Increased Sheep Lung Vascular Permeability Caused by Histamine

By Kenneth L. Brigham and Patty Jill Owen

ABSTRACT
To see whether histamine increases lung vascular permeability to protein, we compared the effects of steady-state histamine infusions on lung vascular pressures, lung lymph flow, and lung lymph protein content with the effects of mechanically elevated lung vascular pressures on these variables in the same unanesthetized sheep. We surgically implanted catheters in the pulmonary artery, the left atrium, the superior vena cava, and a main lung lymphatic. After the sheep had recovered from surgery, we carried out steady-state experiments without anesthesia. Histamine induced a dose-related, quickly reversible increase in lung lymph flow without affecting pulmonary artery pressure, and it caused left atrial pressure to fall. During 4-hour intravenous 4 μg/kg min⁻¹ histamine infusions, lymph flow and lymph protein clearance (lymph flow × lymph-plasma protein concentration ratio) increased more than they did with mechanically elevated pressure even though vascular pressures fell. Lymph-plasma protein ratios decreased linearly with increasing lymph flow during increased pressure experiments, but during histamine infusions the ratios did not decrease even though lymph flow increased 2-6-fold. Lymph clearance and permeability-surface area products (PS) for eight protein fractions with molecular radii ranging from 36 to 100 Å decreased with increasing molecular size in base-line, increased pressure, and histamine studies. PS values for all eight fractions were significantly higher than base-line in histamine experiments but not in increased pressure experiments. Four-hour intravenous histamine infusions caused moderate increases in lung water content. Left atrial infusions had less effect on lymph flow than did intravenous infusions. We conclude that histamine causes pulmonary vascular permeability to protein to increase but that the effects on exchanging vessel porosity are more modest than those suggested for systemic microvessels. Histamine should be considered a possible mediator of increased lung vascular permeability.

Although several human diseases in which respiratory distress and pulmonary edema occur are thought to result from increased lung microvascular permeability to protein (1), studies of possible mediators of this effect in the pulmonary circulation, including studies of histamine, have been largely negative (2-4). Most of these studies have been of short duration, in anesthetized animals or isolated lungs, and the usual criterion for increased transvascular filtration has been the accumulation of excess lung water.

Brigham et al. (5) have reported studies in a chronic unanesthetized sheep preparation which show that lung lymph flow and protein content are more sensitive indexes of increased microvascular permeability than are measurements of lung water. Therefore, we studied the effects of histamine, given parenterally, in the same preparation to further clarify the actions of histamine in the pulmonary circulation.

Histamine caused a dose-related reversible increase in fluid and protein filtration from lung vessels without increasing vascular pressures. The histamine effects were clearly different from the effects of increased lung vascular pressure measured in the same sheep, and vessels supplied by pulmonary artery blood appeared to be affected. We conclude that histamine increases lung microvascular permeability to protein and therefore should be considered a potential mediator in reactions in which such changes occur.

Methods
Experiments were carried out in 12 young female or castrated male sheep weighing 32-58 kg.

EXPERIMENTAL PREPARATION
The sheep were prepared by a series of three thoracotomies as described previously (5). First, to eliminate abdominal contributions to a large elongated lymph node in the posterior mediastinum (caudal mediastinal node), the tail of the node was resected below the inferior pulmonary ligaments through a right thoracotomy. After
4–6 days, through a left thoracotomy, the pericardium was resected, a stainless steel clip was placed at the posterior border of the left atrium, a silicon elastomer-coated Foley balloon catheter (Dow Corning Corp.) was put in the left atrium, and silicone elastomer catheters were positioned in the left atrium and the main pulmonary artery. Finally, 4–6 days later, a small silicone elastomer cannula was placed in the efferent lymph vessel emerging from the head of the caudal mediastinal node through a right thoracotomy. Polyethylene catheters were inserted in the superior vena cava and the thoracic aorta through the neck vessels. Lymph flow from sheep prepared this way increases when left atrial pressure is increased but does not increase when systemic venous pressure is increased and therefore represents mostly lung lymph (5).

