Hysteresivity of the lung and tissue strip in the normal rat: effects of heterogeneities

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Hysteresivity of the lung and tissue strip in the normal rat: effects of heterogeneities. J Appl Physiol 91: 737–747, 2001.—We measured lung impedance in rats in closed chest (CC), open chest (OC), and isolated lungs (IL) at four transpulmonary pressures with a optimal ventilator waveform. Data were analyzed with an homogeneous linear or an inhomogeneous linear model. Both models include tissue damping and elastance and airway inerance. The homogeneous linear model includes airway resistance (Raw), whereas the inhomogeneous linear model has a continuous distribution of Raw characterized by the mean Raw and the standard deviation of Raw (SDR). Lung mechanics were compared with tissue strip mechanics at frequencies and operating stresses comparable to those during lung impedance measurements. The hysteresivity (η) was calculated as tissue damping/elastance. We found that 1) airway and tissue parameters were different in the IL than in the CC and OC conditions; 2) SDR was lowest in the IL; and 3) η in IL at low transpulmonary pressure was similar to η in the tissue strip. We conclude that η is primarily determined by lung connective tissue, and its elevated estimates from impedance data in the CC and OC conditions are a consequence of compartment-like heterogeneity being greater in CC and OC conditions than in the IL.

Hysteresivity (η), introduced by Fredberg and Stamenesic (12), is a material property of the tissue and is defined as the energy dissipated relative to the elastic energy stored in the tissue in a cycle. As such, η should only depend on the material composition of the tissue. The value of η for lung, which is usually obtained in isolated-lung (IL) or open-chest (OC) conditions, is between 0.12 and 0.25 (14–17, 27, 36). In contrast, the values of η for tissue strip range from 0.05 to 0.1 (10, 12, 22, 29, 30, 32, 39). Among the possible reasons for the relatively wide range of η in the lung are 1) configurational differences between OC preparations and ILs; 2) small- vs. large-amplitude oscillations; and 3) lung volume history. The variability of η in the tissue strip may originate from 1) differences in prestress; 2) strain amplitude dependence; and 3) variations in strain history. Despite the relatively large variability of the lung and tissue strip η, the ranges reported do not overlap. This discrepancy between lung and tissue strip η values may be attributed to 1) the lack of surface tension in the tissue strip; 2) differences in mechanical behavior due to three-dimensional uniform stretching of the lung vs. uniaxial stretching of the tissue strip; and 3) heterogeneity of the lung and the respiratory system contributing to η extracted from whole lung measurements but not to η of the tissue strip.

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The purpose of this study was to identify mechanisms that are likely to play a role in the different $\eta$ values obtained from lung and tissue strip measurements. We hypothesized that some form of heterogeneity in the intact lung in the OC condition and in the respiratory system exists, even under normal conditions. Thus we estimated airway properties and $\eta$ from total respiratory impedance and lung impedance in the OC condition and in ILs at several lung volumes. The $\eta$, as a material property of lung tissue, was then directly estimated from tissue strip preparations.

**METHODS**

**Animal preparation.** The study was performed in seven male Sprague-Dawley rats (Charles River Laboratories, Boston, MA) weighing between 280 and 350 g. The protocol was approved by the Boston University and Harvard Medical School Animal Care and Use Committees. Each rat was anesthetized with an intraperitoneal injection of 10–20 mg/kg of xylazine and 30–35 mg/kg of ketamine. Then a dose of 5–10 mg/kg pentobarbital sodium was administered hourly to maintain anesthesia. The rats were tracheotomized, and a tracheal cannula (2-mm ID) was connected to the outlet of a computer-controlled, small-animal ventilator (Flexivent, SCIREEQ).

