 ml of solute-free water, 77 mM NaCl, or 308 mM NaCl into the air spaces, the clearance of HOH from the air spaces to the perfusate was invariably and significantly less than that of 3H2O (see Fig. 1 and Table 2). (P5S) in the control experiments averaged 5.77 ± 0.85 (SE) cm3/s.

Increases in the perfusion rate from 6.45 to 22.1 ml/min increased pulmonary arterial pressures (Fig. 2) and the rates at which both 3H2O and HOH were cleared from the lungs (Table 2). Of the 5 ml of water that had been instilled into the lungs, 1.74 ± 0.30 ml had been recovered in the venous outflow after 4 min of perfusion at 6.45 ml/min. In contrast, only 2 min were required to recover 2.35 ± 0.28 ml of the instilled water when the lungs were perfused at 22.1 ml/min. Increasing flow by a factor of 3.4 (from 6.45 to 22.1 ml/min) was associated with a twofold increase in (P5S) (see Fig. 3).

The calculated value of (P5S) was increased when 77 mM NaCl rather than water was instilled into the lungs (Fig. 3, bar 3 vs. 1). (P5S) was not significantly greater when hypertonic saline was instilled into the lungs rather than water (Fig. 3, bar 4 vs. 1). Cooling of the perfusate and airway instillate to 11°C slightly reduced mean values of (P5S) from control values, but this effect was not significant (Fig. 3, bar 5 vs. 1).

Incorporation of 0.5 mM HgCl2 in the air-space and perfusate fluids decreased mean values of (P5S) by 28% (Fig. 3, bar 7 vs. 6). This effect was significant when the two groups were compared by using a paired t-test (P < 0.05), but it was not significant with use of a Newman-Keuls test, and the effect that HgCl2 had on endothelial filtration was much more pronounced (Fig. 3, bar 9 vs. 8; see (P5S) and inhibition with HgCl2).

Electrolyte concentrations in the perfusate. The inflow of water into the perfusate resulted in similar decreases in EB-labeled albumin concentration, osmolality, and [Na+] in the perfusate at slow flow (Fig. 4A). However, the relative concentrations of K+ were greater than those of the other indicators between 100 and 250 s after the water had been instilled into the lungs. This was particularly evident at slower flows (compare Fig. 4, A and B). [Na+] values appeared to be slightly lower than expected from the osmolality measurements at high flows (Fig. 4B), but this could reflect limits of the precision with which these parameters were measured. Unlike the experiments in which water was instilled into the air spaces at 37°C, no evidence was observed for release of K+ from the lungs into the perfusate after instillation of 77 mM NaCl at 37 or 25°C, 308 mM NaCl at 37°C, or water at 11°C.
Air-space solute concentrations (groups 3 and 4). Because of the rapidity with which water was lost from the lungs when there was no solute in the airway solution (groups 1, 2, and 5), it was not possible to sample the airway fluid at the end of these experiments. However, samples of the air space could be collected after 240 s had elapsed when either 77 or 308 mM NaCl was instilled into the lungs (Fig. 5). When 77 mM NaCl was instilled into the air spaces, both [Na\(^+\)] and osmolality in the air space increased to values approaching those in the perfusate. Similarly, air-space [Na\(^+\)] and osmolality rapidly fell after instillation of 308 mM NaCl into the air spaces and approached those in the plasma. No K\(^+\) was present in the solutions instilled into the lungs, but there was movement of K\(^+\) from the lung to the perfusate during the course of the experiments. Much more K\(^+\) entered the air spaces when hypotonic rather than hypertonic saline was instilled into the air spaces.

