Interstitial albumin concentration measured during growth of perivascular cuffs in liquid-filled rabbit lung

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Moe, Sonja M., Robert L. Conhaim, and Stephen J. Lai-Fook. Interstitial albumin concentration measured during growth of perivascular cuffs in liquid-filled rabbit lung. J Appl Physiol 96: 283–292, 2004.—The growth rate and albumin concentration of interstitial fluid cuffs were measured in isolated rabbit lungs inflated with albumin solution (3 g/dl) to constant airway (Paw) and vascular pressures for up to 10 h. Cuff size was measured from images of frozen lung sections, and cuff albumin concentration (Cc) was measured from the fluorescence of Evans blue labeled albumin that entered the cuffs from the alveolar space. At 5-cmH₂O Paw, cuff size peaked at 1 h and then decreased by 75% in 2 h. The decreased cuff size was consistent with an osmotic absorption into the albumin solution that filled the vascular and alveolar spaces. At 15-cmH₂O Paw, cuff size peaked at 0.25 h and then remained constant. Cc, rose continuously at both pressures, but was greater at the higher pressure. The increasing Cc with a constant cuff size was modeled as diffusion through epithelial pores. Initial Cc-to-airway albumin concentration ratio was 0.1 at 5-cmH₂O Paw and increased to 0.3 at 15 cmH₂O, a behavior that indicated an increased permeability with lung inflation. Estimated epithelial reflection coefficient was 0.9 and 0.7, and equivalent epithelial pore radii were 4.5 and 6.1 nm at 5- and 15-cmH₂O Paw, respectively. The initial cuff growth occurred against an albumin colloid osmotic pressure gradient because a high interstitial resistance reduced the overall epithelial-interstitial reflection coefficient to the low value of the interstitium.

The perivascular interstitial space, situated between the vascular and alveolar spaces of the lung (see Fig. 1), acts as a reservoir in the early stages of pulmonary edema to protect the alveolar space from flooding (36, 38). The storage capacity of the interstitium is, therefore, of interest and has been studied extensively by using liquid-inflated lungs (7, 10, 11, 22, 23). The usual method is to measure perivascular interstitial cuff size in lungs rapidly frozen after liquid inflation for specific time periods. This approach has allowed the investigation of the mechanical properties of the interstitium.

In isolated rabbit lungs inflated with a 3 g/dl albumin solution to a constant airway inflation pressure, interstitial cuffs reached a maximum size by 1–5 h and remained constant in size thereafter (22, 23). The growth of interstitial cuffs was modeled by fluid flow through resistive and compliant elements, analogous to the flow of an electric current through ohmic resistances with shunt capacitors by using electrical analog models. In this analysis, the epithelial resistance was lumped with the interstitial resistance, and cuff growth was assumed to be due entirely to bulk flow driven by the hydrostatic pressure difference between the alveolar space and interstitium. The maximum cuff size was achieved when the interstitial pressure measured by micropuncture at the lung hilum equilibrated to the airway pressure (22, 23).

The growth of interstitial cuffs measured by the interstitial pressure response at the lung hilum was found to be faster with albumin and positively charged solutions and slower with Ringer and negatively charged solutions (23). These responses due to albumin concentration and charged solutions were attributed to the properties of the interstitium because they were consistent with the results from previous measurements of steady flow through lung interstitial segments (32).

Because interstitial albumin concentration was not measured in the foregoing studies, the albumin colloid osmotic (oncotic) pressure acting across the epithelium was not considered in the analysis. A permeable epithelial barrier to protein was implied in isolated lung studies that showed an interstitial tracer protein concentration that was 90% of the instilled airway liquid (17). However, a recent study in rat lung indicated a significant barrier to protein with a relatively high reflection coefficient to albumin of 0.83 (13). This high reflection coefficient of the epithelium implied a relatively low interstitial protein concentration during cuff growth and a significant airway-to-interstitial osmotic pressure gradient that would oppose the hydrostatic pressure gradient. Thus we wondered how interstitial cuffs would form against a relatively large transepithelial osmotic pressure gradient between the alveolar and interstitial compartments.

Accordingly, we studied the change in interstitial cuff albumin concentration and cuff size as a function of inflation time at low and high inflation pressures in liquid inflated rabbit lungs. Our results showed that the growth of interstitial cuffs depended primarily on the airway inflation pressure and was largely independent of the albumin concentration in the airway solution. During cuff growth to maximum size, the cuff-to-airway albumin concentration ratio increased at high airway pressure, which indicated an increased epithelial permeability. At low inflation pressure, we found that the cuffs reached a maximum size early on and then decreased in size thereafter, although cuff albumin concentration continued to rise. This behavior was consistent with reabsorption of cuff liquid by osmotic flow into the vascular and alveolar spaces over time. At high inflation pressure, the increase in cuff albumin concentration with a nearly constant cuff size was consistent with diffusion through restrictive epithelial pores.
To determine the effect of an increased airway osmotic pressure, we increased the albumin concentration in the airway and vessel solutions to 5 g/dl. We used an inflation time of 1 h at airway inflation pressures of 5 and 15 cmH2O.

