Stiffness of peripheral airway folding membrane in rabbits

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Lambert, Rodney K., Peter D. Paré, and Mitsushi Okazawa. Stiffness of peripheral airway folding membrane in rabbits. J Appl Physiol 90: 2041–2047, 2001.—We have observed that small, membranous bronchioles from rabbits, in which the smooth muscle is not activated, experience a critical elastic buckling involving the whole airway wall during deflation of the lung. This implies that, at some point during the deflation, the airway wall goes from being in a state of tension to a state of compression. At the transition, there is neither net tension nor net compression in the wall, and the transmural pressure difference must, therefore, be zero. Thus at this point, the pressure difference across the muscle that results from the passive stress in the muscle is just balanced by the pressure difference across the folded mucosal membrane. We estimated the muscle stress, and hence the pressure across the muscle, from published data on rabbit trachealis (Opazo-Saez A and Paré PD, J Appl Physiol 77: 1638–1643, 1994) and equated this to the pressure across the folded membrane. By using a theoretical prediction of this pressure (Lambert RK, Codd SL, Alley MR, and Pack RJ, J Appl Physiol 77: 1206–1216, 1994), together with the results of our morphometric measurements on these airways, we estimated that the flexural rigidity of the folding membrane in peripheral rabbit airways is of the order of $10^{-12}$ Pa⋅m$^3$. This value implies that, in these airways, membrane folding provides significant resistance to airway smooth muscle shortening.

The fact that airway smooth muscle (ASM) must develop tension to narrow an airway indicates that forces exist in and on the airway wall against which the muscle does work. The most obvious of these is the tension in the alveolar attachments that transfers pleural pressure to the airway wall (19). When the muscle shortens and the airway narrows, local deformation of the parenchyma causes an additional force that is usually referred to as the parenchymal interdependence force. For many years, much interest has centered on this force (5, 6, 8–12, 15–18). The interaction is usually described in terms of classical elasticity theory in which the parenchyma is treated as a continuum and the local distortion is governed by the local shear modulus (17).

It has also been proposed by three different groups that the folding of the epithelial membrane could provide an additional force that opposes the muscle (13, 14, 24, 31). Because it is not clear exactly which structures inside the muscle wall contribute to this force, we will use the term “folding membrane” from now on to denote the total structure that provides the force. In Lambert’s analysis (13), elastic shell theory was used to show that an increased number of folds required an increased pressure difference across the folding membrane. A further development of the analysis showed that, as the airway narrowed, more folds developed (14). The key property of the folding membrane that determines the pressure difference required to produce a given level of folding was shown to be its flexural rigidity, $D$, which in turn depends on both the Young modulus, $E$, and the thickness of the tissue that folded. There are no data for these quantities for airway folding membrane, although there are data for the basement membrane of renal tubules, presumably a similar material (29, 30).

The data in our recent article (21) indicate that this model explains at least some of the observations of membrane folding in airways in which ASM has been activated. In particular, the walls of these airways remained circular as the lung was deflated, and the number of membrane folds ($n$) was shown to increase when the luminal area ($A_i$) was reduced. However, airways in which ASM was not activated collapsed in a different pattern when the lung was deflated; the entire airway wall underwent a buckling into an oval shape (the fundamental mode of collapse of an elastic tube) after the development of only a few folds in the folding membrane. After this buckling, membrane folds continued to increase as the airway flattened further and its long axis shortened.

The relationship of fold development in the membrane to the external load causing the folding is determined by $D$. Thus evaluation of $D$ is essential to an assessment of the mechanical significance of folding. For instance, knowing the value of $D$ for an airway will...
enable us to know whether membrane folding provides significant resistance to muscle shortening. In this study, we will use the observation of elastic buckling of the entire airway wall to obtain a value for the D of the folding membrane.

**METHODS**

The data presented here are taken from those obtained in the previously reported study mentioned above (21). Briefly, we used New Zealand White rabbits divided into two groups: one was exposed to nebulized and infused carbachol (the Carb group), and the other, the control (Cont) group, to saline. After challenge (real or sham), lungs in the open-chest animals were brought to a lung recoil pressure (PL), either by deflation from PL = 4 cmH2O to one of −4, 0, or 2 cmH2O or by holding at a positive end-expiratory pressure of 4, 7, or 10 cmH2O before infusion of Formalin and removal from the animal. After removal, the lungs were immersed in a bath of the same in both groups of rabbits.