Perfusion of the lungs for 2 min with 0.5 mM HgCl\(_2\) decreased the loss of HOH from the perfusate without having a significant effect on peak losses of \(^3\)HOH, and ratio of peak D\(^3\)HOH to peak DTHO fell from 0.68 ± 0.02 to 0.34 ± 0.02 (P < 0.01, Fig. 6, A and B). (P\(_f\)S\(_{\text{endo}}\) after injections of virtually solute-free water averaged 281 ± 61 ml/s and fell to 39 ± 3 ml/s when 0.5 mM HgCl\(_2\) was incorporated in the perfusate. Perfusion with HgCl\(_2\) did not significantly affect pulmonary arterial pressures in these brief experiments, but increases in pressure were observed in more prolonged studies (not shown).

Morphological Studies

Confocal microscopy. Half-micrometer-thick optical sections of aquaporin-1-stained lung were recorded with a \(\times 60\) objective (numeric aperture 1.4) present. Staining for aquaporin-1 was observed on the endothelium of all segments of the pulmonary capillary bed (Fig. 7). At this level of magnification, however, staining of alveolar type I (ATI) and alveolar type II (ATII) cells could not be resolved.

Immunogold labeling. At the ultrastructural level, aquaporin-1 was localized by immunogold labeling on the apical and basolateral surface of endothelial cells, as well as on the membrane of a number of endothelial vesicles (Fig. 8). Aquaporin-1 was not detected in ATI cells, and immunogold labeling for aquaporin-1 in ATII cells was quite variable. When present, label was detected on both apical and basolateral surfaces of ATII cells. However, in comparison to endothelial cells, the degree of labeling was less, and some ATII cells in the
same section remained unlabeled. Sections incubated with preimmune rabbit serum followed by protein A gold were consistently negative.

DISCUSSION

The high incidence and serious consequences associated with edema formation in the lungs have been responsible for continuing interest in the manner in which water moves between the pulmonary compartments. Three basic strategies have been used to study water exchange in the lungs. 1) Injections of labeled water (\(^{3}\)HOH or \(^{2}\)H\(_2\)O) have been made into the pulmonary artery or air spaces, and its exchange with tissues has been measured. 2) Hypertonic or hypotonic solutions have been injected into the pulmonary artery or air spaces, and the movement of HOH has been monitored. 3) The movement of fluid out of the vasculature has been studied after increases in hydrostatic pressure. In this study, a direct comparison was made between tracer and osmotic water fluxes in the lungs.

Loss of Labeled Water From Air Spaces Is Limited by Perfusion

Because labeled water and most gases and lipophilic solutes rapidly cross cell membranes, it is generally assumed that at attainable cardiac outputs, concentrations or partial pressures of these substances are virtually the same in the air spaces and the plasma leaving the pulmonary exchange vessels. For example, similarities in the manner in which deuterated water (\(^{2}\)HOH), \(^{3}\)HOH, \(^{14}\)C butanol, and \(^{14}\)CO\(_2\) emerge from the lungs in the pulmonary venous outflow after they have been instilled into the lungs (4, 9, 10) suggest that equilibration between the alveolar compartment and

---

**Fig. 3.** Calculated values of filtration coefficient (\(P_f\))-surface area (S) products of alveolar-capillary barrier \([P_f(S)]_A\) and of endothelium \((P_f(S))_{endo}\). Calculated values of \(P_fS\) were significantly lower when solute-free water was instilled into air spaces (bars 1 and 5) than after instillation of hypotonic saline (bars 3 and 6) or at increased flow (bar 2). As indicated in text, instillation of water may be associated with a decrease in perfused surface area rather than a change in actual values of \(P_f\). HgCl\(_2\) had a much greater inhibitory effect on filtration across endothelium than across alveolar-capillary barrier (see text). Cold, hypothermia; fast, increasing flow by a factor of 3.4 (from 6.45 to 22.1 ml/min). *Mean values different from respective control values (P < 0.05).