Size and Albumin Concentration of Perivascular Interstitial Cuffs

In the cryostat (−20°C), the frozen lungs were cut into slabs (~1 cm thick) transverse to the cranial-dorsal direction. The surfaces of both (~1 × 1 × 1 cm) cut from the slabs were planed smooth with a microtome. Lung samples and the calibration plate (see below) were kept frozen in a covered glass petri dish containing dry ice. The dish was placed on a micromanipulator attached to the stage of a compound microscope that allowed both fluorescent [Evans blue labeled albumin (EBA)] and white light (color) imaging of blood vessels and their surrounding interstitial cuffs. The fluorescence videomicroscope used in previous studies (45) was modified to measure a color image.

Color images of vessels with interstitial cuffs were examined on a video monitor (model no. PVM 1390, Sony) via a digital camera (Nikon COOLPIX 990) mounted to an eyepiece port of the microscope. Lung samples were illuminated with an external fiber-optic source. Directing a flow of air on the cover eliminated water condensation on the glass cover of the dish. Images were stored on a videocassette recorder (SVHS, Panasonic) coupled to a time-code generator. Image size was adjusted by using various objective lenses (×2.5, ×8, ×25, or ×50, American Optical and Zeiss). A calibration scale (minimum division, 20 μm) was simultaneously imaged. Digital color images were transferred to a computer and analyzed for vessel diameter, vessel area (A), and cuff area (A) by using Adobe Photoshop, Scion Image (National Institutes of Health), and ImageTool (UTHSCSA). The actual A observed on the color image was verified by comparison with the fluorescent image (see below).

Albumin concentration of perivascular interstitial cuffs was measured by using fluorescence videomicroscopy with a video camera (C2400, Hamamatsu, SIT) that allowed both bright and ultra-low-level light imaging. For EBA fluorescent imaging, light from a high-pressure mercury lamp (Zeiss illuminator 100 with HBO 50) was filtered through the following lens system (Zeiss): an infrared absorbing filter (300–750 nm band pass), a bypass filter (BP 510–560 nm), and a dichromatic beam splitter (FT 580). The fluorescent light image was recorded by the video camera through a barrier filter (LP 590) and an adjustable (×2) interface lens (Zeiss), observed on a video monitor (Panasonic WV 5490), and stored in a videocassette recorder (Panasonic NV-8950). A time-code generator superimposed a time signal on the recordings.

After obtaining the color image of a vessel with interstitial cuff, its entire fluorescent image was examined on the video monitor. Figure 1 shows a color image of an artery (dyed red) surrounded by an interstitial cuff and surrounding alveolar space (dyed blue). Figure 2 shows the corresponding black and white image of EBA fluorescence. Note that the cuff observed in both images was not uniform in thickness. The fluorescent image showed a definite demarcation in light intensity that separated the vessel, cuff, and surrounding alveolar region. Note that the artery had the least brightness (least EBA), the interstitial cuff an intermediate brightness, and the surrounding alveolar region the most brightness, an indication of EBA concentration. To obtain the fluorescent image shown in Fig. 2, the entire field (within the outer circle) was subjected to incident light. Light intensity from this image could not be used to determine relative albumin concentration, because light from the brightest regions greatly affected regions with lower intensity. Some fluorescence was observed in vessels because the endothelium was partially permeable to albumin, so that, with absorption of interstitial liquid, some EBA entered the vessels.

To determine albumin concentration in the interstitial cuff, only a small region of interest within the interstitial cuff was illuminated (small circle, Fig. 2). We used the minimum illuminated region...
to 60 μm (45). Thus changes in cuff light intensity due to changes in interstitial depth caused by vessel eccentricity were not observed.

Statistics

The data are reported as mean values ± SD in Table 1 and as mean values ± SE in Figs. 4 and 5. Paired and unmatched Student’s t-tests were used where appropriate to test for significant differences between two groups. Linear and multilinear regression analyses were used to test for correlation between two variables and among three variables, respectively. A P value of <0.05 indicated significance.

RESULTS

Experiments

Figure 3 is a representative calibration curve from a frozen diluted sample of the stock EBA solution used to fill the airways. Table 1 is a summary of the Cc-to-airway albumin concentration (Ca) ratio (Cc/Ca) and A/v measured in lungs inflated with 3 g/dl albumin solution at different inflation times and at airway inflation pressures (Paw) of 5 and 15 cmH₂O. Figure 4, A and B, shows plots of A/v and Cc/Ca, respectively, vs. inflation time at 5- and 15-cmH₂O Paw. These data included only the cuffs in which Cc was measured. Thus A/v at Paw of 5 cmH₂O appeared to reach a minimum value of 0.2 between 2 and 10 h, only because smaller cuffs with no measurement of Cc were excluded. The number of vessels with no measurable cuffs increased with inflation time, which indicated a monotonic decrease in interstitial cuff volume. Vessel diameters ranged from 200 to 3,800 μm. Some cuffs occurred around arteries that adjoined an airway. Airway cuffs were too small for a measure of albumin concentration. Not all vessels observed had cuffs. Vessel of diameter <100 μm produced no observable cuffs, in keeping with previous studies (7, 10, 11, 22, 23). Vessels <200 μm in diameter produced cuffs that were too small for a measurement of albumin concentration (see MATERIALS AND METHODS). The fraction of vessels with cuffs did not vary significantly with inflation time or inflation pressure.