Using standard morphometric techniques, we measured Ai, area inside the basement membrane (Am bm), and the subdivisions of wall area as well as the perimeter of the epithelial basement membrane (Pbm bm). Pbm bm was our reference measure for airway size because it is independent of muscle shortening or lung inflation (7). The distribution of Pbm bm was the same in both groups of rabbits.

The data were pooled at each PL for each treatment to obtain overall trends with PL. They were also put into bins based on Pbm bm at each PL. After some preliminary analysis of the distribution of airway sizes, it was decided to use the bins shown in Table 1. The numbers of airways studied at each PL are given in Table 2. Results for the Carb airways were presented in the previous report (21). Mean values and SE of relevant measures of airway size and deformation were calculated for the pooled data and by bin. Where possible, data were normalized on an appropriate variable. This was often the calculated value of that variable for a fully dilated airway with a circular basement membrane under the assumption that the basement membrane does not stretch. This was done to obtain the greatest possible generalization of the results by removing the size of the airway from the variable being examined.

Results from the previous study that are relevant here are as follows. Carb airways developed epithelial folds at high PL, the number of folds increased with decreasing PL to a maximum at PL = 0 cmH2O, and then the airways underwent whole wall buckling and flattened with further reduction in PL. In contrast, although Cont airways developed a few epithelial folds at high PL, the number increased only slightly with reduction in PL before the entire airway buckled and flattened, after which epithelial fold number increased with further reduction in PL. Larger airways underwent whole wall buckling at higher values of PL than did the smaller airways.

**Table 1. Pbm bm ranges used in determining bins**

<table>
<thead>
<tr>
<th>Label</th>
<th>Pbm bm Range, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>1 mm</td>
<td>0.9–1.1</td>
</tr>
<tr>
<td>1.5 mm</td>
<td>1.4–1.6</td>
</tr>
<tr>
<td>Large</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

Pbm bm, basement membrane perimeter.

**Table 2. Number of airways studied at each PL**

<table>
<thead>
<tr>
<th>PL, cmH2O</th>
<th>No. of Control Airways</th>
<th>No. of Carbachol Airways</th>
</tr>
</thead>
<tbody>
<tr>
<td>−4</td>
<td>68</td>
<td>92</td>
</tr>
<tr>
<td>0</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>105</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>81</td>
</tr>
<tr>
<td>Total</td>
<td>365</td>
<td>549</td>
</tr>
</tbody>
</table>

PL, lung recoil pressure.

**THEORY**

We are concerned with the onset of whole wall buckling in Cont airways. This occurs at a value of PL that is less than that at which epithelial folds first appear when PL is reduced from a high value. We will use this observation of whole wall buckling to evaluate the D of the folding membrane. To do this, we will use a simplified model of the airway wall that consists of a tube of folding membrane internal to, and separated from, a tube of smooth muscle. Any mechanical contribution of the adventitial layer is excluded.

A schematic diagram of a narrowed airway that contains a folded membrane but that has not yet undergone whole wall buckling is shown in Fig. 1. With pleural pressure as reference, the pressures indicated in Fig. 1 are related as follows

\[
\Delta P_{fm} = P_g - PL
\]

\[
\Delta P_m = P_g - Ppb
\]

\[
\Delta P_m - \Delta P_{fm} = Pbb - PL
\]

where \(\Delta P_{fm}\) is the pressure difference across the folding membrane, \(\Delta P_m\) is the pressure difference across the smooth muscle, \(P_g\) is the pressure in the highly deformable region between the ASM and the folding membrane (the “gap”), and Ppb is the peribronchial pressure (or, to put it another way, peribronchial radial stress). We are assuming that there is no change in Ppb across the adventitial tissue.

Elastic buckling of a tube requires an inwardly directed pressure difference (that is, for an airway, Ppb > PL). Thus the question arises as to how such a pressure difference could be generated. In a fully inflated lung, the pressure difference across the wall of an airway is directed outwards (Ppb < PL),