**Measurement protocol.** Rats were mechanically ventilated with a $V_T$ of 10 ml/kg at a frequency of 90 breaths/min. Ventilation was superimposed on a transpulmonary pressure (Ptp) of 0.25–0.3 kPa, which was adjusted by inserting the expiratory port of the mechanical ventilator into water. Impedance measurements were first made in the closed-chest (CC) condition performed at four different Ptp levels (0.3, 0.5, 0.8, and 1.1 kPa). To standardize volume history, each measurement was preceded by two consecutive inflations of the lungs to total lung capacity, defined as a tracheal pressure of 3 kPa. The target Ptp was set only before the measurement. The first Ptp level was the in situ Ptp. Next, the impedance measurements were repeated in the OC and finally in the IL conditions at the same four Ptp levels. After collection of the CC data at the four Ptp levels, a partial thoracotomy was performed by cutting the diaphragm to produce a bilateral pneumothorax. Before opening the chest, we connected the lateral port of the tracheal cannula to another pressure transducer. The ventilator was stopped at functional residual capacity, and the tracheal cannula was blocked. Once the chest was opened, the airway opening pressure jumped up to a value that was taken as the mean in situ Ptp in the CC animal. The impedance measurements were repeated in the OC condition at four levels of Ptp.

After the measurements in the OC condition, the lung was isolated. When the animal’s heart stops, circulation in the lung also stops, and blood in the vessels starts to coagulate. It is likely that the coagulation would make the tissue stiffer and hence affect lung impedance measurements. Thus blood clots would start gathering in the lower parts of the lung, and, as a result, it may induce artificial heterogeneity in the lung. To avoid this problem, the animals were exsanguinated by cutting the portal vein and abdominal aorta, and saline with heparin (1,000 IU/l) was immediately administered to the pulmonary artery under 20- to 30-mmHg pressure for 5 min. This procedure washed the blood out of the lung. The lung was then isolated from the thoracic cavity. During the isolation, dry oxygen was used to ventilate the lungs, because this procedure avoids formation of edematous regions in the lung. Impedance data were collected again at four levels of Ptp using the same procedure as in the OC measurements while the lung was floating in a saline bath.

The Ptp values in the OC and IL conditions were set as follows. The first Ptp was taken as the estimated in situ Ptp as described above. The value of this pressure was between 0.2 and 0.3 kPa in all animals, which, for simplicity, was taken as 0.3 kPa. The second, third, and fourth Ptp levels were simply obtained by adding 0.2, 0.5, and 0.8 kPa, respectively, to the first Ptp. The reason for this setting was to ensure that the distending pressure of the lung was the same during impedance measurements in the CC, OC, and IL conditions. The order of the four different Ptp levels was randomized among rats. The impedance data were then presented in terms of Ptp corresponding to the four different Ptp levels.

**Impedance measurements.** Impedance data collection was made by interrupting mechanical ventilation for 16 s using an optimal ventilation waveform (OVW), which is a broadband waveform containing energy from 0.5 to 15 Hz (24). The frequencies in the OVW are selected according to a nonsum of differences (NSND) criterion, which eliminates harmonic distortion and minimizes cross talk among the frequencies that are present in the input flow waveform and hence provides smooth estimates of the input impedance of the system (35). The OVW method can provide information on the mechanical properties of the lung during conditions mimicking breathing. The OVW technique was developed exactly for this purpose, and hence the volumes delivered are similar to normal spontaneous $V_T$ values but in a closed-circuit, forced-oscillatory system. In our experiment, we matched the OVW amplitude to the $V_T$ delivered by the mechanical ventilator. The corresponding pressure oscillations were also similar to Ptp values (0.8–1.0 kPa) during spontaneous breathing. The ventilator displacement and cylinder pressure signals were low-pass filtered at 30 Hz and sampled at 256 Hz before they were stored. With the use of Fourier analysis, impedance spectra were calculated on overlapping blocks of pressure and flow data as the ratio of the cross-power spectrum of pressure and flow and the autopower spectrum of flow (28). The forced-oscillatory system was calibrated by measuring the input impedance of known analogs including tubes and bottles. The frequency response of the system was obtained, and the measured impedance spectra was off-line corrected for any phase difference between pressure and estimated flow.