At Paw of 5 cmH₂O, the minimum value of Cc/Ca of 0.1 occurred at inflation times of 0.5 and 1 h, which corresponded to the rapid phase of cuff growth during which A/v rose to a value of ~0.8 (Fig. 4). By contrast, at Paw of 15 cmH₂O, the minimum value of Cc/Ca of 0.3 occurred at an inflation time of 0.25 h, which corresponded to a time when the peak value of A/v was measured. Both Cc/Ca and A/v were immeasurable...
at the inflation time of 0.11 h. We associated these minimum values of C/c/C_a during cuff growth with bulk flow of liquid across the epithelium at a high Péclet number so that the epithelial reflection coefficient, α_e = 1 - C_c/C_a, was 0.9 and 0.7 at Paw of 5 and 15 cmH_2O, respectively (see Eq. A13, Appendix).

At Paw of 5 cmH_2O, C_c/C_a increased monotonically from 0.1 at 0.5–1 h to 0.72 at 10 h. The increase in C_c/C_a between 1 and 2 h was accompanied by a rapid fall (75%) in A_c/A_v to a value of 0.2. This was attributed to the absorption of cuff liquid with a reduced albumin concentration into the vascular and alveolar spaces. Between 2 and 10 h, A_c/A_v remained constant at 0.2, whereas C_c/C_a increased. The replacement of the 3 g/dl albumin solution in the vessels with Ringer solution (Table 1, groups F–H, and Fig. 5) eliminated the absorption of cuff liquid into the vessels. These experiments showed a relatively small but significant increase in C_c/C_a from 0.11 at 1 h to 0.22 at 3 h and a relatively small (not significant) reduction in A_c/A_v (25% at 3–7 h). This behavior was attributed to reabsorption of cuff liquid into the alveolar space (see below). However, the 75% reduction in cuff size measured by both vascular and alveolar absorption, compared with the smaller reduction measured by alveolar absorption alone, implied that vascular absorption was the major route of clearance of cuff liquid.

In contrast to the behavior at 5-cmH_2O Paw, C_c/C_a at 15-cmH_2O Paw increased more rapidly from 0.3 at 0.25 h to 0.74 at 5 h. No measurement of C_c/C_a was obtained at 0.11 h because A_c/A_v was too small (<0.2). The increase in C_c/C_a after 0.25 h occurred in conjunction with a nearly constant A_c/A_v. This implied that liquid absorption into the vessels was negligible, consistent with an increased endothelial permeability at high inflation pressure (31). Accordingly, the increase in C_c/C_a that occurred after 0.25 h at 15-cmH_2O Paw was modeled as passive restricted diffusion through epithelial pores (see below).

Airway and vessel inflation to 5-cmH_2O Paw at 1 h with 5 g/dl albumin solution produced values for C_c/C_a and A_c/A_v that were not significantly different from those measured with 3 g/dl albumin solution (Table 1, groups B and N). Thus the rate of cuff growth was largely independent of the C_a and depended only on the Paw-to-interstitial hydrostatic pressure difference. This was attributed to a much larger hydraulic conductivity of the epithelial barrier than that of the interstitial cuffs, as discussed below. However, at 15-cmH_2O Paw, C_c/C_a decreased significantly from 0.48 at 1 h after airway and vessel inflation, with a 3 g/dl albumin solution in the airway, to 0.34 with a 5 g/dl albumin solution, whereas A_c/A_v was unchanged (Table 1, groups K and O). This behavior might have been caused by a slower rate of cuff growth with the higher albumin concentration.

**Data Analysis**

**Effect of interstitial resistance on cuff formation and reabsorption.** We developed an analysis to show the reasons why the growth of interstitial cuffs to maximum size depends...
primarily on the hydrostatic pressure difference. The important results are presented here with details in the APPENDIX. Consider transport across a two-membrane system, namely the epithelium in series with the interstitial cuff. The epithelial-interstitial reflection coefficient ($\sigma_{ec}$) is related to the epithelial ($K_e$)-to-interstitial conductance ($K_c$) ratio ($K_e / K_c$) and the $\sigma_e$-to-$\sigma_c$ ratio ($\sigma_{ec} / \sigma_c$)

$$\sigma_{ec} / \sigma_c = [1 + (K/K_c)]/[1 + (K_e / K_c)(\sigma_e / \sigma_c)]$$

Figure 6 shows the variation of $\sigma_{ec}$ vs. $K_e / K_c$ for the values of $\sigma_e$ of 0.9 and $\sigma_c$ of 0.1. For an interstitial resistance (reciprocal of conductance) that was much greater than epithelial resistance during the initial phase of cuff growth, both $\sigma_c$ and $\sigma_{ec}$ were near zero, so that the bulk flow was determined primarily by the hydrostatic pressure difference and was largely independent of the osmotic pressure difference (Table 1, groups B and K and groups N and O). As the cuffs grew, interstitial resistance was reduced, and $\sigma_{ec}$ increased above $\sigma_c$. When $\sigma_{ec}$ became large enough so that the effective osmotic pressure difference exceeded the hydrostatic pressure difference, alveolar reabsorption of cuff liquid occurred (Fig. 4A).

The reduced interstitial cuff size, together with an increased interstitial protein concentration between 2 and 10 h at 5 cmH$_2$O Paw (Figs. 4 and 5), was attributed, in part, to vascular absorption and, in part, to alveolar reabsorption of the interstitial fluid.