EXPERIMENTAL PROTOCOLS

General.—Sheep were unanesthetized, standing in a cage, during all of the experiments. Before studies were begun, the left atrial clip was located by fluoroscopy with the sheep standing in its experimental cage; this position was used as the zero reference level for all vascular pressures. During each experiment, vascular pressures were measured continuously with miniature strain gauges (Micron Instruments, Inc.) and an electronic recorder (Hewlett-Packard Co.). Lung lymph flow was measured at 15-minute intervals by recording the volume drained from the cannula into a graduated tube, and protein concentrations were measured in plasma from blood drawn each hour and in lymph pooled at 30-minute intervals.

Increased Pressure Studies.—Once in each of six sheep, responses to increased lung vascular pressures were measured. After at least 2 hours of base-line observation, the left atrial balloon was inflated enough to increase left atrial pressure by 15–20 cm H2O; the pressure was then kept stable for 4 hours. Lymph flow and lymph protein concentration were stable after 2 hours of balloon inflation (5), and overt pulmonary edema did not occur.

Histamine Studies.—Histamine was studied by three different protocols. First, we examined the effects of steady-state intravenous histamine infusions. Twelve steady-state histamine infusions were carried out in the same six sheep that had been subjected to the increased pressure studies. After at least 2 hours of base-line observation, 4 μg/kg min⁻¹ of histamine phosphate (Eli Lilly Co.) was infused through the superior vena caval catheter for 4 hours using a constant-rate infusion pump (Harvard Apparatus Co., Inc.). This dose produced a substantial increase in lymph flow without causing the preparation to become unstable. Vascular pressures, lymph flow, and lymph and plasma protein concentrations reached a stable level by 2 hours after the beginning of the histamine infusion. Then, we determined histamine dose-response relationships. After at least 2 hours of base-line observation, histamine was infused at increasing rates, starting with 1–2 μg/kg min⁻¹. We waited for a plateau in lymph flow and vascular pressure responses, then increased the infusion rate to 3–4 μg/kg min⁻¹, waited for a plateau, again increased the infusion rate to 5–6 μg/kg min⁻¹, and continued the infusion until a response plateau was reached. Higher doses caused severe respiratory distress and marked fluctuations in vascular pressures. Finally, we made comparisons between left atrial and intravenous histamine infusions. To try to separate systemic and pulmonary vascular effects, on consecutive days, in the same sheep, 4 μg/kg min⁻¹ of histamine phosphate was infused for 4 hours through the superior vena caval catheter on one day and through the left atrial catheter on the other. To avoid bias, the order of the studies was varied.

OTHER METHODS

Protein Analyses.—Total protein concentrations in lymph and blood plasma were measured with an automated system (AutoAnalyzer, Technicon Instruments Corp.) by a modified Biuret method (6); duplicate determinations differed by less than 5%. Protein fractions were separated in steady-state base-line and experimental lymph and blood plasma samples by polyacrylamide gradient gel electrophoresis using 4–30% gradient gel slabs (Pharmacia Fine Chemicals) and Tris-barbital buffer at pH 8.0 and ionic strength 0.06. Electrophoresis was carried out for 16.5 hours at 125 v (constant voltage), and the gels were stained with 0.5% Ponceau S in 7.5% trichloroacetic acid, destained electrophoretically in 7% acetic acid, and scanned spectrophotometrically at 510 nm. Using the measured total protein concentrations, the concentrations of each of eight protein fractions consistently identified in plasma and lymph samples were calculated. To estimate the effective molecular radius for each of the eight fractions, gel slabs were run with both lymph and plasma samples and five proteins of known molecular weight and free diffusion coefficient. Using the Einstein-Stokes equation (7), the effective molecular radius was calculated for each of the known proteins and plotted as a function of migration distance. The effective molecular radius of the eight plasma and lymph protein fractions was estimated from this standard curve. Figure 1 is a plot of the curve showing the known proteins and the locations of the eight plasma and lymph fractions. Fraction I is albumin. Total globulin concentrations in Table 2 are the sums of fractions II–VIII.