**Tissue strip measurement.** Lung parenchymal strips were obtained after the completion of the lung impedance measurements. The experimental setup was described in detail by Yuan et al. (39). Briefly, after the lung was degassed in a suction chamber, tissue strips 4.5 × 4.5 × 10 mm in size were prepared with the use of a ruler and a razor. Each end of the tissue strip was fixed by cyanoacrylate glue to small metal clips attached to straight steel wires. The assembly was placed in a vertical glass tissue bath (Wilbur Scientific, Boston, MA) filled with saline, with the upper wire attached to a force transducer (model 400A, Cambridge Technologies) and the lower wire to a computer-controlled lever arm (model 300H, Cambridge Technologies) of a displacement generator. The servo-controlled lever arm was driven by a displacement signal generated by a computer. The signal was sent out from the digital-to-analog port of a data-acquisition board (DT2812, Data Translation) and then smoothed with a low-pass filter (8-pole, R858L8EX, Frequency Devices, Haverhill, MA) with a cutoff frequency of 15 Hz. Both displacement and force signals were low-pass filtered at 15 Hz and then sampled at 60 Hz. Linearity and hysteresis of the measurement system were tested with a steel spring of known stiffness (0.2
N/cm²). The spring was attached to the apparatus, and the measured spring stiffness showed neither frequency nor amplitude dependence, and the hysteresis of the stress-strain loop was negligible.

The experiments were performed at room temperature (24°C). The force-length relationship of the strips was displayed on an oscilloscope during sinusoidal oscillations, and the hysteresis area between force and displacement was minimized by adjusting the horizontal and vertical positions of the device. The strips had an initial length of 10 mm in the stress-free condition. The strips were preconditioned by performing a single slow stretch to a stress of ~2 kPa. The sample was then stretched to a given prestress (operating stress), and the corresponding length of the sample was measured in the organ bath. The dynamic properties of the strips (see below) were measured around this operating stress level using a peak-to-peak strain amplitude of 8% of the actual length of the tissue strip at that operating stress. A similar preconditioning procedure was applied again, and the measurements were repeated at around one of the four mean operating stresses (0.35, 0.5, 0.65, and 0.95 kPa). These stresses likely surround the distending stress of the lung in situ. The order of the stress levels was randomized in the different tissue samples. Instead of the traditional sinusoidal oscillation approach, we used a broad-band pseudorandom displacement input signal. The signal was the sum of seven sinusoids chosen according to the NSND frequency composition (35). The signal had a flat power spectrum and random phases and was used as the displacement input signal. The length of the NSND sequence was 4,096 points, so that using a sampling rate of 60 Hz corresponded to a time period of 68 s. The dynamic moduli of the strips defined as the ratio of the cross-power spectrum of stress and strain and the auto-power spectrum of strain was calculated on overlapping blocks of data using Fourier analysis.

**Modeling.** Data were analyzed in terms of two models: a homogeneous linear (HL) model and an inhomogeneous linear (IHL) model. The HL model was introduced by Hantos et al. (17), whereas the IHL model was developed by Suki et al. (37). Briefly, for the HL model, the airway tree was modeled by a linear airway compartment and a linear viscoelastic (37). Briefly, for the HL model, the airway tree was modeled by a set of airway pathways arranged in parallel. The resistance in each pathway is connected in series, with Iaw and the linear viscoelastic tissue impedance given by

\[
Z_{\text{Iaw}} = \frac{Z}{\text{R}_{\text{a}} + \frac{Z}{\text{R}_{\text{b}}}}
\]

where \(\text{Z}_{\text{Iaw}}\) is the linear impedance of the tissues (Zlti) is given by

\[
\frac{\text{Z}_{\text{Iaw}}}{\text{Z}_{\text{Iaw}}} = \frac{\text{G}}{\text{H}} - \frac{\text{jH}}{\text{G}}\alpha
\]

where \(\text{G}\) and \(\text{H}\) are the coefficients of tissue damping and elastance, respectively. The exponent \(\alpha\) governs the degree of the frequency dependence of \(\text{R}_{\text{t}}\) (\(\text{G} = \text{G}(\omega)\)) and \(\text{E}_{\text{t}}\) (\(\text{E}_{\text{t}} = \text{H}(\omega)^{-1}\)). This model is called the constant-phase model because the phase of \(\text{Z}_{\text{Iaw}}\) is independent of frequency (17). The \(\eta\) coefficient (12) is \(\text{G}/\text{H}\) in this model representation. The combination of Eqs. 1 and 2 provides a four-parameter model (Raw, Iaw, G, H) to fit lung impedance data.

