Effective permeability of hydrophilic substances through walls of lymph vessels: roles of endothelial barrier

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Ono, Nobuyuki, Risuke Mizuno, and Toshio Ohhashi. Effective permeability of hydrophilic substances through walls of lymph vessels: roles of endothelial barrier. Am J Physiol Heart Circ Physiol 289: H1676–H1682, 2005. First published June 17, 2005; doi:10.1152/ajpheart.01084.2004.—The wall effective permeability of hydrophilic substances labeled with fluorescent dyes was evaluated in an isolated cannulated rat single lymph vessel through a videomicroscope system. Sodium fluorescein (NaFl; 332 mol wt) and FITC-dextrans (4,400, 12,000, and 71,200 mol wt) were administered into the intraluminal space of the lymph vessels and then excited by a Xenon lamp. Changes in the fluorescence intensity of the dyes were continuously measured by a silicon-intensified target camera through appropriate filters. The net flux of each dye in the wall of the lymph vessels was calculated by the relationship between the fluorescence intensity and the concentration of the dyes used in the present study. After administration of 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate into the lymph vessels, the net flux of FITC-dextran 4,400 and 12,000 but not 71,200 was augmented significantly. These results suggest that in the efferent lymph of the popliteal lymph node compared with that in the afferent lymph (4, 22).

Materials and Methods

Seven- to eight-week-old male Wistar rats (200 g body wt, n = 29, SLC, Hamamatsu City, Japan) were used for the present study. The rats were housed in an environmentally controlled vivarium and fed a standard pellet diet and water ad libitum. All experimental protocols were approved by the Animal Ethics Committee of Shinshu University School of Medicine in accordance with the principles and guidelines of the Japanese Physiological Society.

Isolation and cannulation of lymph vessels. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and exsanguinated. After an incision of the abdomen, the iliac lymph nodes and their afferent lymph vessels were excised and placed on a Petri dish containing cold Krebs bicarbonate solution (−4°C). The Krebs solution contained (in mM) 120.0 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 NaH2PO4, 5.5 glucose, and 25.0 NaHCO3. With the use of microsurgical instruments and an operating microscope, the lymph vessels (n = 29, 173.5 ± 6.7 μm in diameter, 3 mm in length) were isolated and transferred to a vessel chamber (12 ml) containing two glass micropipettes and the Krebs solution.

After each lymph vessel was mounted on a pipette (proximal) and secured with a 10-μm nylon suture, the perfusion pressure was raised to 3 cmH2O to flush out and clear the lumen from the vessel. The other end of the vessel was then mounted to the outflow micropipette. The proximal (inflow) micropipette was connected with a double-lumen Tygon tubing and a 50-ml syringe (inflow pressure column). The distal (outflow) micropipette was connected to the Tygon tubing and a 50-ml syringe (outflow pressure column). The Krebs solution, bubbled with a gas mixture of 5% CO2-95% N2 to give a pH 7.40 ± 0.01 and a pO2 60 mmHg, was superfused over the vessel. The flow rate of the superfusion solution was 18 ml/min throughout the experiments. After cannulation of the lymph vessel, the chamber was transferred to the stage of an inverted microscope (Olympus IMT-2). The lymph vessels were then warmed slowly to 37°C and allowed to equilibrate for 30 min. During the equilibration period, the inflow and outflow perfusion pressure of the vessels was set at 6.5 and 5.5 cmH2O (mean intraluminal pressure of 6 cmH2O), respectively.