Indicator-Dilution Lung Water Measurements.—Extravascular lung water was measured during steady-state base-line and experimental periods by indicator dilution. Both 51Cr-labeled erythrocytes and 125I-labeled albumin were used as intravascular indicators to avoid errors due to red blood cell–plasma transit time differences (8, 9). Red blood cells from the sheep were labeled by incubating a blood sample for 1 hour with 51Cr-sodium chromate at room temperature and washing the cells three times with 0.89% NaCl solution. For each study, a mixture of 15-μc 51Cr-labeled erythrocytes, 10-μc 125I-labeled albumin, and 30-μc 14C-labeled water was injected as a bolus through the superior vena caval catheter, and arterial blood samples were taken at 1.0-second intervals by allowing blood to flow from the aortic catheter into heparinized tubes on a rotating disk collector. Radioactivity was measured in 0.5 ml portions of each arterial

1 The standard proteins used were: albumin (bovine albumin fraction V, Technicon Instruments Corp.), ceruloplasmin (Behring Diagnostics), α₂-macroglobulin (Behring Diagnostics), catalase (Worthington Biochemicals), and β-lipoprotein (human, purified by ultracentrifugation in Dr. Virgil LeQuire's laboratory, Department of Anatomy, Vanderbilt University).
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Results

INCREASED PRESSURE STUDIES

The effects of a 4-hour increase in lung vascular pressures were similar to those reported previously (5). As shown in Table 1, hematocrit and arterial pH increased slightly, but arterial blood gases did not change. Cardiac output decreased as a result of inflation of the left atrial balloon, and there was a small but significant increase in extravascular lung water measured by indicator dilution.

Table 2 summarizes the vascular pressures and lymph data for all of the lymph studies. As reported previously (5), lymph flow always increased when pressure was increased. Lymph flow began to increase by 15 minutes after pressure had been elevated and reached a stable level by 2 hours after the pressure elevation. Increased pressure did not affect plasma protein concentration, but lymph concentrations of both albumin and globulin decreased.

HISTAMINE STUDIES

Steady-State Histamine Infusion.—Figure 2 shows the effects of a 4-hour intravenous infusion of 4 \( \mu \)g/kg min\(^{-1}\) of histamine phosphate on vascular pressures and lymph flow in one experiment. Immediately after the infusion was begun, left atrial pressure fell and within 15 minutes lymph flow increased. The sheep developed slightly labored respiration, and the conjunctivae and mucus membranes became erythematous. Heart rate increased, and aortic pulse pressure widened with a slight decrease in mean aortic pressure. Vascular pressures and lymph flow reached a plateau in 30–45 minutes and remained at that level for the duration of the infusion, returning to base line over 60–90 minutes after the histamine was stopped. As shown in Table 1, by the end of a 4-hour histamine infusion, arterial blood hematocrit, \( P_{\text{CO}_2} \), and pH had increased slightly. Arterial blood \( P_{\text{O}_2} \) and cardiac output fell from base line, and lung water

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measured by indicator dilution increased. As summarized in Table 2, histamine consistently caused left atrial pressure to fall and lymph flow to increase without affecting pulmonary artery pressure significantly. The average steady-state lymph flow increase was over twice the baseline value, although there was considerable variability among sheep. During the histamine response, plasma albumin and globulin concentrations and lymph albumin concentrations were significantly lower than baseline. However, during the histamine response, while lymph flow was increased, lymph-plasma albumin and globulin concentration ratios were not significantly different from baseline values.