**Fig. 4.** Changes in solute concentrations. A: group 1 experiments. B: group 2 experiments. C: group 3 experiments. D: group 5 experiments. Instillation of hypotonic solutions into airways resulted in similar dilution of Evans blue-labeled albumin, Na\(^+\), and osmolality in perfusate emerging from lungs. There appeared to be release of K\(^+\) from tissues into perfusate when solute-free water was instilled into lungs (A and B). This was not seen when hypotonic NaCl was used as an instillate (C) or at low temperatures (D). Conc, concentration; T, temperature.
blood leaving the exchange vessels is complete for each of these indicators. Perhaps the most convincing evidence that \(^3\)HOH transport is limited by flow rather than permeability was provided by experiments in which the rates of transport of \(^3\)HOH and \(^{14}\)CO\(_2\) from the air spaces to the perfusate were directly compared (10): when mixtures of \(^3\)HOH, \(H^{14}CO_3\), and \(^{14}\)CO\(_2\) are instilled with carbonic anhydrase into the airways, the quantity of \(^{14}\)C appearing in the outflow remains the same as that of \(^3\)HOH between a pH of 7.4 and 8.0. The fraction of the \(^{14}\)C label in the form of the more diffusible species (\(^{14}\)CO\(_2\)) is 5% at a pH of 7.4 and 1.25% at pH 8 so that the gradient for diffusion is reduced by 75% at the more alkaline pH. Because the rate of diffusion of \(^{14}\)CO\(_2\) relative to that of \(^3\)HOH is uninfluenced by this change in the concentration gradient between the air spaces and perfusate, losses from the air spaces of both \(^3\)HOH and \(^{14}\)CO\(_2\) must be limited by flow rather than by diffusion.

When indicator losses from the air spaces are limited by the rate of tissue perfusion, the permeability of the alveolar-capillary membranes cannot be measured. It is for this reason that the recent observation of Schnitzer and Oh (31) that uptake of \(^3\)HOH by perfused rat lungs was limited by perfusing them with \(HgCl_2\) may reflect decreased tissue perfusion rather than inhibition of
In a recent study by Carter et al. (2), diffusion of $^2$HOH rather than the filtration coefficient of the endothelium. rapid, exchange with the tissues is limited by flow movement across cell membranes by labeled water is very are presumably not inhibited by HgCl$_2$. Because move- branes occurs primarily through the cell lipids, which in a recent study by Carter et al. (2), diffusion of $^2$HOH into the air spaces of mouse lungs was monitored with an intra-alveolar fluorescence technique. They reported that increasing flow rates from 1.7 to 2 ml/min accelerated movement of $^2$HOH into the air spaces, but a further increase to 2.3 ml/min was without effect, and they concluded that exchange was diffusion rather than flow limited at these higher flows. However, increases in flow in the latter experiments may have been accomplished by vascular recruitment rather than accelerate- water channels. Diffusion of $^3$HOH through cell mem- branes occurs primarily through the cell lipids, which are presumably not inhibited by HgCl$_2$. Because move- ments across cell membranes by labeled water is very rapid, exchange with the tissues is limited by flow rather than the filtration coefficient of the endothelium. In a recent study by Carter et al. (2), diffusion of $^2$HOH into the air spaces of mouse lungs was monitored with an intra-alveolar fluorescence technique. They reported that increasing flow rates from 1.7 to 2 ml/min accelerated movement of $^2$HOH into the air spaces, but a further increase to 2.3 ml/min was without effect, and they concluded that exchange was diffusion rather than flow limited at these higher flows. However, increases in flow in the latter experiments may have been accomplished by vascular recruitment rather than acceleration of the velocity of flow in the monitored vessels.