In the IHL model, we represented the airway tree by a set of airway pathways arranged in parallel. The resistance in each pathway is connected in series, with Iaw and the linear viscoelastic tissue impedance given by Eq. 2. These pathways are then combined in parallel. Suki et al. (37) used a variation in Raw from path to path, implemented in a probabilistic manner. If one distributes the pathway resistances according to a distribution function \(n(\text{R})\), one can calculate the total admittance of the system, which is the summation of the conductances of the individual branches. Suki et al. suggested the following form for the resistance distribution

\[
n(\text{R}) = \left\{ \begin{array}{ll}
\frac{K}{\text{R}^2}, & \text{if } \alpha \leq \text{R} \leq \text{R}_{b} \\
0, & \text{otherwise}
\end{array} \right.
\]

where \(\mu\) is a shape parameter of \(n(\text{R})\) and the parameters \(\alpha\) and \(\text{R}_{b}\) define the limits of the distribution. A simple solution is obtained when \(\mu\) takes integer values. When \(\mu = 1\), \(n(\text{R})\) decreases hyperbolically with resistance, and the corresponding impedance of the model is given by (37)

\[
Z_{\text{Iaw}} = \frac{Z}{\ln \left( \frac{\text{R}_{b} \text{R}_{a} + Z}{\text{R}_{a} \text{R}_{b} + Z} \right)}
\]

where

\[
K = \frac{1}{\ln \text{R}_{b} - \ln \text{R}_{a}}
\]

The Raw of the model can be obtained by calculating the expected value of the random variable \(\text{R}\)

\[
\text{Raw} = \int_{\text{R}_{a}}^{\text{R}_{b}} \text{R} n(\text{R}) \text{dR} = \text{K}(\text{R}_{b} - \text{R}_{a})
\]

The heterogeneity in the model can be characterized by the standard deviation of Raw (SDR)

\[
\text{SDR} = \sqrt{\int_{\text{R}_{a}}^{\text{R}_{b}} (\text{R} - \text{Raw})^2 n(\text{R}) \text{dR}}
\]

Inserting Eqs. 3 and 6 into Eq. 7, we obtain

\[
\text{SDR} = \sqrt{\text{K}(\text{R}_{b}^2 - \text{R}_{a}^2)/2 - \text{K}^2(\text{R}_{b} - \text{R}_{a})^2}
\]

The HL and IHL models were fit to measured data using a global optimization method (9), which minimizes the root-mean-square error (RMSE) between data and model.

**Statistical analysis.** The statistical differences among model parameter at different Ptp levels and conditions were tested using ANOVA (Fisher paired least-significant difference test). If significant differences were found among the groups, a paired t-test (Wilcoxon signed-rank statistics) was also applied. \(P < 0.05\) shows statistical difference. StatView 4.0 (Abacus Concepts, Berkeley, CA) software was used for all statistical analyses.

**RESULTS**

Figure 1 shows a representative case of the real and imaginary parts of lung impedance and the fits of the HL and IHL models in the CC, OC, and IL conditions in the same rat. The imaginary parts are generally equally well fit by both models. However, in case of the real parts, the IHL model provides visually better fits than the HL model, especially in the CC and OC conditions. Comparison of the RMSE values of the IHL
and HL models shows a lower fitting error with the IHL than with the HL model in all conditions (CC, OC, and IL) and nearly all Ptp levels (Fig. 2). The RMSE values of the IHL model are statistically significantly lower than those of the HL model at nearly all Ptp levels and in all three conditions (Fig. 2). In short, the improvement in error by the IHL model in Fig. 2 indicates the existence of significant heterogeneities in the normal rat lung.

Figures 3, 4, and 5 show the parameters G, H, \(\eta = G/H\), and Raw in the CC, OC, and IL conditions, respectively, using both models. The G and \(\eta\) from the HL model were almost always statistically significantly higher than those from the IHL model. The H showed little difference between the IHL and the HL models. The G and H first decreased and then increased with Ptp in the CC conditions, whereas they both increased in the OC and IL conditions. The \(\eta\) was nearly independent of Ptp.

The SDR from the IHL model was the lowest in the IL (Fig. 6), suggesting that heterogeneity in the CC and OC conditions is higher than in IL. Also, heterogeneity appeared to be the smallest at Ptp levels of 0.3 and 0.5 kPa and increased with Ptp, except in the IL condition.