The different effects of mechanically increased pressure and histamine on lymph-plasma protein ratios are illustrated in Figure 3 where total protein lymph-plasma concentration ratios are plotted as a function of lymph flow, with both sets of values normalized to the baseline value in each experiment. Over the range of pressures studied, lymph flow increased to 1.5–2.5 times baseline. During increased pressure, lymph-plasma protein ratios always decreased from baseline, and the magnitude of the decrease correlated well with the magnitude of the increase in lymph flow. During the steady-state histamine response, lymph flow was 2–6 times baseline, but, with the exception of two experiments with large increases in lymph flow, lymph-plasma protein ratios did not decrease substantially from baseline. The lymph flow change caused by histamine in six experiments was in the range of that seen during increased pressure, but the effects of the two interventions on lymph-plasma protein ratios were clearly different.

Table 3 shows the average steady-state lymph-plasma concentration ratios for eight protein fractions for baseline, increased pressure, and histamine experiments. Although there was considerable variability, the ratios tended to decrease with increasing molecular size in all three groups.
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TABLE 2

Summary of Steady-State Data from Increased Pressure and Histamine Infusion Experiments in Six Sheep (Mean Body Weight 43 ± 3 kg)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>P\textsubscript{n} (cm H\textsubscript{2}O)</th>
<th>P\textsubscript{a} (cm H\textsubscript{2}O)</th>
<th>(\hat{Q})\textsubscript{lym} (ml/hour)</th>
<th>Plasma albumin (g/100 ml)</th>
<th>Plasma globulin (g/100 ml)</th>
<th>Lymph albumin (g/100 ml)</th>
<th>Lymph globulin (g/100 ml)</th>
<th>Lymph-plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line</td>
<td>19 ± 1</td>
<td>5 ± 1</td>
<td>6.1 ± 1</td>
<td>2.68 ± 0.35</td>
<td>3.75 ± 0.22</td>
<td>2.19 ± 0.34</td>
<td>2.20 ± 0.16</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>(n = 6)</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Increased pressure</td>
<td>32 ± 2*</td>
<td>20 ± 3*</td>
<td>11.6 ± 2.3*</td>
<td>2.71 ± 0.34</td>
<td>4.02 ± 0.23</td>
<td>1.83 ± 0.34*</td>
<td>1.80 ± 0.10*</td>
<td>0.63 ± 0.07*</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base line</td>
<td>18 ± 1</td>
<td>5 ± 1</td>
<td>6.6 ± 1.4</td>
<td>2.28 ± 0.18</td>
<td>3.81 ± 0.15</td>
<td>1.75 ± 0.21</td>
<td>2.19 ± 0.09</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>(n = 12)</td>
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<td></td>
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</tr>
<tr>
<td>Histamine</td>
<td>17 ± 1</td>
<td>-3 ± 1*</td>
<td>14.2 ± 1.7*</td>
<td>2.03 ± 0.09*</td>
<td>3.37 ± 0.12*</td>
<td>1.47 ± 0.09*</td>
<td>2.09 ± 0.09</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>(n = 12)</td>
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</table>

All values are means ± SE. In each experiment, base-line values from a 2-hour observation period were averaged, increased pressure values were averaged over the last 60 minutes of a stable 4-hour elevation of left atrial pressure (see Methods), and histamine values were averaged over the last 60 minutes of a 4-hour intravenous infusion of 4 \(\mu\)g/kg min\(^{-1}\) of histamine. \(P\textsubscript{n}\) and \(P\textsubscript{a}\) are average pulmonary artery and left atrial pressures, respectively, referred to the posterior border of the left atrium, and \(\hat{Q}\)\textsubscript{lym} is lung lymph flow. Globulin includes all proteins except albumin.

*Significantly different from base line \((P < 0.05)\).