Osmotic Flow of Water Across the Alveolar-Capillary Barrier Is Limited by Permeability

The clearance of HOH among the air-space, tissue, and vascular compartments of the lungs in the present experiments was invariably slower than that of $^3$HOH, indicating that equilibrium of the unlabeled species is limited by the permeability of the membranes separating these volumes. This observation might appear paradoxical because 1) the label does not appear to influence the rate of water exchange and 2) estimates of $P_f$ of cell membranes measured with osmotic gradients are from 3 to 10 times greater than tracer permeability to labeled water ($P_d$) (38). The explanation for this observation can be understood in terms of the Fick relationship, which indicates that the rate of water diffusion should be directly proportionate to the concentration gradient between compartments. After instillation of HOH and $^3$HOH into the air spaces, the concentration of water in the air spaces is 55 mol/l, whereas that in the vasculature is only 0.3 mol/l less (54.7 mol/l). There is consequently considerable back diffusion of HOH molecules immediately after instillation of the water. In contrast, there is initially no $^3$HOH in the perfusate, and consequently equilibration of $^3$HOH proceeds much more rapidly than that of HOH.

As indicated in Fig. 1, $^2$HOH fell to values close to those of $^3$HOH after 120 s. This suggests that samples collected at these times are derived from exchange vessels with slower flow, in which the removal of HOH as well as $^3$HOH is limited by capillary flow rather than by diffusion.

$(P_f S)_a$ and $(P_f S)_{endo}$

Calculation of filtration coefficients of the pulmonary membranes are complicated by uncertainties concerning the surface area involved in these experiments. Although estimates are available concerning the total endothelial and epithelial surface areas of intact lungs (39) and of alveoli near the surface of the lungs (2), there is no way to be sure either what fraction of the alveoli are filled with fluid or whether they are uniformly perfused. We have therefore chosen to express our data in terms of $P_f \times S$ products (in cm$^2$/s, Fig. 3). For the control experiments (group 1), $(P_f S)_a = 5.77 \pm 0.85$ cm$^2$/s. If a value of 6,080 cm$^2$ is assumed for $S$ of these lungs (37), then $P_f A$ would be equal to $9.4 \pm 1.4 \times 10^{-4}$ cm/s. This value is significantly less than that estimated from fluorescent studies in mouse lungs by Carter et al. (2) (0.17 \pm 0.001 cm/s at 25°C) and may reflect differences in the surface area actually perfused, the species studies, and the experimental approaches used in these studies. It is possible that the capillaries near the surface of the lungs visualized by Carter et al. are more permeable to water than the remainder of the vessels of the lungs or that the vessels in the mouse lungs have become more permeable because of experimental conditions.

Values of $(P_f S)_a$ were increased by a factor of 2 when perfusion rates were increased by a factor of 3.4. This increase could reflect an increase in the effective surface area of the vascular bed because of the tendency of increased intravascular pressures to recruit additional vessels or distend vessels that are already perfused. $(P_f S)_a$ was also increased when either 77 or 308 mM NaCl was instilled into the air spaces rather than water. It is possible that instillation of water into the air spaces causes sufficient cellular swelling to reduce local perfusion and the surface area of exchange. Cooling the perfusate, instillate, and lungs to 11°C did not have a significant effect on either $^2$CHOH or $^3$CHOH compared with experiments conducted at 37°C but did reduce the peak $C_{HOH}$-to-peak $C_{^3HOH}$ ratio by 25%.
Calculated values of $(P_f S)_A$ were not significantly different from those of the control experiments. This relatively small effect of cooling is consistent with an activation energy of $<4$ kcal/mol and is compatible with movement through pores rather than lipid membranes (38).

Mercurials inhibit transport through both aquaporin-1 and -5 channels. The action of HgCl$_2$ appeared to
have a much greater effect on filtration through the endothelium than through the alveolar-capillary barrier as a whole (Fig. 3). The simplest explanation for this observation would be that 1) there are more water channels on the endothelium that can be inhibited by HgCl₂ than those that are present on the epithelium and 2) PₛS product of the epithelium is significantly less than (PₛS)endo. (PₛS)endo values were greater than PₛS values of the epithelium in these experiments but, once again, direct comparisons are difficult to make because of the different experimental approaches used in these studies, including different methods of calculating filtration in the fluid-filled and air-filled lungs (see Appendix).