Fig. 4. Interstitial cuff-to-vessel area ratio ($A_c / A_v$; A) and interstitial cuff-to-airway albumin concentration ratio ($C_c / C_a$; B) vs. time of inflation for inflation pressures (airway pressure (Pa)) of 5 and 15 cmH$_2$O. The curved line passing through the data of $C_c / C_a$ at 15-cmH$_2$O airway pressure is the solution to the diffusion equation, as described in the text. Both airways and vessels were inflated with 3 g/dl albumin solution. Values are means ± SE.

Fig. 5. Comparison between $A_c / A_v$ (A) and $C_c / C_a$ (B) vs. time of inflation between the vessels filled with 3 g/dl albumin solution and the vessels filled with Ringer solution. $C_a$ was 3 g/dl. Inflation pressure was 5 cmH$_2$O. Values are means ± SE.

Fig. 6. Theoretical curve (linear-log) of epithelial-interstitial reflection coefficient ($\sigma_{ec}$) vs. epithelial-to-interstitial conductance ratio ($K_e / K_c$) for $\sigma_e$ of 0.9 and $\sigma_c$ of 0.1.
found that interstitial Cc/Ca was 0.1 at 5-cmH2O Paw and increased to 0.3 at 15 cmH2O. Based on a bulk flow of high Péctel number (see APPENDIX), the σe was 0.9 and 0.7 at 5 and 15 cmH2O inflation pressure, respectively. The initial cuff growth to maximum size was determined by the airway-to-interstitial hydrostatic pressure difference and was largely independent of osmotic pressure gradients. We attributed this behavior to a relatively high interstitial resistance that reduced the effective σe to that of the interstitium. At 5-cmH2O inflation pressure, after the initial cuff growth, the decrease in cuff size in conjunction with an increase in Cc was attributed to an osmotic flow of cuff liquid of reduced albumin concentration into the vascular and alveolar spaces. At 15-cmH2O inflation pressure, interstitial cuff concentration increased with time with little change in cuff size, consistent with a diffusion process.

Method

We modified the method used previously to study interstitial cuff growth in liquid-filled rabbit (22, 23), dog (7, 11), and sheep lungs (10). There are several advantages in using liquid-filled rather than air-filled lungs. First, filling the lung with liquid eliminated the effect of alveolar surface tension so that the pressure acting on the epithelial surface was the inflation pressure. Second, submerging the lung in saline eliminated the effect of gravity on vascular pressure (46). Third, we subjected the vessels and airways to the same hydrostatic pressure and albumin concentration solution to eliminate any airway-to-vessel hydrostatic and osmotic pressure gradients. Using similar vascular and Paw values (zone 1) reduced the surface area for vascular-to-interstitial flow (12, 19, 46). Accordingly, the growth of interstitial cuffs occurred predominantly by flow from the alveolar space. Thus the albumin concentration measured in the interstitium reflected predominantly the sieving properties of the alveolar epithelium.

Because of the presence of lung parenchymal tissue, light intensity measured from the alveolar space was ~70% of that measured from the stock solution. Thus the light measured in interstitial cuffs might have underestimated actual Cc. However, we believe this effect was small because the tissue in the interstitial cuff was diluted at least fivefold based on cuff size (22, 23).

Comparison with Previous Studies

In our laboratory’s previous experiments in isolated rabbit lungs inflated with albumin solution (22, 23), the Aa/Ae increased monotonically to a plateau that depended on the size of the vessels and the airway inflation pressure. In our present study, in addition to measuring cuff size, we also measured the increase in albumin concentration of the interstitial cuffs as a function of inflation time.

In the present study, the number of vessels examined was ~100, which was considerably less than that measured in the previous studies (~1,000; Refs. 22, 23). The number of cuffs examined was relatively small, mainly because of the time required to measure Cc. The small number of cuffs examined might account, in part, for the following differences between the present and previous studies. First, because of the small number of cuffs examined, no effect of vessel size on Aa/Ae or Cc/Ca was observed. Second, the time required to reach a
plateau in cuff size was shorter in the present study (~0.5 vs. 1–5 h). No reduction of cuff size was observed in the previous study (22), which required 3–5 h to reach its maximum size. The reduction of cuff size also implied an interstitial cuff pressure that was below the airway inflation pressure (7).

**Relation to Other Studies of Alveolar Clearance**

Our results at the low inflation pressure confirmed our laboratory’s previous observation (22) that cuff growth in rabbit lungs was slower than in dog (7) or sheep lungs (10) inflated to a higher inflation pressure (~14 cmH₂O). However, at the high inflation pressure, cuff growth rate was comparable to that measured in dog and sheep lungs. We attributed the growth of interstitial cuffs in our previous measurements to passive transport properties of the interstitium and ignored the effects of the epithelium. The slower cuff growth in the isolated rabbit lung contrasts to in vivo studies showing that clearance of liquid instilled into lobular regions was fastest in the rabbit and human and slowest in the dog (27). In these studies, clearance of alveolar liquid was accompanied by an increase in protein concentration in the remaining alveolar liquid. Active transport of liquid was postulated as the primary mechanism of clearance of airway liquid.