Figure 7 shows the tissue strip parameters during pseudorandom oscillations at frequencies and operating stresses similar to those occurring during lung impedance measurements. The values of H tended to increase with stress, and the value of tissue strip \(\eta\) was between 0.04 and 0.11 at all stress levels. Table 1 compares the lung parameters and their Ptp dependence for the CC, OC, and IL conditions with the stress dependence of the tissue strip parameters.

Figure 8 compares the Ptp dependence of the lung parameters from the IHL model in the three different conditions with the corresponding dependence of the tissue strip parameters on the operating stress level. The values of H tended to increase with Ptp except in the CC condition. The values of \(\eta\) in the CC and OC conditions were higher than in the IL condition. The values of tissue strip \(\eta\) at low stresses were similar to those in the IL condition at low Ptp levels. The discrepancy between lung and tissue strip \(\eta\) was the smallest for the IL condition at low Ptp levels. The Raw was smallest in the IL condition, especially at Ptp levels of 0.5, 0.8, and 1.1 kPa. However, there were no statistically significant differences in the Raw at different Ptp levels.

**DISCUSSION**

Contradicting values of lung tissue \(\eta\) have been reported in the literature for \(\eta\) extracted from lung impedance measurements (14–17, 27, 36) and from tissue strip measurements (10, 12, 22, 29, 30, 32, 39). The primary aim of this study was to study the mechanical properties of the airways and tissues in the normal lung and to uncover the underlying mechanisms responsible for the differences in \(\eta\) of the lung and lung tissue strips. Among the possible reasons are the lack of surfactant in the tissue strip, three-dimen-
rtional uniform stretching of the lung vs. uniaxial stretching of the tissue strip, and heterogeneities due to airways and/or configurational differences among IL, OC, and CC preparations. To identify which of these mechanisms are likely to play an important role in this controversy, we measured total respiratory impedance and lung impedance in OC and IL conditions as a function of Ptp. The \( \eta \) was estimated using a detailed analysis of the mechanical impedance spectra and compared with that obtained in the tissue strip from the same rat. Model analysis indicated that 1) heterogeneities existed in the CC and OC lung and, to a smaller extent, in the IL; 2) the \( \eta \) in the CC and OC conditions was higher than that in the IL; and 3) the \( \eta \) of IL at low Ptp values was similar to that of the tissue strip at low distending stresses.

**Heterogeneity in the respiratory system.** In an attempt to understand these results, we first compare mechanics in the CC, OC, and IL conditions. A surprising finding of this study is that the IHL model fits the data significantly better than the HL model under all conditions. Owing to the increased complexity of the IHL model compared with the HL model, the improvement in fit by the IHL model appears to be guaranteed because the number of parameters in the IHL model is five, whereas in the HL model it is only four. Thus one needs to critically examine whether the added complexity of the IHL model was necessary. The amount of improvement by the IHL model over the HL model was large (20–60%) and statistically significant in the CC and OC conditions at all Ptp levels (Fig. 2). However, the improvement in error was less important (10–30%) in the IL condition, reaching statistical significance only at two of the four Ptp levels (Fig. 2). The improvements between population averages are in accord with the fact that the improvements with the IHL model were statistically significant on an individual basis in 40, 30, and only 10% of the cases for the CC, OC, and IL conditions, respectively. In these comparisons, we used the \( F \) test, which takes into account the fact that the two models have different numbers of parameters. These findings imply the presence of significant heterogeneities in the CC and OC conditions but not in the IL. The corresponding mechanical parameters obtained from the two models were also different. For example, \( G \) was higher and \( R_{aw} \) was lower in the HL model than in the IHL model in most conditions and Ptp levels (Figs. 3–5). Because the IHL model includes some form of structural heterogeneity, the estimation of \( G \) should be more reliable from the IHL than from the HL model. Also, from the IHL model analysis arises a quantitative index of heterogeneity via the estimated SDR, rather than just a single \( R_{aw} \) from the HL model. The values of SDR were the lowest in the IL condition (Fig. 6). Hence our findings suggest that \( G \) is overestimated and \( R_{aw} \) is underestimated by the HL model compared with the IHL model. Compared with the IHL model, this overestimation of \( G \) was substantial in the CC and OC conditions at all Ptp levels. In short, the HL model analysis may lead to biased estimates of tissue properties, especially those of \( G \).