Evidence for inhibition of filtration between the vasculature and air spaces of lungs by HgCl₂ has been reported by Folkesson et al. (12) and Carter et al. (2). No attempt was made in these experiments to distinguish between endothelial and epithelial effects of this inhibitor. Folkesson et al. (12) found that HgCl₂ slows filtration in the fluid-filled and air-filled lungs (see Appendix).

Distribution of Aquaporin-1 in the Alveolar-Capillary Barrier

Confocal microscopy showed the presence of aquaporin-1 antigen along the endothelium, but it was not possible at the resolution of these studies to tell whether there was any present in the epithelial cells. The immunogold studies demonstrated aquaporin-1 along both the basolateral and apical surfaces of the endothelium. Lesser quantities were observed on some of the type II cells, but none was associated with the type I cells, which cover most of the epithelial surfaces of the alveoli.

Evidence for the presence of aquaporin-1 on continuous endothelial cell membranes has been reported by a number of laboratories by using either in situ hybridization or specific antibodies (17, 37, 40). Schnitzer and Oh (31) found that expression of this molecule on the luminal surfaces of rat lung endothelium is comparable to that present on rat blood cell plasma membranes. Concentrations of aquaporin-1 were particularly high in the caveolae of these cells.

Although there appear to be relatively few aquaporin-1 molecules in the epithelium, other aquaporins such as the mercury-insensitive water channels (16) and aquaporin-5 (28), which is sensitive to HgCl₂, may promote the movement of water between the air spaces and the vasculature of the lungs. The relatively slight effect that cooling has on the transport of HOH relative to 3H0H from the air spaces to the perfusate that we observed in the group 4 experiments is consistent with the presence of such channels. It is therefore likely that much of the osmotic water transport between the air spaces and perfusate occurs through some sort of channels, though there appears to be less aquaporin-1 in the epithelium than the endothelium.

Effect of Osmolality on (PₛS)ₐ

When 77 mM NaCl rather than water was instilled into the lungs, the calculated value for (PₛS)ₐ exceeded that observed after instillation of solute-free water (see Fig. 3). This difference in the calculated value of (PₛS)ₐ may better reflect tissue perfusion (increased S) rather than a difference in P, because the average peak CTHO was greater after instillation of 77 mM NaCl than after instillation of water, although this difference was not quite significant (Table 2). (PₛS)ₐ was also greater when hypertonic saline rather than water was instilled into the lungs, causing water to move from the perfusate to the air spaces. Once again, this may indicate better perfusion because the average peak C₃HOH may have been greater when hypertonic saline rather than water was instilled into the lungs. It is possible that tissue perfusion was reduced by cellular swelling after instillation of solute-free water (see below).

As indicated in Fig. 5, air-space [Na⁺] had reached 124 meq/l 4 min after instillation of 5 ml of 77 mM saline into the air spaces. This is equivalent to the loss of 1.59 ± 0.08 (SE) ml from the air-space compartment and is ~0.3 ml greater than that which reached the perfusate, suggesting retention of a small volume of water in the tissues. When 5 ml of 308 mM NaCl were instilled into the air spaces, concentrations in the air spaces fell to 183 meq/l when air-space fluid was collected after 4 min. This is equivalent to the movement of 3.43 ± 0.26 ml of water into the air spaces, a value that did not differ significantly from that which was lost from the perfusate.

Movement of Solute After Osmotic Challenges

After instillation of water into the air spaces, decreases in [Na⁺] and [K⁺] were initially similar to decreases in protein concentrations in the perfusate leaving the lungs (Fig. 4A). As in our previous studies, this suggests that αₛ values of the endothelium and epithelium to these solutes are ~1.0 (7, 11). It is difficult to compare this αₛ value with previous estimates of solute reflection coefficients (34, 26) because the latter values were on the basis of what may be inappropriate comparisons of changes in weight induced by increases in hydrostatic pressures, which tend to increase interstitial volumes, and decreases in weight caused by hypertonic infusions, which decrease cellular volumes (7).