Whatever the role of active mechanisms in the absorption of alveolar liquid into the circulation, the results of the present study indicated that the clearance of alveolar liquid can occur via the interstitium into the vasculature by passive mechanisms. Intersitial clearance of alveolar liquid has been demonstrated in unanesthetized sheep that showed significant interstitial cuffs 4 h after the instillation of 100 ml of Evans blue dyed serum into the airways (26). The observed interstitial cuffs contained little albumin, which was evident from the small Cc measured in the present study. The increasing protein concentration in the liquid instilled in sheep lungs, as the instilled liquid volume was reduced (26), might be brought about by the formation of interstitial cuffs of low-protein concentration or in high-permeability-type pulmonary edema, when interstitial and alveolar liquid have relatively high but equal protein concentrations, or in high-permeability-type pulmonary edema, when interstitial and alveolar liquid have relatively high but equal protein concentrations (43, 44). Both situations demand a low σec. This behavior is obtainable with an increased Ke that produces an Ke/Kc of 1:3 with an σec of 0.3 (Fig. 6). Alveolar flooding in vivo occurs after the cuffs are formed (36) when the interstitial-to-airway hydrostatic pressure gradient just offsets the interstitial-to-airway osmotic pressure gradient. Under these conditions, Péclet numbers are relatively small, and the airway-to-interstitial protein concentration ratio approaches 1 (Eq. A11, Appendix).

**Differences Between the Isolated Lung and the In Vivo Lung**

There are major differences between the isolated lung as used in the present experiments and the in vivo lung. First, the isolated lung experiment was designed to study the growth of interstitial cuffs by transport of alveolar liquid across the epithelium. In vivo, the normal capillary pressure results in filtration in the interstitium, whereas a relatively high plasma protein concentration results in osmotic absorption of alveolar liquid into the circulation. Microvascular filtration in volume overloaded lambs (33) produced interstitial-to-plasma albumin concentration ratios of 0.3–0.7 that were similar to values measured after vascular and alveolar reabsorption in the present study. Second, in the isolated lung, clearance by interstitial lymphatics was absent so that any absorption of interstitial liquid by a restrictive endothelial and epithelial barrier resulted in an increase in interstitial albumin concentration. In vivo, the...
Mechanisms Producing Cuff Growth and Reabsorption

We considered passive forces rather than active transport to be the predominant mechanism for the growth of interstitial cuffs. Vesicular transport, although a possibility, was considered unlikely in view of studies showing that the vesicular inhibitor nocodazole had no effect on alveolar protein clearance in the in vivo rabbit lung (18). Moreover, vesicular inhibitors increased tissue permeability in many isolated organ systems (6, 28, 29, 34). However, the role of active transport mechanisms in attenuating the growth and resolution of interstitial fluid cuffs needs to be specifically addressed in further studies.

APPENDIX

The \( \sigma_{ec} \) as a Function of Interstitial Resistance

The following analysis illustrates the reasons why the growth of interstitial cuffs to maximum size depends primarily on the hydrostatic pressure difference. The Starling equation relates bulk flow (\( Q_b \)) across a membrane to the differences in hydrostatic pressure (\( \Delta P \)) and protein colloid osmotic (oncotic) pressure (\( \Delta \pi \)) acting across the membrane (36, 40)

\[
Q_b = K(\Delta P - \sigma \Delta \pi)
\]  

\( (A1) \)

where \( K \) is the hydraulic conductance (filtration coefficient) of the membrane. We apply Eq. A1 to the two-membrane system, consisting of the alveolar epithelium in series with the perivascular interstitium. The conductance of the epithelial-interstitial barrier (\( K_{ec} \)) is related to the \( K_e \) and \( K_c \) from Eq. A1, with \( \Delta \pi = 0 \), as follows

\[
1/K_{ec} = 1/K_e + 1/K_c
\]  

\( (A2) \)

The \( \sigma_{ec} \) is related to the \( \sigma_e \) and \( \sigma_c \), obtained from Eq. A1, with \( \Delta P = 0 \)

\[
1/(K_{ec}\sigma_{ec}) = 1/(K_e\sigma_e) + 1/(K_c\sigma_c)
\]  

\( (A3) \)

The following equation is obtained from Eqs. A2 and A3

\[
\sigma_e/\sigma_c = [1 + (K_e/K_c)]/[1 + (K_e/K_c)(\sigma_e/\sigma_c)]
\]  

\( (A4) \)

This equation provides an estimate of the \( \sigma_{ec} \) in terms of the \( K_e/K_c \). Figure 6 is a linear-log plot of \( \sigma_{ec} \) vs. \( K_e/K_c \) for the values of \( \sigma_e \) of 0.9 and \( \sigma_c \) of 0.1. For an interstitial resistance (reciprocal of conductance) that is much greater than epithelial resistance (\( K/K_c \) \( \gg \) 1), \( \sigma_{ec} \) \( \rightarrow \) \( \sigma_c \). This case was applicable to the initial phase of cuff growth when both \( \sigma_c \) and \( \sigma_{ec} \) were near 0, so that the \( Q_b \) was determined primarily by \( \Delta P \) and was largely independent of \( \Delta \pi \) (Eq. A1). As the cuff grows, interstitial resistance was reduced and \( K/K_c \) decreased, so that \( \sigma_{ec} \) increased above \( \sigma_c \). When \( \sigma_{ec} \) became big enough so that \( \sigma_{ec} \) \( \Delta \pi > \Delta P \), alveolar reabsorption of cuff liquid occurred. In the limit, as \( K/K_c \) \( \ll \) 1, \( \sigma_{ec} \) \( \rightarrow \) \( \sigma_c \). The condition during cuff growth, \( K/K_c \) \( \gg \) 1, \( \sigma_{ec} \) \( \rightarrow \) \( \sigma_c \), implied that \( K_e \) was much greater than \( K_c \), as the following estimates during cuff growth showed.