Perfusion of fluorescent dyes into intraluminal space of lymph vessels. After the equilibration period, each dye was administered into the intraluminal space of the lymph vessels. In the present study we...
used three sets of mean intraluminal perfusion pressure for the administration of the dyes as follows: 4 cmH₂O (4.5 cmH₂O inflow pressure and 3.5 H₂O outflow pressure), 6 cmH₂O (6.5 cmH₂O inflow pressure and 5.5 H₂O outflow pressure), and 8 cmH₂O (8.5 cmH₂O inflow pressure and 7.5 H₂O outflow pressure). In these pressure studies, we performed at a random order of the pressure. After the intraluminal space was completely filled with the dyes for 5 min, the outflow was stopped by closure of the stopcock while the inflow pressure simultaneously adjusted to each intraluminal pressure and continued pressurizing the lymph vessels throughout the experiments. In the present study, we could not measure an intraluminal pressure of the lymph vessels directly. However, we measured indirectly an intraluminal pressure via the inflow pressure column that was connected to a pressure transducer (Becton Dickinson) and an amplifier (6M52, Sanei) through a MacLab data acquisition system (AD Instruments) and a personal computer (Power Macintosh 8500/1200, Apple). In the organ chamber used in the present study, the preparations were exposed by an extraluminal hydrostatic pressure (0.5 cmH₂O) kept constant throughout the experiments. In addition, we used a large inflow pressure column (50 ml) for preventing loss of pressure head. Thus transmural pressure in the present study depends on the intraluminal pressure through the input column and is constant throughout the experiments (4, 6, or 8 cmH₂O). These ranges of the pressure are suitable for producing stable spontaneous beatings of isolated rat lymph vessels (19).

Measurements of intensity of dye and spontaneous beatings of lymph vessels. The optical system contains a Xenon lamp (Olympus), a 50% neutral density filter (Olympus), an electrically controlled shutter (Sigma Kikai) and a dichroic mirror (420 nm). A fluorescence image was obtained through a barrier filter (520 nm) with the use of a silicon-intensified target camera (C-2400, Hamamatsu Photonics), an objective ×10 lens (numerical aperture 0.30, Olympus), and a photo ×3.3 eyepiece lens (NFK, Olympus). The images were displayed on both a video monitor (Hamamatsu Photonics) and an image monitor (Nemco) through a video timer (For A, VTG-33). The images were also recorded on a videocassette recorder (BR-S611, Victor). The video monitor was used for measuring the maximum diameter (Dₘₐₓ, in micrometers), minimum diameter (Dₘᵢₙ, in micrometers), and frequency (in minutes) of spontaneous beatings of the lymph vessels by a video caliper (21). The changes in the intensity of the fluorescence images were measured by an image analyzer (Epson personal computer/AT compatible). The focus of the images was adjusted onto the central plane of each lymph vessel. To measure the changes in the intensity of fluorescence images, the image was digitized (512 × 512 pixels and 8 bits density in each pixel) every 500 ms. A window displayed on the monitor with the fluorescence image was adjusted to the center of each lymph vessel corresponding to the longitudinal axis of the vessel and kept constant throughout each experiment (Fig. 2). The width of the window was set at 30% to 60% of the maximum diameter of the lymph vessels. The averaged intensity of the digitized images in the window was calculated by computer and then recorded on a pen recorder.

Experimental protocol. All experimental protocols were conducted in a dark room.

Relationship between concentrations of dyes and intensities of digitized images. To determine an optimal concentration of the dyes used in the present study, the relationships between the concentrations of the dyes and the intensities of the digitized images were evaluated. The dyes used were sodium fluorescein (NaFl; from 0 to 0.5 μmol, 328 mol wt), FITC-dextran 4,400 (hereinafter referred to as 4kD; 4,400 mol wt, from 0 to 10 μM), FITC-dextran 12,000 (hereinafter referred to as 12kD; 12,000 mol wt, from 0 to 1 μM), and 71,200 (hereinafter referred to as 70kD; 71,200 mol wt, from 0 to 0.5 μM). Each dye was continuously administered dose dependently through a glass pipette (250 μm in intraluminal diameter) and then excited. The intensities in the pipette were measured to evaluate the correlation.

Effects of photobleaching on fluorescent dyes. To determine the effects of photobleaching, the dyes (in μM: 0.5 NaFl, 10 4kD, 1 12kD, and 0.5 70kD) were put into a glass pipette (250 μm in intraluminal diameter) and then excited. After the maximal intensity of the dye in the glass pipette was reached, the administration of the dye was terminated. The changes in the intensity of the dyes in the glass pipettes were evaluated with or without the shutter. Activation of the shutter for 20 min allowed exposure of the dyes for 5 s in intervals of 5 s.