Previous experimental evidence has strongly suggested that inhomogeneous airway constriction in response to bronchoconstrictor stimulus is the dominant alteration in the rat lung (26). By using a mixture of neon and oxygen (NeOx), Lutchen et al. (26) reported...
that inhomogeneities significantly contribute to an overestimation of G during lung constriction. However, in contrast to our findings, they also reported the lack of heterogeneities under baseline conditions. There are several important methodological differences between the two studies. First, in the Lutchen et al. study, the thoracotomy was a wide midsternal opening, whereas, in this study, we cut an opening on the diaphragm. Thus, in the former study, most of the lung surface was exposed to atmosphere, and hence the measuring condition may have been more similar to our IL condition where heterogeneities were not judged important. To test this, in two rats impedance was also measured after a full thoracotomy. The impedance spectra and parameters were more similar to our OC condition than to the IL condition. Second, Lutchen et al. measured transfer impedance using body surface oscillations, whereas we measured input impedance. Although Hantos et al. (15) found virtually no difference between input and transfer impedances of the rat up to

Fig. 3. The parameters damping (G; A), elastance (H; B), airway resistance (Raw; C), and hysteresivity (η; D) at different Ptp levels in the CC condition. Values are means ± SD. ▲, IHL model parameters; ●, HL model parameters. Significant difference, *P < 0.05 (Mann-Whitney’s U-test).

Fig. 4. The parameters G (A), H (B), Raw (C), and η (D) at different Ptp levels in the OC condition. Values are means ± SD. ▲, IHL model parameters; ●, HL model parameters. Significant difference, *P < 0.05 (Mann-Whitney’s U-test).
10 Hz, they used small-amplitude oscillations. Thus it is likely that, during the OVW measurements, which apply normal tidal-breathing amplitudes and rates, input and transfer impedances may be different due to tissue nonlinearities and due, potentially, to compartmental behavior. In this respect, the body surface oscillations mimic the natural breathing conditions more than forcing at the airway opening and hence appear to be a more homogeneous condition, in agreement with Lutchen et al. (26).

Our results indicate that the CC and OC conditions are more heterogeneous than the IL condition. This is evident from the fact that the estimated SDR was statistically significantly lower by at least 50% in the IL than in the CC and OC conditions at three of the four Ptp levels (Fig. 6), which resulted in lower G, \( \eta \), and Raw values in the IL than in the CC and OC conditions. There are several mechanisms that could potentially contribute to the estimated values of SDR. The most important factor is perhaps the heterogeneity of the airway tree itself. In the normal lung, this would be due to the asymmetric branching structure of the airways. This inherent heterogeneity is reflected in the SDR that we obtained in the IL condition. In the OC and CC conditions, the presence of the chest wall and the abdominal pathways likely increases the heterogeneity and SDR. Additionally, the chest wall is known to have a Newtonian resistance (3). Despite the contribution of the chest wall to the data in the CC condition, the Raw and \( \eta \) were very similar in the CC and OC conditions, suggesting that the OC condition may still be influenced by the presence of the chest wall. Additionally, the coefficient of variation of resistance, \( CV = SDR/Raw \), from the IHL model would also be influenced by the presence of chest wall resistance. The chest wall resistance shifts the Ra and Rb as obtained from the model fitting. The corresponding Raw will also be shifted by the same amount, but SDR is invariant to a constant shift. Thus the effect of the Newtonian chest wall resistance is to decrease CV in the CC and OC conditions. Despite this effect, the CV was the smallest in the IL condition.

We also note that, even with the IHL model, we were unable to recover \( \eta \) values from the CC and OC conditions similar to those in the tissue strip measurements (Fig. 8). In contrast, \( \eta \) values in the IL were nearly identical to those in the tissue strips (Fig. 8). This suggests that the type of heterogeneity built into the IHL model may be appropriate to describe the heterogeneity of the airway tree in the IL condition, but it
may not be appropriate to account for the compartmental heterogeneity in the respiratory system. In other words, the IHL model assumes a parallel set of resistors leading to identical tissue impedances. The respiratory system in the CC condition, on the other hand, may behave more like a compartmental system where the compartments correspond to the chest wall, rib cage, and abdominal pathways with different resistances as well as different compliant elements. Thus the significant amount of heterogeneity in the CC and OC conditions may be due to compartmental-like behavior of the respiratory system. In the OC measurement, the lung was not completely exposed to atmospheric pressure, and hence a similar compartmental behavior may have been sustained as in the CC condition.