First, we estimated the \( V_e \) relative to the value of 5% of air space volume measured in sheep lungs, where maximum \( A_e/A_c \) was 2.4 (10). Based on the maximum \( A_e/A_c \) of 0.7 measured in our present study (Fig. 4A), \( V_e \) was 1.5% of air space volume. Because rabbit air space volume was \( \approx 100 \) ml (35 ml/kg; Ref. 47), \( V_e \) was, therefore, 1.5 ml.

We obtained an estimate of \( K_{ec} \) from the results of electrical analog models of cuff growth measured in previous studies (22, 23). Fluid resistance (\( R \)) of the \( K_{ec} \) in those studies was defined by using Darcy’s law for a porous material

\[
R = \Delta P/(Q/V_e) = (vL^2/K_{ec})/(A_e/A_c), \quad V_e = A_eL
\]  

\( (A5) \)

Resistance was defined as \( \Delta P \) per unit flow (\( Q \)) normalized by dividing by vessel volume (\( V_e \)). Here \( K_{ec} \) is a permeability constant and \( v \) is the fluid viscosity; and \( L_e \) is the lumped vessel length. Note that, with cuff growth, \( R \) decreases as \( A_e/A_c \) increases. \( R \) at the start of cuff growth was \( \approx 35 \) cmH\(_2\)O/ml/h (23). From the maximum \( V_e \) value of 1.5 ml and maximum \( A_e/A_c \) of 0.8, \( V_e \) was \( \approx 2 \) ml. \( K_{ec} \), equal to \( V_e/L_e \), was, therefore, \( 6 \times 10^{-2} \) ml/h/cm\(^2\)-cmH\(_2\)O\(^{-1}\).

Epithelial conductivity in the rat lung (13) was estimated to be \( 2 \times 10^{-2} \) ml/h/cm\(^2\)-cmH\(_2\)O\(^{-1}\). For \( K_{ec} \) of \( 6 \times 10^{-2} \) ml/h/cm\(^2\)-cmH\(_2\)O\(^{-1}\) (above), \( K_e/K_c \) was 16 (Eq. A2). With the use of typical values of \( \sigma_e \) of 0.9, \( \sigma_c \) of 0.1 (42), and \( K_e/K_c \) of 16 in Eq. A4, \( \sigma_{ec} \) was 0.11; that is, \( \sigma_{ec} \) is a good approximation of \( \sigma_{ec} \) during cuff growth.

Intersitial Cuff Liquid Reabsorption by Osmotic Flow

We used the Starling equation to estimate the absorptive pressure from interstitial cuffs into vessels after the cuffs had grown to maximum size

\[
\dot{Q}_c = K_e[(P_e - P_v) - \sigma_{ec}(\pi_c - \pi_v)]
\]  

\( (A6) \)

where \( \dot{Q}_c \) is the bulk flow of liquid across the interstitial-endothelial barrier; \( K_e \) is interstitial-endothelial barrier conductance; \( P_e \) is interstitial hydrostatic pressure; \( P_v \) is vascular hydrostatic pressure; \( \sigma_{ec} \) is interstitial-endothelial barrier reflection coefficient; \( \pi_c \) is interstitial protein colloid osmotic pressure; and \( \pi_v \) is vascular protein colloid osmotic pressure. We used an equation analogous to Eq. A4 to evaluate the \( \sigma_{ec} \)

\[
\sigma_{ec}/\sigma_c = [1 + (K_e/K_c)]/[1 + (K_e/K_c)(\sigma_{ec}/\sigma_c)]
\]  

\( (A7) \)

where \( \sigma_{ec} \) is the endothelial reflection coefficient, and \( K_{ec} \) is endothelial conductance. Because the vascular pressure was equal to or slightly below the Paw (zone 1), the entire capillary bed was collapsed so that osmotic reabsorption occurred across precapillary corner vessels (12, 19). Under these conditions, \( K_e \) was much greater than \( K_{ec} \) (\( K_e/K_c \ll 1 \)), so that \( \sigma_{ec} \) was associated with the endothelium. In other words, \( \sigma_{ec} = \sigma_e \).

At 5-cmH\(_2\)O Paw, after the cuffs have grown to maximum size so that \( P_e = Paw = P_v \), the absorptive pressure from the cuffs into the vessels was proportional to the difference in the effective osmotic pressure between the cuff and vessel, \( \sigma_{ec}(\pi_c - \pi_v) \). We used Landis and Pappenheimer’s equation (20) relating albumin osmotic pressure (cmH\(_2\)O) to albumin concentration (C; g/dl): \( \pi = 1.36 \times (2.8C + 0.18C^2) \). With \( C/C_e \) of 0.1, \( C_e \) (and \( C_v \)) of 3 g/dl, and, assuming \( \sigma_{ec} \) for the endothelium to be 0.7 (40), \( \sigma_{ec}(\pi_c - \pi_v) \) was 8.2 cmH\(_2\)O. This absorptive osmotic pressure was reduced to 6.4 and 2.7 cmH\(_2\)O as \( C/C_e \) was increased to 0.3 and to 0.7, respectively. The reduction of the \( A_e \) from the maximum value of \( A_e/A_c \) of 0.8 to 0.2 as cuff liquid was absorbed into the vessel resulted in a reduction of \( P_v \) from 5 to 1.3 cmH\(_2\)O, based on an initial value of \( 0 \) cmH\(_2\)O for \( P_v \) at the start of cuff growth (4) and a constant interstitial compliance. Accordingly, the net absorptive pressure into the vessels was reduced from its maximum value of 8.2 cmH\(_2\)O (with a \( \Delta P \) of \( 0 \) cmH\(_2\)O) at 1 h to \( -1 \) cmH\(_2\)O