Effects of intraluminal pressures on changes in intensity after administration of dyes into lymph vessels. To determine the effects of intraluminal pressures on changes in the intensity of each dye in the intraluminal space of lymph vessels, NaFl (0.5 μM), 4kD (10 μM), 12kD (1 μM), and 70kD (0.5 μM) were put into the lymph vessels for 5 min and then excited for 20 min. After the maximal intensity of the dye in the lymph vessels was reached, the outflow was stopped by closure of the stopcock while the inflow pressure simultaneously adjusted to each intraluminal pressure (4, 6, or 8 cmH₂O) and continued pressurizing the lymph vessels throughout the experiments. The changes in the intensity of the images were evaluated for 20 min with the shutter at 5 s exposure in intervals of 5 s.

Effects of 3-{[3-cholamidopropyl]dimethylammonio}-1-propanesulfonate on changes in intensity after administration of dyes into lymph vessels. The changes in the intensity of the dyes in the lymph vessels were also measured before and after treatment with 3-{[3-cholamidopropyl]dimethylammonio}-1-propanesulfonate (CHAPS, 0.3%) at an intraluminal pressure of 6 cmH₂O. CHAPS, which is a detergent that can disrupt or eliminate the barrier of endothelial cells without changing the responsiveness of smooth muscle cells (15, 16), was perfused into the intraluminal space of the lymph vessels for 10 min at a mean intraluminal perfusion pressure of 6 cmH₂O (6.5 cmH₂O inflow pressure and 5.5 H₂O outflow pressure). After CHAPS was rinsed out for 5 min, the changes in the intensity of each dye were also evaluated. At the end of each experiment, the intraluminal space was rinsed with the Krebs bicarbonate solution without the dyes to confirm a return to the image obtained before the administration of the dyes.

Calculation for net flux of dyes through wall of lymph vessels. The relationships between the concentrations of the dyes and the intensities of the digitized images enabled us to evaluate the concentrations of the dyes in the intraluminal space of the lymph vessels. The changes in the fluorescence intensities in the intraluminal space of the lymph vessels reached a plateau after the perfusion of the dyes was stopped at 5 min. Thus the net flux of the dyes from the intraluminal to the extraluminal space of the lymph vessels was calculated with the use of the equation

\[
\text{Net flux (for 5 min) = } (C_0 - C_i) \pi d \text{ (μM/μm)}
\]

where \(C_0\) is the initial concentration of each dye in the intraluminal space of the lymph vessels, \(C_i\) is the concentration of each dye in the intraluminal space of the lymph vessels after the perfusion of dyes was stopped at 5 min, and \(d\) is the diameter of the lymph vessels. We also calculated the percent net flux as follows: percent net flux = 100 \times \left[\frac{(C_0 - C_i)}{C_0}\right].

A pressure gradient is maintained across the lymphatic wall in the present study. Thus the net flux calculated in the isolated lymph vessels reflects an effective permeability rather than permeability of the solutes.

Drugs. All the salts for the Krebs bicarbonate solution (Wako), NaFl (Alcon), FITC-dextran (Sigma), and CHAPS (Dojindo) were used in the present study. The dyes and CHAPS were directly diluted into the Krebs bicarbonate solution. All salts and drugs were prepared on the day of the experiment.

Statistics. The ejection fraction, a parameter of the spontaneous beatings of the lymph vessels, was calculated as follows: \(\frac{\pi(D_{\text{max}}/2)^2 - \pi(D_{\text{min}}/2)^2}{\pi(D_{\text{max}}/2)^2}\) (21). The data are presented as means ± SE, and \(n\) indicates the number of vessels. Regression analysis was used.
to determine the relationship between the concentration of the dyes and the intensity of the digitized images. Significant differences ($P < 0.05$) were determined by ANOVA, followed by Scheffé’s post hoc test or paired Student’s $t$-test, as appropriate.