After instillation of water into the air spaces, [K⁺] in the perfusate initially fell by approximately the same amount as [Na⁺], osmolality, and concentrations of EB-labeled albumin. However, [K⁺] subsequently increased relative to [Na⁺], concentrations of EB-labeled albumin, and osmolality. Because there were no red blood cells in this preparation, it can be concluded that aspiration of water resulted in the release of K⁺ from the pulmonary tissues. This could be related to normal volume control mechanisms that result in loss of K⁺ from cells after cellular swelling in hypotonic media (33) or to cell damage caused by this very hypotonic exposure. Movement of K⁺ into the perfusate was not
observed when less hypotonic solutions (77 mM NaCl) or hypertonic solutions (308 mM NaCl) were instilled into the lungs or when the lungs were cooled to 11°C. However, considerably more K⁺ entered the air spaces when 77 mM NaCl rather than 308 mM NaCl was instilled into the lungs (Fig. 5). Rapid increases in air-space [K⁺] have been observed when a variety of isotonic solutions have been instilled into the lungs (6, 8), and [K⁺] in the alveolar epithelial lining fluid is significantly greater than in plasma (23). It is therefore likely that factors in addition to cellular swelling are involved in the movement of K⁺ into the air spaces. Nevertheless, considerably less K⁺ entered the air spaces when hypertonic saline was instilled into the air spaces, suggesting that cellular dehydration may have reduced K⁺ losses from pulmonary tissues.

Role of Aquaporin-1 in Osmotic and Hydrostatic Movement of Water in Lungs

Both the physiological and morphological data obtained in this study are consistent with the presence of more aquaporin-1 on the endothelium than on the epithelium. This distribution of water channels would tend to link the osmolality of the subepithelial tissues more closely to that of the blood than any solutions that may be aspirated. The presence of fewer aquaporin-1 molecules in the epithelium may also slow evaporative water losses from the alveoli. However, recent evidence suggests that the absence of aquaporin-1 from red blood cells does not result in any clinical disorders (27), and the exact role of this membrane protein remains to be determined. It is possible that, in some tissues, water conduction can occur through alternative aquaporins. Some water transport across the epithelium may also be mediated by aquaporin-5 channels, which are sensitive to HgCl₂ and have been detected in the type I epithelial cells.

Aquaporin-1 is found in considerable abundance on many epithelial surfaces that conduct large amounts of water, such as the renal epithelia of both proximal tubules and the thin descending loop of Henle (37), the colonic crypts, choroid plexus, ciliary body, iris, sweat gland, and gall bladder (15, 16, 37, 38). However, as indicated above, aquaporin-1 also appears to be present on most continuous capillary beds (e.g., lung, skeletal muscle, and heart) aside from the brain and is present on both the basolateral and apical membranes of these cells (31). In contrast, it is absent on discontinuous endothelial membranes, such as those of the liver (31). Although the present study is consistent with the conclusion that some of the water crossing the endothelial barrier in response to osmotic gradients traverses mercury-sensitive aquaporins, it cannot be concluded that these structures limit the rate at which filtration occurs across the capillary walls in response to increased hydrostatic pressure gradients. Because water moving through aquaporins is not accompanied by solute, filtration through these channels would be associated with the development of an osmotic gradient that would slow further filtration. Thus the rate of filtration that occurs when intravascular pressures are increased may be limited by the rate of solute movement rather than the number of aquaporins present or their permeability to water.