Fredberg and Stamenovic (12) summarized measured in lungs and tissue strips under baseline conditions and in various species (15). The values obtained from lungs of different species were compared by reanalyzing the pressure-volume loops of Bachofen and Hildebrandt (1, 18). These values ranged from 0.12 to 0.3 in normal condition (12). Whereas these values are from different species, they are similar to our CC and OC results from rat lung impedance data. The from dynamic measurements on tissue strips ranged from 0.05 to 0.1 (10, 12, 22, 29, 30, 32, 39), similar to the data obtained here. Thus, whereas our results are similar to several earlier data in the literature, they also allow us to address the question of the discrepancy between the values of from whole lung and tissue strip measurements.

First, the from the CC and OC conditions is higher than that from the IL and tissue strip. Hantos et al. (16) reported that for the CC condition was higher than that for the OC condition in the cat. Hirai et al. (19) also reported that depended slightly on lung volume in CC and OC conditions. Both are similar to our present results. Interestingly, however, the of the tissue strip at low distending stresses and was from the IL condition at low Ptp levels are almost the same. This implies that, after accounting for heterogeneities by using the IHL model, the mechanism of dissipation as inferred from the IL condition is similar to that determining of the tissue strip. Because the tissue strip measurements were done in a water-filled organ bath without the influence of the surface tension of the air-liquid interface, surfactant appears to contribute very little to . Indeed, surface tension is known to contribute little to at low Vt values, but it is significant at higher Vt values (34). Additionally, the tissue strip measurements were done during uniaxial stretching, whereas the IL condition represents three-dimensional isotropic expansion of the lung. Thus surface film properties and structural differences between IL and tissue strip, including three-dimensional geometry and boundary conditions, have little contribution to during tidal-like oscillations at physiological Ptp values and at frequencies surrounding the normal breathing rate. This is important because it allows us to study the mechanisms contributing to the value of of the lung without the confounding influence of heterogeneities in the tissue strip. Accordingly, our data suggest that, because the tissue strip properties are only moderately affected by cell contractility (39), from the IL condition primarily reflects the dynamic properties of the connective tissues.

Fig. 7. Lung tissue strip mechanical parameters: tissue G (Gtis; A), tissue H (Htis; B), and of mean operating stress. Values are means ± SD.