**RESULTS**

*Relationship between concentrations of dyes and intensities of digitized images.* Figure 1A shows the relationships between concentrations of the dyes and the intensities of the digitized images of the glass pipette. After the administration of the dyes into the glass pipette, the fluorescence intensities increased, ranging in gray scale value from 50 to 240. The regression analyses demonstrate that the relationships were dose dependent and linear to the concentrations of NaFl ($y = 188x + 66$, $P < 0.05$, $r^2 = 0.99$), 4kD ($y = 12x + 62$, $P < 0.05$, $r^2 = 0.99$), 12kD ($y = 111x + 53$, $P < 0.05$, $r^2 = 0.98$), and 70kD ($y = 269x + 88$, $P < 0.05$, $r^2 = 0.99$). Thus we determined optimal concentrations of the dyes for evaluating changes in the fluorescence intensities of the intraluminal space of lymph vessels as NaFl (0.5 μM), 4kD (10 μM), 12kD (1 μM), and 70kD (0.5 μM) in the present study. The digitized fluorescence intensity of the glass pipette after the administration of each dye was kept in a range from 150 to 240 on the gray scale because this was a submaximal and suitable value for analysis in the optical system. According to the regression analyses, we could determine the concentration of dyes in the intraluminal space of the lymph vessels and could calculate net flux of the dyes.

*Effects of photobleaching on dyes.* Figure 1B shows the effects of photobleaching on percent changes in the fluorescence intensity of the glass pipette with (closed symbols) or without (open symbols) use of the shutter. Without use of the shutter, the percent changes in the fluorescence intensities of NaFl (0.5 μM), 12kD (1 μM), and 70kD (0.5 μM) were <5% during the 20 min. Without the shutter, the administration of 4kD (10 μM) results in a >5% reduction of percent changes in the fluorescence intensity after administration of the dye is stopped. There was no significant photobleaching in the percent changes of the fluorescence intensity with use of the shutter (closed symbols).

**Table 1. Effects of excitation of dye on frequency and ejection fraction of spontaneous beatings of lymph vessels at 6 cmH$_2$O intraluminal pressure**

<table>
<thead>
<tr>
<th>Dye</th>
<th>n</th>
<th>Excitation</th>
<th>Frequency, min$^{-1}$</th>
<th>Ejection Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaFl</td>
<td>4</td>
<td>Before</td>
<td>21.3 ± 1.5</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>NaFl</td>
<td>4</td>
<td>After</td>
<td>20.8 ± 1.5*</td>
<td>0.71 ± 0.04*</td>
</tr>
<tr>
<td>4kD</td>
<td>4</td>
<td>Before</td>
<td>19.4 ± 1.1</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>4kD</td>
<td>4</td>
<td>After</td>
<td>19.8 ± 0.8*</td>
<td>0.69 ± 0.02*</td>
</tr>
<tr>
<td>12kD</td>
<td>5</td>
<td>Before</td>
<td>19.5 ± 0.5</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>12kD</td>
<td>5</td>
<td>After</td>
<td>19.4 ± 0.8*</td>
<td>0.66 ± 0.04*</td>
</tr>
<tr>
<td>70kD</td>
<td>4</td>
<td>Before</td>
<td>17.5 ± 0.8</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>70kD</td>
<td>4</td>
<td>After</td>
<td>18.0 ± 0.9*</td>
<td>0.73 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, number of vessels; NaFl, sodium fluorescein; 4kD, FITC-dextran 4,400; 12kD, FITC-dextran 12,000; 70kD, FITC-dextran 71,200; *No significant difference from before excitation.

Changes in fluorescence intensities of lymph vessels after *administration of dyes.* The frequency and ejection fraction of the spontaneous beatings in isolated lymph were not significantly different before and after the excitation of each dye. Table 1 shows the results of the frequency and ejection fraction of spontaneous beatings in isolated lymph at an intraluminal pressure of 6 cmH$_2$O.

Figure 2 shows representative transilluminated and fluorescence images of the intraluminal space of lymph vessels before and after perfusion with 0.5 μM NaFl (Fig. 2, A–C) and 0.5 μM 70kD (D–F). The fluorescence intensities of the lymph vessels increased after the administration of NaFl (Fig. 2B) and 70kD (Fig. 2E). After the administration of NaFl was stopped, the fluorescence intensity decreased (Fig. 2C). There was, however, no reduction in the fluorescence intensity of the lymph vessel after the administration of 70kD (Fig. 2F) was stopped. These results indicate that NaFl permeated the wall of the lymph vessels, whereas 70kD stayed in the intraluminal space.