![Fig. 1. Schematic diagram of constricted airway showing pressures (peribronchial (Ppb), gap (Pg), lung recoil (PL)) discussed in text. Other symbols refer to morphometric parameters. WAo, cross-sectional wall area of gap; Pbm bm, perimeter of epithelial basement membrane; Am bm, area inside basement membrane; Pm, luminal perimeter; Ai, luminal area.](http://jap.physiology.org/)
resisting the tendency of the airway to narrow under the influence of the elastic forces in its wall. Our data show that, as the lung is deflated from high volumes, the Cont airways narrow less than would a circular hole in the parenchyma (21). Thus in the vicinity of such an airway, the parenchyma is stretched less than it would be in the vicinity of the circular hole, and Ppb must be greater than pleural pressure. At sufficiently small lung volumes, Ppb could be greater than Pt, thus providing the necessary condition for whole wall buckling. The buckling that occurred at Pt = ~2 cmH₂O for the smaller airways indicates that, at about this pressure, Ppb exceeded Pt and the airways were being compressed from outside. Thus at some point in the transition from the airway being held open to its being compressed, Ppb = Pt (at which point the local tension in the parenchymal tissue is zero) and (from Eq. 1)

$$\Delta P_{m} = \Delta P_{w}$$

Another way of obtaining this result is to think of the tension in the airway wall when Ppb = Pt. At this point, the airway wall must be in a state of zero net tension (by the Laplace law) because, if the net tension were greater than zero, the airway wall would remain circular and the airway would narrow as the lung deflated until the tension became zero. Thus at the Pt at which net tension is zero, the tendency of the passive stress in the ASM to narrow the airway is exactly counterbalanced by the “springiness” of the folding membrane, which tends to keep the airway open. At this Pt, the airway would still be circular.

$$\Delta P_{m}$$ (Eq. 2) can be related to the wall tension and thus wall circumferential stress ($$\sigma$$) and muscle thickness ($$T_{m}$$) through the Laplace equation (Eq. 3)

$$\Delta P_{m} = \frac{\sigma T_{m}}{r_{m}}$$

Assuming that the only significant source of tension in the airway wall is the ASM and because the ASM in these Cont airways has not been challenged, $$\sigma$$ is the passive stress in the ASM. Thus $$\sigma T_{m}$$ is the circumferential tension/unit axial length in the airway wall; $$r_{m}$$ is the radius of the muscle layer. $$T_{m}/r_{m}$$ can be evaluated from our morphometric measurements, and $$\sigma$$ can be estimated from published data for rabbit trachealis (22). Thus by calculating $$\Delta P_{m}$$ using Eq. 3, we can obtain $$\Delta P_{w}$$ from Eq. 2 for the circular airway on the verge of whole wall buckling.

Finally, the theory of membrane folding that we are using (13, 14) relates $$\Delta P_{w}$$ to the D of the folding membrane (Eq. 4)

$$\Delta P_{w} = \frac{D}{r_{m}} P_{th}$$

where $$P_{th}$$ is the theoretical, nondimensional value of the transmembrane pressure difference used in the published analysis of folding (14). Increases in $$P_{th}$$ lead to deeper folds and, when the folds encounter the geometric constraint of the muscle wall, to increases in fold number. As can be seen from Eq. 4, $$\Delta P_{w}$$ depends inversely on the cube of the radius of the membrane tube ($$r_{m}$$). This value is calculated when the membrane is circular in shape. Its value can be estimated from the morphometric data

$$r_{m} \approx \frac{P_{bm}}{2\pi}$$

Combining Eqs. 2, 4, and 5 yields Eq. 6 from which D will be evaluated

$$D = \frac{P_{bm} \Delta P_{m}}{8\pi P_{th}}$$

RESULTS

The aim in presenting the following sequence of results is to use Eq. 6 to calculate a value for D. To do this, the value of $$P_{th}$$ at which the wall first buckles must be identified. The histological evidence showed that whole wall buckling occurred between 4 and 0 cmH₂O (21). This corresponds to the steeply sloping part of the normalized $$A_{i}$$ vs. Pt curve (Fig. 2, in which $$A_{i}$$ represents luminal area in a fully dilated airway). We will assume that the small airways and the 1-mm airways commence buckling at Pt = ~2 cmH₂O, because there is a large decrease in $$A_{i}$$ between Pt = 2 and 0 cmH₂O. By using the same criterion, the 1.5-mm airways appear to buckle at ~4 cmH₂O. However, the size of the error bar shows that there is substantial heterogeneity in $$A_{i}$$ values at this value of Pt, indicating that many of these airways are already buckled. We chose not to use the data at Pt = 7 cmH₂O because the values for $$T_{m}/r_{m}$$ at this Pt and at Pt = 10 cmH₂O appear to be anomalous (Fig. 3). We have no explanation for these anomalous values. This consideration also rules out using data for the large airways. Thus we will evaluate D from Eq. 6 only for the small and 1-mm airways. The steps in the calculation are as follows.

$$P_{bm}$$ was obtained morphometrically for every airway.