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(with a $\Delta P$ of 3.7 cmH$_2$O) at 10 h. Thus net absorption at 1 h diminished with inflation time, becoming a small net filtration at 10 h. The increase in $C_c/C_a$ between 1 and 10 h was caused by an osmotic flow of cuff liquid with a reduced C into the vascular and alveolar spaces that was maximal at 1 h and became smaller as the increasing $C_c/C_a$ reduced the effective osmotic pressure difference.

Our results with Ringer solution in the vessels (Table 1, groups F–H, Fig. 5) indicated that reabsorption of cuff liquid also occurred into the alveolar space between 1 and 3 h, because $C_c/C_a$ increased from 0.1 at 1 h to 0.22 at 3 h. This was associated with a small (insignificant) decrease in $A_c/A_o$. Reabsorption of cuff liquid into the alveolar space required that $\sigma_{ce}$ increase above $\sigma_e$ during cuff growth, as interstitial resistance decreased with cuff growth (Eq. A5). After cuff growth to maximal size, an $\sigma_{ce}$ value of $\sim 0.3$ would be sufficient to produce alveolar reabsorption. This could be obtained with a reduction in $K_{f}/K_{t}$ to 1.3 (Eq. A4, Fig. 6); that is, with an interstitial resistance that was threefold smaller than the epithelial resistance. For $\sigma_{ce}$ of 0.3, the effective osmotic pressure difference for alveolar reabsorption $[\sigma_{ce}(\sigma_e - \sigma_{il})]$ was 2.5 cmH$_2$O at 1 h and was reduced to 1.8 cmH$_2$O at 7 h. Accordingly, net absorption into the airways occurred at 1 h, with a maximal absorptive pressure of 2.5 cmH$_2$O ($\Delta P$ of 0 cmH$_2$O), and changed to net filtration with an absorptive pressure of $\sim 1.9$ cmH$_2$O ($\Delta P$ of 3.7 cmH$_2$O) at 7 h. In the foregoing analysis, we neglected the effects of diffusion. We showed later that diffusion was negligible at 5-cmH$_2$O Paw based on the estimated $R_p$.

At Paw of 15 cmH$_2$O, an effective osmotic pressure difference of 3 cmH$_2$O would reduce $A_c/A_o$ by 20% and would be offset by a reduction of $P_f$ by 3 cmH$_2$O. The small decrease in $A_c/A_o$ was not detected in our measurements and was consistent with previous measurements of perivascular interstitial pressure by micropuncture at the lung hilum that equilibrated to the inflation pressure of 15 cmH$_2$O (23). Thus any opposing osmotic force due to reabsorption might have been too small to be detected or might be masked by the much larger number of cuffs with a slower growth rate that was measured in the previous study (see DISCUSSION; Refs. 22, 23). However, studies in dog lungs showed that hiliar interstitial pressure equilibrated to 11.5 cmH$_2$O with a lung inflation pressure of 14 cmH$_2$O and suggested a small absorptive pressure of 2.5 cmH$_2$O (7).

Modeling Diffusion Across the Epithelium

At 15-cmH$_2$O Paw, because $A_c/A_o$ remained constant after cuff growth, the increase in $C_c/C_a$ with inflation time was modeled as a diffusion process to determine the $t_o$ for diffusion across epithelial pores. Diffusion from the cuffs into the vessels was neglected because of the small endothelial surface area associated with the zone 1 conditions (Paw = $P_f$; Refs. 12, 19, 46). We used the differential form of Fick’s law to describe the time ($t$) rate of transfer of a solute mass ($M_t$) into the interstitial cuff volume (solute concentration $C_v$, volume $V_v$, $C_v = M_v/V_v$) across the alveolar membrane from the alveolar space (solute concentration $C_a$)

$$dM_t/dt = (C_v - M_v/N_v)D_vA_v/L_v$$

(A8)

With constant $C_v$, the solution of this linear first-order differential equation is

$$C_c/C_a = 1 + B \exp(-t/t_o), \quad t_o = V_v/L_v(D_vA_v)$$

(A9)

where $B$ is an arbitrary constant to be determined from the initial conditions. With the initial conditions, $t = 0$, $C_c = C_a$, Eq. A9 becomes

$$C_c/C_a = 1 + (C_c/C_a - 1) \exp(-t/t_o)$$

(A10)

where $C_c/C_a$ is 0.33. For Paw of 15 cmH$_2$O, we fit Eq. A10 (Fig. 4B, solid line) to the part of the $C_c/C_a$ vs. $t_c$ curve after the maximum $A_c/A_o$ was reached ($t > 0.25$ h, Fig. 4A). The part of the $C_c/C_a$ vs. $t_c$ curve for $t < 0.25$ h was attributed to the cuff growth by $Q_a$. A linear regression fit to the values of $\ln(1 - C_c/C_a)$ vs. $t_c$ data produced a slope of $-1/t_o$.