*Effects of intraluminal pressure on net flux of dyes.* With the elevation of intraluminal pressure ranging from 4 to 6 to 8 cmH$_2$O, the frequency and ejection fraction of the beatings significantly increased and decreased, respectively. The frequency values at intraluminal pressures of 4 and 8 cmH$_2$O were 16.2 ± 0.7 min$^{-1}$ ($n = 17$) and 21.6 ± 0.6 min$^{-1}$ ($n = 17$).
17, $P < 0.05$ vs. 4 cmH$_2$O), respectively. The ejection fraction values at intraluminal pressures of 4 cmH$_2$O and 8 cmH$_2$O were 0.78 ± 0.02 ($n = 17$) and 0.59 ± 0.02 ($P < 0.05$ vs. 4 cmH$_2$O; $n = 17$), respectively.

Figure 3 shows the effects of intraluminal pressure (4, 6, and 8 cmH$_2$O) on the net flux of the dyes (Fig. 3A, NaFl, 0.5 µM, $n = 4$; Fig. 3B, 4kD, 10 µM, $n = 4$; Fig. 3C, 12kD, 1 µM, $n = 5$; Fig. 3D, 70kD, 0.5 µM, $n = 4$). The net flux of NaFl, 4kD, 12kD, and 70kD at an intraluminal pressure of 6 cmH$_2$O was $0.56 ± 0.11 \times 10^{-3}$, $3.43 ± 0.33 \times 10^{-3}$, $0.09 ± 0.03 \times 10^{-3}$, respectively.
10^{-3}$, and $0.00 \pm 0.01 \times 10^{-3}$ $\mu$M/$\mu$m, respectively. The percent net flux of NaFl, 4kD, 12kD, and 70kD at an intraluminal pressure of 6 cmH$_2$O was 52.3 $\pm$ 8.1%, 21.2 $\pm$ 1.3%, 5.1 $\pm$ 1.5%, and $-0.4 \pm 0.9\%$, respectively. These results indicate that NaFl and 4kD significantly (>5% of the initial concentration) penetrated the wall of the lymph vessels and that 12kD slightly (~5% of the initial concentration) penetrated the wall. On the other hand, 70kD did not pass through the lymphatic walls (~0% of the initial concentration). In addition, there were no significant differences in the net flux of NaFl, 4kD, or 12kD among the intraluminal pressures (Fig. 3, A–C).

Effect of CHAPS on net flux of dyes in lymph vessels. There were no significant differences in the frequency and ejection fraction of spontaneous beatings in the isolated lymph vessels at an intraluminal pressure of 6 cmH$_2$O before and after an administration of 0.3% CHAPS. Thus the frequency and ejection fraction after the treatment with CHAPS were 23.0 $\pm$ 1.7 min$^{-1}$ ($n=12$, not significant vs. before 23.6 $\pm$ 1.6 min$^{-1}$) and 0.68 $\pm$ 0.03 ($n=12$, not significant from before 0.67 $\pm$ 0.03), respectively.

Figure 4 shows the net flux of the dyes in the lymph vessels at an intraluminal pressure of 6 cmH$_2$O after stopping the administration (Fig. 4A, 10 $\mu$M 4kD; Fig. 4B, 1 $\mu$M 12kD; Fig. 4C, 0.5 $\mu$M 70kD) obtained before (open columns) and after (shaded columns) the treatment with 0.3% CHAPS. After the treatment with CHAPS, the net flux of 4kD (Fig. 4A) or 12kD (Fig. 4B) was significantly augmented. Thus the percent net flux of 4kD and 12kD after the treatment with 0.3% CHAPS was 29.9 $\pm$ 2.2% ($P<0.05$ vs. before treatment; 19.2 $\pm$ 1.9%) and 18.6 $\pm$ 1.4% ($P<0.05$ vs. before treatment; 4.2 $\pm$ 1.8%), respectively. On the other hand, the treatment with 0.3% CHAPS did not increase net flux of 70kD in the wall of the lymph vessels (Fig. 4C).