APPENDIX

Calculation of the $P_{fS}$ Product of the Alveolar-Capillary Barrier ($[P_fS]_A$)

Unless information is available concerning the osmolality of perfusate in the pulmonary exchange vessels, no estimate can be made of the $P_fS$ product of the membranes separating the alveolar and vascular compartments. Because the perfusate leaving alveoli that contain the solution instilled into the air spaces becomes mixed with perfusate from air spaces not exposed to this solution, it cannot be assumed that the outflow osmolality ($c_v$) is the same as the osmolality of the fluid leaving the pulmonary capillaries ($c_{vasc}$). Three factors could contribute to differences between the osmolality of the perfusate collected from the venous outflow and that of the perfusate leaving the capillaries: 1) the outflow from some regions of the lung that became filled with the instilled fluid may have not reached the venous samples at the time they were collected; 2) some alveoli may not been filled with fluid; or 3) the water from some portions of the lung may have been absorbed into the vasculature at an earlier time.

To calculate the average osmolality present in the vessels that exchanged water with the air spaces, the model shown in Fig. 9 was used. As indicated in this figure, it was assumed that both $^3$HOH and HOH entered the exchange vessels of the air spaces represented at the top of the diagram. It was assumed that there was no exchange of $^3$HOH or HOH in the remaining air spaces (bottom of the diagram). As explained in DISCUSSION, it was assumed that $^3$HOH concentrations in the perfusate leaving the exchanging alveoli were the same as that in the air spaces ($c_{vasc}$)

$$c_{vasc} = c_v$$  \hspace{1cm} (A1)

Once $^3$HOH leaves the microvasculature, it becomes mixed with perfusate that has not been exposed to the instilled fluid. Consequently, the concentration of $^3$HOH in samples of the venous blood collected from the left atrium (i.e., $[^3$HOH]ₐ) is less than that in the microvascular or air spaces. If conservation of $^3$HOH is assumed

$$[^3$HOH]ₐF_v = [^3$HOH]ₐF_v$$  \hspace{1cm} (A2)

Furthermore, if conservation of the solutes in the pulmonary vasculature is assumed

$$c_{vasc}F_v + c_v(F_v - F_{vasc}) = c_vF_v$$  \hspace{1cm} (A3)

Combining Eqs. A2 and A3 yields

$$c_{vasc} = c_v - (c_v - c_{vasc}) \frac{[^3$HOH]ₐ}{[^3$HOH]ₐ}$$  \hspace{1cm} (A4)

where $c_v$ designates the osmolality of the perfusate entering the pulmonary arteries.

Once $c_v$ and $c_{vasc}$ are known, the average osmolality in the capillary was calculated by an approximation, by which it was assumed that flow was constant between the arteries and veins. Under these circumstances, the average osmolality of the perfusate in the capillaries ($c_c$) falls exponentially (5) and can be calculated from the equation

$$c_c = \frac{c_v - c_{vasc}}{\ln c_v - \ln c_{vasc}}$$  \hspace{1cm} (A5)

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The assumption of an exponential decline in concentration represents an approximation in concentrations because there is a small change in the flow that occurs between the artery and veins, a factor that was not considered in the model of Crone (5). The value of \( P_f S \) was calculated in Eq. 14.

Calculation of the \( P_f S \) Product of the Microvascular Barrier \([P_f S]_{endo}\)

Calculation of the \( P_f S \) of the pulmonary exchange vessels was based on 1) the rate of movement of water (\( \text{CHOH} \)) from the capillaries to the lung tissues after 0.7 ml of water had been introduced into the arterial inflow and 2) the concomitant difference in microvascular and tissue osmolalities, \( \Delta c \). As described in METHODS, both the perfusate and injection solution contained EB-labeled albumin. In contrast, only the injection solution contained \([^{14}\text{C}]\text{dextran}\) and \( \text{CHOH} \). A simple model of the lungs was considered in which the extravascular space of the lung was considered to be a single compartment in which concentrations fell uniformly as some of the injected water entered the lung tissues. Because the transit times of the water and each of the indicators through the lungs were heterogeneous, the venous samples contained a mixture of perfusate that contained the injected-water bolus and perfusate that did not contain the injectate. Venous concentrations, consequently, did not equal concentrations in the capillaries. However it was assumed that there was conservation of osmoles and volume in the inflow tubing, and then