Renkin (13) has suggested that if protein transport is principally by diffusion then the permeability-surface area product \((PS)\) for a solute can be calculated by the formula:

\[ PS = LR/(1 - R), \]

where \(L\) is lymph flow and \(R\) is the lymph-plasma protein ratio. This formula can be used to calculate permeability-surface area product \((PS)\) for all of the studies, in all three groups.
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FIGURE 6

Effects of step increases in the histamine infusion rate on lung vascular pressures and lymph flow. Each increase in the histamine infusion rate caused an additional decrease in left atrial pressure and an additional increase in lymph flow.

was not significant for seven of the eight fractions. PS was significantly elevated from base line during histamine infusion for all of the fractions, but PS still declined with increasing molecular size.

The ratio of extravascular lung water to dry weight of bloodless lung averaged $5.30 \pm 0.24$ (SD) in six sheep killed at the end of a 4-hour intravenous 4-$\mu$g/kg min$^{-1}$ histamine infusion and $4.36 \pm 0.16$ in six sheep prepared identically and killed under base-line conditions. The difference was significant ($P < 0.05$).

**Histamine Dose-Response Relationships.**—Figure 6 shows the lymph flow and lung vascular pressure responses to step increases in the histamine infusion rate. Each increase in the histamine infusion rate caused an additional increase in lung lymph flow and an additional fall in left atrial pressure. Lymph flow responses during six experiments like the one illustrated in Figure 3 done in four sheep are shown in Figure 7. Lymph flow during the plateau at each histamine infusion rate is expressed relative to steady-state base-line lymph flow. Increasing the histamine infusion rate always caused lymph flow to increase. The response was reproducible in a single sheep, but there was considerable variability in the responses among sheep.

**Left Atrial Versus Intravenous Histamine Infusions.**—Five times in two sheep, we compared the responses to left atrial 4-$\mu$g/kg min$^{-1}$ histamine infusions with those to identical intravenous infusions. The studies were done on consecutive days. Figure 8 shows the total quantity of lymph in excess of base line collected during the 4-hour histamine infusions in these studies. Regardless of the order of the studies, the lymph flow response to the intravenous infusion was always greater. Total excess lymph during intravenous infusion averaged twice that during left atrial infusion.

**Discussion**

Since lymph protein concentration does not change in transit through peripheral lymphatics (14) or lymph nodes (15) and since fluid filtered from the microcirculation which does not accumulate in the interstitial space is drained away by lymphatics (16), under steady-state conditions lymph flow and lymph protein concentration should reflect the net volume and the average protein concentration of exchanging vessel filtrate. Several investigators have applied this concept to the study of microvascular filtration in the lung (5, 17, 18) and other organs (13, 19, 20). Based on this concept, the results of the studies reported in the present paper clearly demonstrate that histamine increases lung vascular permeability to protein.

![Graph showing lymph flow and histamine infusion rate](image)

**FIGURE 7**

Lung lymph flow expressed relative to base-line flow as a function of the histamine infusion rate in six experiments in four sheep. Different symbols are used for each sheep. Lines connect points obtained in a single experiment. Lymph flow always increased when the histamine infusion rate was increased. The response was reproducible in a single sheep but variable among sheep.

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Histamine infusions resulted in a dose-related increase in lung lymph flow which was maintained for the duration of the infusion. This lymph flow increase occurred while pulmonary artery pressure was unchanged and left atrial pressure fell. In dogs, histamine appears to increase pulmonary venous (postcapillary) resistance more than arteriolar (precapillary) resistance (21). If this phenomenon occurred in our experiments, then pressure in exchanging vessels could have increased during histamine infusion even though pulmonary artery and left atrial pressures did not. However, in four histamine experiments lymph flow was higher than it was in any of the increased pressure studies and too high to be explained by an increase in microvascular pressure, even if pulmonary artery pressure (the maximum possible value) is taken as the best estimate of pressure in exchanging vessels. As suggested by others in reference to the peripheral circulation (22, 23), increased pressure may contribute to the increased lymph flow caused by histamine, but the magnitude of the lymph flow increase in several studies cannot be explained by increased pressure alone.