DISCUSSION

The major findings of the present study are summarized as follows: 1) NaFl and 4kD in the intraluminal space of isolated rat lymph vessels significantly penetrated the wall of lymph vessels; 2) 12kD in the intraluminal space of isolated rat lymph vessels slightly permeated the lymphatic wall, whereas 70kD did not penetrate the wall; 3) intraluminal pressure ranging from 4 to 8 cmH$_2$O did not significantly affect the net flux of the dyes used in the present study; and 4) after the administration of CHAPS into the lymph vessels, the net flux of 4kD and 12kD but not 70kD was augmented significantly. These results suggest that hydrophilic substances with smaller molecular weight (4,400) are able to permeate from the intraluminal to the extraluminal space through the walls of lymph vessels and that the endothelial cell layer may be a barrier for hydrophilic substances with larger molecular weight (4,400 to 12,000) in small-sized lymph vessels.

Development of technique for evaluating permeability of hydrophilic substances through walls of isolated lymph vessels. Several experimental approaches have been made to analyze macromolecular transport and permeability through the walls of blood vessels and lymph vessels (6, 20, 26, 27) in vivo with the use of techniques with fluorescent dyes. Although these in vivo studies seem to demonstrate functions under physiological conditions, it is difficult to identify the roles of neural, humoral, and local metabolic factors. The normal environment and pressures of efferent lymph vessels in vivo changed rapidly due to endogenous nitric oxide by lymphatic flow or chemical mediators by lymphocytes in the lymph. In the in vivo condition, it is possible to apply fluorescent dyes into the intraluminal space of lymph vessels via the interstitial space. However, it is quite difficult for us to directly infuse the dyes into the intraluminal space of rat lymph vessels in vivo. On the other hand, isolated preparations of blood and lymph vessels enabled us to directly administer the dyes into the intraluminal space of the vessels and to quantitatively evaluate the mechanisms for wall permeability in detail under controlled conditions (15, 16, 33). Therefore, we first attempted to construct an isolated small-sized lymph vessel preparation suitable for evaluating effective permeability of hydrophilic substances through the wall of lymph vessels.

In the present adopted technique, the relationship between the concentrations of the dyes and the intensities of the images in the glass pipettes were dose dependent and linear for all dyes used. Thus the changes in the fluorescence intensities indicate changes in the concentrations of the dyes. It is also known that the dye has a photobleaching effect that results in a decrease in fluorescence intensity. Figure 1B shows there is no significant reduction in the intensity through the photobleaching effect with use of the shutter. The photactivation of the dyes causes an oxygen free radical-mediated inhibition of spontaneous beatings of rat mesenteric lymph vessels in vivo (34). In the present study, however, there were no significant effects of
excitation of the dyes on the frequency and ejection fraction of the lymphatic beatings. Thus these results indicate that the changes in the fluorescence intensity reflect changes in the concentrations of the dyes in the intraluminal space of lymph vessels and that the present experimental conditions have no significant photobleaching effect and no significant effect on the spontaneous beatings of the lymph vessels.

In the present study, the values of frequency and ejection fraction of the lymphatic beatings in the control condition and the response of the lymphatic pump activity to increasing intraluminal pressure were quite similar to those obtained with previous studies (19). We believe that the mechanical properties of isolated rat lymph vessels of iliac lymph nodes have been maintained during the preparation. Minor damage, however, to the vessel may be involved in the permeability of the dyes in the wall of isolated lymph vessels. Thus further methodological improvement including more intact in vivo study will be needed to evaluate the permeability of solutes in the walls of lymph vessels as well as initial lymphatics.

The spontaneous changes in the diameter of lymph vessels may affect the intensity of the dyes. In fact, the intensity of the fluorescence dyes in the intraluminal space of lymph vessels was reported to be higher during the dilated phase than during the constricted state (20). Thus, in the present study, we evaluated the fluorescence intensity of the lymph vessels obtained in the maximal dilated phase only. The shutter use condition, in which the vessels are exposed for 5 s in intervals of 5 s, enabled us to measure the fluorescence intensity obtained in the maximum dilated phase of spontaneous beatings.