Modeling Transport During Cuff Growth: Relationship Among $C_c/C_a$, $\sigma_e$, and Solute-to-Rp Ratio

We modeled the epithelium as a membrane to determine its $\sigma_e$ from measured values of $C_c/C_a$. The equilibrium solution for $C_c/C_a$ for solute flow across a membrane with uniform cylindrical pores is as follows (40)

$$C_c/C_a = (1 - \sigma_e)(1 - \sigma_e \exp(-x))$$

(A11)

Here $\sigma_e$ is due to solute drag for the epithelium. The parameter $x$ is the Péctel number, defined as the ratio of the solute flux due to $Q_o$ to that due to diffusion ($D_vA_v/L_v$)

$$Péctel \text{ number} = (1 - \sigma_e)Q_o/(D_vA_v/L_v)$$

(A12)

For a large $Q_o$, relative to diffusion (Péctel number $> 4$), $\exp(-x) \rightarrow 0$, and Eq. A12 becomes

$$C_c/C_a = (1 - \sigma_e)Q_o/(D_vA_v/L_v)$$

(A13)

Reflection coefficient is related to $a/R_p$, the solute-to-Rp ratio, as follows (2)

$$\sigma_e = (1 - \phi)^2, \quad \phi = (1 - a/R_p)^2$$

(A14)

where $\phi$ is the solute distribution function, the core-to-pore area ratio. The core area is the area within one solute radius of the pore wall. We assumed that the initial cuff growth to maximal size occurred by $Q_o$ with high Péctel number so that $\sigma_e = 1 - C_c/C_a$ (Eq. A13). This was justified below. From the minimum values of $C_c/C_a$ of 0.1 and 0.33, $\sigma_e = 0.9$ and 0.67 for Paw of 5 and 15 cmH$_2$O, respectively. The $a/R_p$ values were 0.77 and 0.57 for Paw of 5 and 15 cmH$_2$O, respectively (Eq. A14). With an albumin molecular radius ($a$) of 3.5 nm, $R_p$ was 4.5 and 6.1 nm for Paw of 5 and 15 cmH$_2$O, respectively (Table 2).

Transport Properties of the Epithelium

The $t_o$ at 15-cmH$_2$O Paw allowed the determination of $A_c/A_o$ for the epithelial membrane by specifying values for $V_v$ and $D_v$. We modeled the diffusion through cylindrical pores of uniform diameter. The diffusion of a solute through a cylindrical pore is as follows (35)

$$D_v = D_v/\phi$$

(A15)

where $D_v$ is the free diffusion coefficient of albumin ($6 \times 10^{-7}$ cm$^2$/s; Ref. 41); and the parameter $k$ is the drag coefficient of a spherical solute molecule moving along the pore centerline and is obtained from tables as a function of $a/R_p$ (30). For Paw of 15 cmH$_2$O, with $a/R_p$ of 0.57, $k$ from tables was 9.1, $\phi = 0.19$, and $D_v = 0.02D_v$ was 0.12 $\times 10^{-7}$ cm$^2$/s. We assumed an interstitial $V_v$ of 1.5 ml. From $t_o = V_v/(L_v(D_vA_v)$, $A_o/L_o$ was then 7,400 cm. At Paw of 5 cmH$_2$O, $k$ was 55, $\phi = 0.053$, and $D_v$ was 0.001$D_v$. $A_o/L_o$ was 4.000 (proportional to $R_p^2$). We assumed that $V_v$ was similar at both 5- and 15-cmH$_2$O Paw, based on the maximum lung volume at Paw of 6 cmH$_2$O measured in saline-filled lungs (3). We verified that the Péctel number was in the range for which Eq. A14 was valid, as follows. With flows of 1.5 and 6 ml/h (maximum $V_v$ of 1.5 ml divided by time of 1 and 0.25 h) at 5- and 15-cmH$_2$O Paw, respectively, the Péctel numbers were 17 and 6, respectively, justifying the assumption used to determine Eq. A13.

The $t_o$ at 5-cmH$_2$O Paw, with an $A_c/A_o$ of 4.000, $V_v$ of 1.5 ml, and $D_v$ of $6 \times 10^{-10}$ cm$^2$/s, was 174 h. Thus the diffusive flux at 5-cmH$_2$O Paw was too small to explain the increase in $C_c/C_a$ observed over the 10 h (Fig. 4B). This supported our explanation that the increase in $C_c/C_a$ was due to absorption by an osmotic flow into the vascular and alveolar spaces.

For an epithelial thickness ($L_e$) of 0.5 $\mu$m and $A_c/L_c$ of 4.000 cm at 5-cmH$_2$O Paw, the total area pore was 0.20 $cm^2$. Total alveolar
surface area was $7.4 \times 10^4$ cm$^2$, based on an alveolar diameter of 80 μm and total alveolar volume of 100 ml (47). The pores numbered $3.1 \times 10^{11}$, based on a $R_t$ of 4.5 nm. The number of pores was 4.2 × 10^6/cm^2 alveolar surface area. The number of pores per alveolus was 840, based on the number of alveoli of $3.7 \times 10^8$, equal to the total alveolar volume divided by the volume of an alveolus. The inter pore spacing for a square lattice distribution was 5 μm. A summary of the transport parameters is given in Table 1.

REFERENCES