We evaluated $$P_{th}$$ using the published theoretical results and our morphometric data. $$W_{A}$$ represents the cross-sectional area of the “gap”. This area, normalized on $$A_{bm}$$ (the $$A_{bm}$$ in a fully dilated airway) was denoted $$A_{sub}$$ in the theoretical paper (14). Figure 9A of that paper is a graph of $$A_{sub}/A_{bm}$$ against $$P_{th}$$ for three values of $$W_{A}/A_{bm}$$: 0.05, 0.1, and 0.15. A value of 0.05 lies within the observed range for rabbits (21). Thus from this graph, choosing $$W_{A}/A_{bm}$$ to be 0.05 and using the mean morphometric values for $$A_{bm}$$, we obtained the values for $$P_{th}$$ given in Table 3. These values are nondimensional and thus have no units.

$$P_{th}$$ is luminal area in fully dilated airway.

- small
- 1mm
- 1.5mm
- large

![Fig. 2. Normalized luminal area (A/A*) vs. P_L/cm water](http://jap.physiology.org/Downloaded_from_10.230201 March 31, 21 2017)
Fig. 3. Muscle thickness ($T_m$)-to-muscle radius ($r_m$) ratio vs. $P_l$. Values are means ± SE. Binned data, control airways, and airway size categories are given in Table 1.

$\Delta P_m$ can be calculated from Eq. 3 if $\sigma$ and $T_m/r_m$ are known. We calculated $T_m/r_m$ from the measured area of smooth muscle and the smooth muscle perimeter at the $P_l$ of interest. The results for all airways are shown in Fig. 3 for all $P_l$ values. We used the values for small and 1-mm airways at $P_l = 2$ cmH$_2$O. The rest of the data set is included to provide a context for the values that we used.

To obtain a value for the muscle $\sigma$ from the published data, we need to know muscle length ($L$). Because the muscle is the outer boundary of the combined areas of the gap and the $A_{bm}$, $L$ normalized on $P_{bm}$ is given by Eq. 7

$$\frac{L}{P_{bm}} = \frac{A_{bm}}{A_{bm} + \frac{W A_2}{A_{bm}}}$$

$L$ values are usually reported normalized on the length at which the muscle develops maximal isometric stress, $L_{max}$. We believe it is reasonable to expect that, in vivo, $L_{max}$ occurs at a reasonably high value of $P_l$. We chose to use the length at $P_l = 7$ cmH$_2$O as $L_{max}$. The exact value of $P_l$ is not very important because $L$ changes very little at high values of $P_l$. Thus $L/L_{max}$ can be estimated by taking the value of $L/P_{bm}$ at the $P_l$ of interest and dividing by the value at $P_l = 7$ cmH$_2$O (Table 3).

In a study conducted in our laboratory, the stress generated by rabbit tracheal smooth muscle as a function of $L$ was measured for the trachealis muscle (22). We assumed that those results were applicable to the ASM and, using $T_m/r_m$, the value of $\Delta P_m$ (Table 3).

$D$ can be calculated (Eq. 6) from the values thus obtained. The results are shown in Table 3. Although $D$ is the basic property of the membrane that determines its buckling and folding behavior, it is not a familiar quantity, and its significance is, therefore, difficult to assess. A better sense of its significance can be obtained from its use in evaluating the $\Delta P_m$ (Eq. 4). This equation relates theoretical, nondimensional pressure to dimensioned pressure through the conversion factor $D/r_m^2$. Values of this factor for the two airway sizes are given in Table 3. Thus a value of $P_{th}$ of 99 (the critical pressure for developing 10 folds) corresponds to ~0.5 cmH$_2$O for the 1-mm airways.