Size-limited effective permeability of dyes through walls of isolated lymph vessels. It is well known that lymph protein increases during the transport of the lymph from peripheral lymph vessels to thoracic ducts (7, 10, 12). Heterogeneity in the concentration of lymph protein between the afferent and efferent lymph vessels of the regional lymph nodes has also been reported (1–3, 24). These results suggest that the concentration of lymph protein is actively and/or passively modulated while lymph returns to the systemic circulation. These results of the changes in the concentration of lymph protein are based on in vivo studies and the collection of lymph fluids and could not demonstrate the possibility of a single lymph vessel condensing the concentration of lymph albumin. According to the regression analyses of the relationship between concentrations of the dyes and the intensities of the digitized images in the glass pipettes, we could determine the concentration of the dyes of the intraluminal space of the lymph vessels and could calculate the net flux of the dyes.

The present study indicates that NaFl and 4kD significantly (>5% of the initial concentration) passed through the wall of lymph vessels, and 12kD slightly (~5% of the initial concentration) penetrated the wall. On the other hand, 70kD did not permeate the lymphatic walls (~0% of the initial concentration). Macromolecules with a molecular weight as high as or higher than 6,000 remained in the lymph vessels and are returned to systemic circulation via the regional lymph nodes and thoracic ducts. On the other hand, smaller molecules including water, sodium, and urea leave from the intraluminal space to extracellular matrix tissues. Thus the limit of permeability through the walls of the lymph vessels is considered to be between 2,300 and 6,000 mol wt (17, 18). These results strongly support the present findings that NaFl and 4kD but not 12kD and 70kD could significantly permeate from the intraluminal to the extraluminal space of the lymph vessels. The present study is the first demonstration of the transport of small molecular hydrophilic substances through the walls of a single isolated lymph vessel. In addition, the nonpermeability of 70kD in the walls of the lymph vessels may reflect the nature of albumin (69,000 mol wt) collection in the lymphatic system. In conclusion, the walls of small-sized lymph vessels may play a crucial role in condensing the concentration of lymph protein, which seems to affect the release of lymphocytes from regional lymph nodes.

Intraluminal pressure ranging from 4 to 8 cmH2O did not significantly affect the net flux of the dyes used in the present study. However, the net flux values of 12kD at an intraluminal pressure of 8 cmH2O were slightly higher than those obtained with pressures of 4 or 6 cmH2O (Fig. 3C). These results suggest that higher intraluminal pressures (>8 cmH2O) may facilitate the increase in the net flux of the dyes (12,000–70,000 mol wt) in the lymph vessels. A pressure gradient is maintained across the lymphatic wall in the present study. Thus we suggest that the net flux calculated in isolated lymph vessels reflects an effective permeability rather than permeability of solutes. It is also still unclear that the main transport mechanism of the dyes in the walls of the lymph vessels is diffusion, which is the concentration gradient driving transport in the present study. We used one concentration in each dye being available for evaluating changes in the intensity because our experimental condition used in the present study has a limitation for intensity range. Thus the driving forces for the net flux across the lymphatic wall, be it a concentration or a pressure gradient, do not control the effective permeability but do control the net flux. The present study, however, at least shows there is a size-limited effective permeability of the dyes in the lymph vessels.

Role of endothelium in transport of hydrophilic substances through walls of isolated lymph vessels. It is well known that the permeability of macromolecule substances depends on the structure of the capillary endothelium in the organs and tissues (14, 25). No information exists regarding the permeability of hydrophilic substances through the walls of lymph vessels and the crucial role of the lymphatic endothelium in the permeability of the substances. CHAPS is known to be a detergent that can disrupt or eliminate the endothelial barrier without changing the responsiveness of smooth muscle cells (15, 16). In fact, treatment with CHAPS did not affect the spontaneous beatings of the lymph vessels in the present study. The treatment with CHAPS significantly increased the net flux of 4kD. Interestingly, although the net flux of 12kD was not identified and was slight in the control, the treatment with CHAPS significantly increased the net flux of 12kD. On the other hand, 70kD did not permeate the wall of lymph vessels before or after the treatment with CHAPS. These results suggest that an endothelial barrier in the lymph vessels may be disrupted by the treatment with CHAPS, resulting in the enhancement of effective permeability for hydrophilic substances in the lymphatic wall. The treatment with CHAPS (the concentration used was the same as in the present study) influenced the intimal permeability of the dyes in the isolated arterioles (15).

Medium-sized FITC-dextran (50.7kD) slowly penetrated the endothelial cell layer of the mesenteric artery but did not
REFERENCES