The differences between increased pressure and histamine are even more dramatic when comparisons of the effects on steady-state lymph protein concentrations are made. In all of the experiments reported previously (5) and in this study, when lung vascular pressure was increased mechanically, lung lymph flow increased and lymph-plasma protein concentration ratios decreased. The relationship between lymph-plasma protein ratios and lymph flow was linear in the increased pressure studies (Fig. 3). However, when lymph flow increased during histamine infusion, lymph-plasma protein ratios did not fall. Thus, the histamine effect cannot be explained by an increase in transmural pressure in exchanging vessels.

Even though our preparation effectively excludes systemic lymph (5), it is possible that fluid filtering from bronchial vessels is drained away by pulmonary lymphatics (24). If this phenomenon occurs, the changes we saw could have resulted from increased permeability in the bronchial circulation (25). However, when we compared the responses to left atrial histamine infusions with those to intravenous infusions in the same sheep, intravenous infusions always caused a greater increase in lymph flow, regardless of the order in which the studies were done. If the observed increase in lymph flow reflected primarily increased filtration from the bronchial (or any other systemic) vascular bed, the response should have been at least as large (perhaps larger) with left atrial histamine infusion. Although histamine may have increased permeability in the bronchial circulation, as reported by others (25), such an effect does not appear to be the main cause of the increase in lung lymph flow which we saw. The smaller increase in lung lymph flow with left atrial infusion may have resulted from rapid histamine metabolism and excretion in the systemic circulation, which has been demonstrated in sheep (26).

Several previous studies have failed to demonstrate an effect of histamine on permeability in the pulmonary circulation. Goetzman and Visscher (2) have shown that alloxan, but not histamine (0.02-0.5 μg/ml perfusate), causes an increase in the rate of radiolabeled albumin transport from alveoli to blood in isolated, perfused, fluid-filled dog lungs. Since lymph reflects only what happens to vascular endothelium, our studies are not incompatible with their findings. Pietra et al. (25) have shown bronchial edema and leakage of 250-500 Å diameter carbon particles from bronchial venules but not from pulmonary vessels after 90-minute intravenous infusions of 7 μg/kg min⁻¹ of histamine base in anesthetized dogs. Our steady-state experiments were done with slower histamine infusion rates than those used by Pietra et al. (25). Although we continued our infusions for 4 hours,
maximum lymph flow and vascular pressure responses occurred by 90 minutes after the beginning of histamine administration. The physical changes in pulmonary microvascular endothelium necessary to explain our observations may be too modest to permit substantial leakage of 250-500 Å radius particles. Grega et al. (3) could see no effect of histamine infusion (2-10 μg base/min) on the rate of weight gain in isolated perfused dog lungs. However, lymph flow and protein content are more sensitive indexes of increased filtration than are measurements of fluid accumulation. In addition, their experiments were of short duration compared with ours, and the concentration of histamine reaching the lung microvascular endothelium may have been higher in our studies. There may be differences among animal species, and living unanesthetized animals may respond differently than do isolated perfused lungs. It is also possible that prolonged histamine infusions cause release of other vasoactive substances that can increase vascular permeability.

On the basis of morphological evidence, it has been suggested that histamine causes large (5000-8000 Å radius) gaps to appear between venular endothelial cells, perhaps as a result of endothelial cell contraction (25, 27-29). However, others have questioned the relationship of these morphological changes to the histamine effect on transmicrovascular exchange (30, 31). Haddy et al. (32) saw increased protein concentrations in lymph from dog forelimbs during infusions of as little as 3 μg/min of histamine, but lymph-plasma protein ratios did not approach unity until high histamine doses (40 μg/min) were used. The permeability increase was dose related. If the main increase in filtration caused by histamine were through numerous very large endothelial gaps, the protein composition of lymph should equal that of plasma. Recently, Carter et al. (33) have reported effects of 10-100 μg of histamine injected subcutaneously on dog leg lymph. They measured lymph flow and lymph-plasma concentration ratios for five protein fractions and found that histamine caused a dose-related increase in lymph flow, lymph-plasma protein ratios, and protein permeability-surface area products (Eq. 1). During the histamine effect, microvascular sieving still occurred as indicated by decreasing lymph-plasma ratios and PS values with increasing protein molecular size.