Knowledge of $D/r_m^2$ enables the calculation of dimensioned $A_t$-transmural pressure ($P_{tm}$) curves for a model airway that stays circular with reductions in $P_{tm}$ difference (Fig. 5). To obtain this curve, we used our simple model of a 1-mm airway consisting of a muscle layer separated from a folding membrane by the gap. There is neither parenchyma nor adventitia in this model. The pressure difference across this airway wall ($P_{tm}$) was calculated as follows

$$P_{tm} = \Delta P_m - \Delta P_{fm}$$

Positive values of $P_{tm}$ indicate that luminal pressure is greater than $P_{pb}$. $\Delta P_{fm}$ was calculated by taking the

Table 3. Calculation of flexural rigidity for folding membrane from data for control airways

<table>
<thead>
<tr>
<th>$P_{bm}$ Bin</th>
<th>Small</th>
<th>1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{bm}$, mm</td>
<td>0.66</td>
<td>1.0</td>
</tr>
<tr>
<td>$P_{bm}^3$, mm$^3$</td>
<td>0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>$T_m/r_m$</td>
<td>0.023</td>
<td>0.022</td>
</tr>
<tr>
<td>$A_{bm}/A_{fm}$</td>
<td>0.80</td>
<td>0.72</td>
</tr>
<tr>
<td>$P_{th}$</td>
<td>630</td>
<td>840</td>
</tr>
<tr>
<td>$L/L_{max}$</td>
<td>0.92</td>
<td>0.87</td>
</tr>
<tr>
<td>$E$, kPa</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>$\Delta P_{bm}$, Pa (from Eq. 3)</td>
<td>510</td>
<td>400</td>
</tr>
<tr>
<td>$D$, Pa·m$^{-3}$</td>
<td>$1 \times 10^{-12}$</td>
<td>$2 \times 10^{-12}$</td>
</tr>
<tr>
<td>$D/r_m^2$, Pa</td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$T_m$, muscle thickness; $r_m$, radius of muscle layer; $A_{bm}$, area inside basement membrane; $A_{fm}$, area inside basement membrane in fully dilated airway; $P_{th}$, theoretical, nondimensional value of transmembrane pressure; $L$, muscle length; $L_{max}$, length at which muscle develops maximal isometric stress; $\Delta P_m$, pressure difference across smooth muscle; $D$, flexural rigidity; $r_{tm}$, radius of membrane tube; $E$, Young modulus.

Fig. 4. Trachealis stress vs. normalized muscle length ($L/L_{max}$, where $L$ is muscle length and $L_{max}$ is length at which muscle develops maximal isometric stress) for passively generated stress (22). Continuous line is fitted cubic equation.
AIRWAY FOLDING MEMBRANE STIFFNESS

This is the first time that a value for $D$ of airway folding membrane has been reported. Our estimated values for $D$ and $D/r_{fm}^{3}$ show that physiologically significant pressure differences are required to cause folding of the airway folding membrane in peripheral airways. Thus membrane folding provides significant opposition to ASM shortening in small airways. These data and analyses prompt us to suggest that airway membrane folding acts to prevent excessive narrowing and that the airway wall thickening which occurs in disease may, by increasing $D$, be a protective mechanism.

We are not aware of reports of $D$ for any other biological membrane with which we could make comparisons. However, $D$ can be calculated from the formula for a tube (27) if the $E$ and folding membrane thickness ($T_{fm}$) are known (Eq. 8)

$$D = \frac{ET_{fm}^{3}}{12(1 - \nu^{2})}$$

where $\nu$, the third physical quantity, is the Poisson ratio of the material. Its value is not known for collagenous membranes but is usually assumed to be 0.5, the value for incompressible materials. Values of $E$ and $T_{fm}$ (~5 MPa and 100 nm, respectively) have been published for renal tubules (29, 30). These yield a value for $D$ of $6 \times 10^{-16}$ Pa·m$^{3}$, a value much less than our value for airway folding membrane. However, bigger tubes (airways) need stiffer walls to resist the same $P_{tm}$ values as smaller tubes (renal tubules). Thus a fairer comparison is between values of $D/r_{fm}^{3}$, which is ~0.6 Pa for renal tubules and thus lies between our two airway estimates.

Our estimates of the value of $D$ depend crucially on the trachealis passive stress curve that we have used. As far as we know, these are the only published data on passive stress in rabbit trachealis. When we used the dog data of Gunst and Stropp (4), our values for $D$ were approximately the same as reported in Table 3. However, use of the dog data of Okazawa and colleagues (20) resulted in values that were a factor of 10 smaller.