\[
caVa = cpVp = cp(Va - Vinj) \quad (A7)
\]

where \( V_a \) and \( V_p \) are volumes of water in perfusate and pulmonary artery, respectively. Furthermore, it is assumed that there is conservation of \([^{14}\text{C}]\text{dextran}\) in the injection solution

\[
\text{[dexam]}_{\text{inj}}V_{\text{inj}} = \text{[dexam]}Va = \text{[dexam]}_p(Vp + Vi) \quad (A8)
\]

where \( \text{[dexam]}_{\text{inj}} \) is the concentration of dextran in the injectate. Combining Eqs. A7 and A8 yields

\[
c_a = \left( 1 - \frac{\text{[dexam]}_p}{\text{[dexam]}_{\text{inj}}} \right)c_p \quad (A9)
\]

An estimate of the average osmolality of perfusate in the capillaries \( c_c \) was made by assuming that there was an exponential increase in osmolality between the arterial and venous ends of the capillaries

\[
c_c = \frac{c_v - c_a}{\ln c_v - \ln c_a} \quad (A10)
\]

Calculation of the osmolality of the tissue \( c_t \) was based on a simple model in which it was assumed that, on entering the capillaries, concentrations of \( \text{H}_2\text{O} \) in the perfusate immediately equilibrated with that in the tissue and \( \text{[^{3}]H}_2\text{O} \).

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Fig. 9. Schematic diagram of movement of water that occurs after instillation of hypotonic fluid into air spaces. It is assumed that there is mixture of perfusion fluid from alveoli that contain instillate with perfusate that traversed alveoli that did not contain instilled fluid. It was assumed that \( \text{[^{3}]H}_2\text{O} \) concentrations at venous end of capillaries equilibrated with air-space concentrations of \( \text{[^{3}]H}_2\text{O} \). This assumption permitted calculation of vascular \( (c_{vasc}) \) and capillary \( (c_c) \) osmolalities of perfusate (see text for explanation and definition of symbols).
\[[{^{3}HOH}]_{a}\text{.}\] The concentration of \([^{3}HOH]\) entering the capillaries ([\([^{3}HOH]_{i}\)]) can be calculated from the equation

\[
[{^{3}HOH}]_{a} = [{^{3}HOH}]_{i} \frac{[\text{dex}]_{i}}{[\text{dex}]_{a}} \tag{A11}
\]

The decrease in \([^{3}HOH]\) concentrations between the artery and capillary were determined by the relative volumes of the capillary space \((V_{c})\) and the extravascular tissue \((V_{ti})\)

\[
\frac{V_{ti}}{V_{c}} = \frac{{^{3}HOH}}{i} - 1 \tag{A12}
\]

These equations assume flow-limited distribution of \([^{3}HOH]\) in the tissues, with instantaneous equilibration of \([^{3}HOH]\) with the extravascular compartment of the lungs. The change in the tissue osmolality relative to that of the capillary that was due to the flow of water from the capillaries to the tissue should be inversely proportionate to the size of these compartments

\[
\frac{c_{i,n-1} - c_{i,n}}{c_{i} - c_{a}} = \frac{V_{c}}{V_{ti}} \tag{A13}
\]

where \(c_{i,n}\) designates the osmolality in the tissue perfused by the \(n\)th sample, and \(c_{i,n-1}\) represents the osmolality of the tissue perfused by the previous sample. Then

\[
c_{i,n} = c_{i,n-1} - \frac{V_{c}}{V_{ti}} (c_{c} - c_{a}) \tag{A14}
\]

The flow of water from the capillary into the tissue was estimated from the equation

\[
CHOH = F_{a} - F_{v} = F_{a} \frac{[EB]_{v} - [EB]_{a}}{[EB]_{v}} \tag{A15}
\]

and the \(P_{S}\) product was then determined (see Eq. 15).

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