Our data for the lung in awake sheep are qualitatively similar to those of Carter and his associates in the dog leg (33). Histamine caused marked increases in lymph protein clearance and PS for eight protein fractions, but the decline in lymph protein clearance and PS with increasing molecular size seen under base-line conditions and during mechanically increased pressure was preserved during histamine infusion. Histamine clearly caused lung vascular permeability to increase, but selectivity on the basis of molecular size was preserved. We believe this fact indicates that the structural changes in microvascular endothelium were more modest than those that have been suggested by morphological data in systemic vascular beds (25, 27-29).

Other investigators have reported that lymph-plasma concentration ratios and permeability-surface area products decrease with increasing molecular size in the lung (17) and other organs (14, 33). Our base-line lymph-plasma ratios for large proteins are higher than those reported in systemic vascular beds (14, 33); this fact is consistent with the concept that lung microvessels are leakier than muscle microvessels (5, 17). Boyd et al. (17) have also found that lymph-plasma ratios for large proteins in sheep lungs are higher than those reported in other organs (17).

Some workers have suggested that increased vascular pressure in the lung (34) and other organs (35) causes microvascular permeability to increase (pore "stretching"). We saw significant increases in lymph protein clearance during increased pressure, but this finding does not mean that permeability was increased. Both the equivalent pore model presented earlier (5) and the diffusion model of Renkin (Eq. 1 [13]) predict an increase in lymph protein clearance with increasing lymph flow even if the permeability-surface area product does not change. Our findings during steady-state increased pressure that lymph-plasma protein concentration ratios declined linearly with increasing lymph flow and that PS values estimated by a diffusion model for seven of the eight measured protein fractions were not significantly different from base line are consistent with the idea that increased vascular pressure in the range we studied does not markedly increase microvascular permeability. However, we elevated pressure only moderately, and it is possible that higher vascular pressures cause permeability to increase (34).

We found that histamine caused extravascular lung water measured both by indicator dilution and by postmortem desiccation to increase. In previous studies, we found no increase in lung water in sheep when vascular permeability was increased following Pseudomonas bacteremia even though the increases in lung lymph flow were larger.
than those we saw with histamine (5). The difference between these findings is consistent with the idea that the pulmonary edema caused by histamine in the doses we studied is mainly bronchial edema (25) and that the lung lymph we collected reflects primarily filtration from pulmonary vessels and not bronchial vessels. It is also possible that histamine interferes in some unknown way with lung lymphatic drainage. We have demonstrated that histamine infusion results in a dose-related, reversible increase in the permeability of pulmonary microvessels to protein. The histamine effect appears to involve primarily vessels supplied by pulmonary artery blood. The lymph protein clearance and the permeability-surface area products for eight protein fractions decreased with increasing molecular size under baseline conditions and during mechanically increased pressure. Although both values were markedly elevated from baseline during histamine infusion, the decline with increasing molecular size was preserved. This finding and the relatively small increase in lymph flow suggest more modest changes in pulmonary microvascular endothelium than others have suggested in systemic vascular beds. Several acute (1, 36, 37) and some subacute and chronic (38) human lung diseases appear to result from increased lung microvascular permeability to protein. It is likely that these changes are mediated by release of one or several endogenous vasactive substances (39). Our studies suggest that histamine should be considered a possible mediator.

References

2. GOETZMAN B, VISSCHER M: Effects of alloxan and histamine on the permeability of the pulmonary alveolo-capillary barrier to albumin. J Physiol (Lond) 204:51–61, 1969
31. DIANA J, LONG S, YAO H: Effect of histamine on equivalent


33. CARTER RD, JOYNER WL, RENKIN E: Effects of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvasc Res 7:31-48, 1974


Increased sheep lung vascular permeability caused by histamine.

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