The muscle strip experiments that provided the data that we used for passive muscle stress were “static” in that the muscle was stretched and allowed to come to a steady value of stress. It is now well established that cycling greatly reduces the active stress that the muscle can generate (2, 3, 23, 25, 26). There appear to be no data on the effect of cycling on passive stress. In the experiments described here, the rabbits were tidally ventilated at 40 breaths/min until death. Thus the airway $L$ and stress were also cycled. However, the cycling was stopped before fixation was started. If some of what we are referring to as passive stress arises from spontaneous muscle tone, then it would be reasonable to expect that this would have been reduced by respiratory cycling, thus leading to an overestimate in our values for $D$ if the lower level of $\sigma$ persisted until fixation prevented any further changes in geometry. A curve for challenged airways similar to that shown in Fig. 5 can be calculated. However, its accuracy depends crucially on the value for the maximal stress of the ASM. Because the $L$ and force were being cycled by tidal breathing, we cannot use the published value of maximal stress and have no reasonable way of estimating the reduction in this value caused by the cycling. Therefore, we chose not to present such a curve.

We cannot reliably evaluate $E$ for airway folding membrane from our results because we could not obtain a value for $T_{fm}$. However, if we assume that the membrane has the same value of $E$ as renal tubules, then, using Eqs. 4 and 8, we estimate the value of $T_{fm}/r_{fm}$ to be ~0.01 in the small and 1-mm airways. This is about the same value reported for renal basement membrane (30). Thus it appears possible that the folding membrane has a similar value of $E$ as that of basement membrane in renal tubules.

A value for $E$ on the order of 5 MPa is in poor agreement with data obtained from mucosal strips taken from tracheas of both sheep (1) and rabbits (28), which were in the range of 3–24 kPa. A value for $E$ of 24 kPa would require $T_{fm}/r_{fm}$ to be ~0.06, which is twice the maximum possible value based on the observed values of $WA/A_{bm}$ in these airways. The difficulty with the mucosal strip data appears to lie in...
identifying the load-bearing tissue and assessing its cross-sectional area to convert the measured tension to stress. It would appear either that both mucosal strip experiments greatly overestimated the amount of tissue that is significant in membrane folding or that the properties of tracheal mucosal tissue are very different from those of folding membrane in membranous bronchioles. Our estimates for the value of $D$ depend on knowing the critical buckling pressure of the airway wall. Because we did not design the experiment to evaluate this quantity, we can make only an educated guess as to its value. However, most of the parameters that contribute to the calculation of $D$ are insensitive to the exact value of $P_\infty$ at which they are evaluated. We estimated $P_{th}$ using $W_{A_d}/A_{bm}$ and $A_{bm}/A_{bm}$ because there is a published relationship between these quantities. We could also have used the number of folds in conjunction with either of these normalized areas. Doing so yields values of $D$ that are up to 10 times larger than those reported here. One of the reasons is that the mean values of these morphometric quantities taken together do not yield a point on the surface predicted by theory [Fig. 3A in the theoretical paper (14)]. It would be unreasonable to expect that the simple averages that we took should yield such a point because the relationship between the quantities is nonlinear and it is not known how to average the data in an appropriate fashion. In addition, the theory is highly idealized in its representation of the material in the gap as being incapable of sustaining shear forces. The effect of fixation shrinkage on the results should be minimal because an attempt was made to correct for this before data analysis took place.

The folding membrane analysis ignored the epithelium. Whereas the presence of epithelium is not expected to have any significant effect on the initiation of elastic collapse, it could have a significant effect on area reduction at large transmembrane pressure differences because it would prevent the close juxtaposition of opposite walls of folding membrane. We believe that the theoretical results are valid at the transmembrane pressures and small fold numbers that existed under the conditions analyzed here.

In summary, we have used the observation of elastic buckling of the entire airway wall to open a window on the stiffness of one of the components of that wall: the folding (epithelial) membrane. We have deduced that the $D$ of the folding membrane in small membranous bronchioles from rabbits is of the order of $10^{-12}$ Pa·m$^2$. This value leads to the conclusion that folding of the epithelial membrane provides a significant load for ASM in small membranous bronchioles, especially at small values of $P_\infty$. Unloaded ASM is capable of shortening to 20% of its starting length, a magnitude of shortening that would result in complete airway closure. These data lead us to suggest that at least one of the reasons that airways do not easily close when the smooth muscle is stimulated is the load provided by the folding of the mucosal membrane. To the extent that the thickening of this membrane, which occurs in diseases such as asthma and COPD, is associated with a preservation of the mechanical properties of the materials that make up the wall, thickening will increase the $D$ and serve as a protective mechanism against excessive airway narrowing.

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