Ptp and stress dependence of lung parameters. Previous studies that used the alveolar capsule technique have shown that the Newtonian resistance of the lung tissue is negligible (19, 22, 27), implying that the values of Raw in rats that we found in the present study can be ascribed to the airways. If we use the HL model,
Table 1. Mechanical properties of the respiratory system, the lung, and tissue strip analyzed by the inhomogeneous linear model at different transpulmonary pressure levels and conditions and different operating stresses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>CC</th>
<th>OC</th>
<th>IL</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw, kPa·s·ml⁻¹</td>
<td>0.3</td>
<td>0.12 ± 0.04</td>
<td>0.12 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.35</td>
</tr>
<tr>
<td>Ra, kPa/ml</td>
<td>0.35</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.0020 ± 0.00063</td>
</tr>
<tr>
<td>Rb, kPa/ml</td>
<td>0.32</td>
<td>0.29 ± 0.07</td>
<td>0.18 ± 0.08</td>
<td>0.0223 ± 0.0088</td>
<td></td>
</tr>
<tr>
<td>G, kPa/ml or kPa</td>
<td>0.25</td>
<td>0.21 ± 0.05</td>
<td>0.14 ± 0.05</td>
<td>0.092 ± 0.013</td>
<td></td>
</tr>
<tr>
<td>H, kPa/ml or kPa</td>
<td>2.35</td>
<td>1.43 ± 0.28</td>
<td>1.38 ± 0.32</td>
<td>0.62 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Hysteresivity</td>
<td>0.15</td>
<td>0.15 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.052 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Raw, kPa·s·ml⁻¹</td>
<td>0.5</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.08 ± 0.04</td>
<td>0.65</td>
</tr>
<tr>
<td>Ra, kPa/ml</td>
<td>0.30</td>
<td>0.26 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.0021 ± 0.00085</td>
<td></td>
</tr>
<tr>
<td>Rb, kPa/ml</td>
<td>0.27</td>
<td>0.26 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.0023 ± 0.0003</td>
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<tr>
<td>G, kPa/ml or kPa</td>
<td>0.30</td>
<td>0.24 ± 0.05</td>
<td>0.11 ± 0.01</td>
<td>0.027 ± 0.003</td>
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</tr>
<tr>
<td>H, kPa/ml or kPa</td>
<td>1.62</td>
<td>1.44 ± 0.44</td>
<td>1.33 ± 0.17</td>
<td>0.09 ± 0.01</td>
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<tr>
<td>Hysteresivity</td>
<td>0.18</td>
<td>0.17 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.04 ± 0.003</td>
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<tr>
<td>Raw, kPa·s·ml⁻¹</td>
<td>0.8</td>
<td>0.13 ± 0.05</td>
<td>0.12 ± 0.03</td>
<td>0.06 ± 0.03</td>
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<tr>
<td>Ra, kPa/ml</td>
<td>0.02</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.09 ± 0.01</td>
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</tr>
<tr>
<td>Rb, kPa/ml</td>
<td>0.38</td>
<td>0.36 ± 0.09</td>
<td>0.19 ± 0.06</td>
<td>0.0023 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>G, kPa/ml or kPa</td>
<td>0.23</td>
<td>0.28 ± 0.12</td>
<td>0.20 ± 0.05</td>
<td>0.027 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>H, kPa/ml or kPa</td>
<td>1.36</td>
<td>2.31 ± 1.32</td>
<td>3.16 ± 1.05</td>
<td>0.09 ± 0.01</td>
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</tr>
<tr>
<td>Hysteresivity</td>
<td>0.17</td>
<td>0.15 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.003</td>
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<tr>
<td>Raw, kPa·s·ml⁻¹</td>
<td>1.1</td>
<td>0.14 ± 0.05</td>
<td>0.12 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Ra, kPa/ml</td>
<td>0.02</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Rb, kPa/ml</td>
<td>0.46</td>
<td>0.39 ± 0.06</td>
<td>0.19 ± 0.07</td>
<td>0.0023 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>G, kPa/ml or kPa</td>
<td>0.23</td>
<td>0.39 ± 0.12</td>
<td>0.31 ± 0.14</td>
<td>0.04 ± 0.004</td>
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</tr>
<tr>
<td>H, kPa/ml or kPa</td>
<td>1.98</td>
<td>3.78 ± 0.70</td>
<td>4.95 ± 2.19</td>
<td>0.03 ± 0.004</td>
<td></td>
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<tr>
<td>Hysteresivity</td>
<td>0.13</td>
<td>0.10 ± 0.02</td>
<td>0.06 ± 0.003</td>
<td>0.09 ± 0.005</td>
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Values are means ± SD. CC, closed chest; OC, open chest; IL, isolated lung; Ptp, transpulmonary pressure; Raw, airway resistance; Ra and Rb, parameters; G, damping; H, elastance.
the interpretation of our finding is that Raw slightly decreases with increasing lung volume, as has been previously reported in humans (31) and rats (19, 22). This would be expected given that the airways increase in caliber with lung volume. Our IHL model results suggest that Raw first decreases slightly at low Ptp values and then starts increasing at high Ptp levels except for the IL condition. The trends seen in Figs. 3 and 4 are similar to that reported by Hantos et al. (16). However, there was no statistical difference in Raw between Ptp levels. The increase in Raw with Ptp may reflect the contribution of the chest wall and an increased heterogeneity due to the compartmental behavior of the respiratory system at higher Ptp values. This explanation is plausible, because, in the IL, Raw decreases steadily with increasing Ptp. Another fact supporting this argument is that the SDR appeared to increase with Ptp in the CC and OC conditions but not in the IL condition (Fig. 6).

The Ptp dependence of the respiratory and lung mechanical parameters G and H shows different patterns in the CC condition than in the OC and IL conditions. For example, G decreases statistically significantly with Ptp in the CC condition (P < 0.05), whereas G steadily increases in the OC (P < 0.05), and it first decreases at Ptp = 0.5 kPa and then increases for higher Ptp values in the IL condition (P < 0.05). The increasing behavior of G and H with Ptp is not likely related to compartmental heterogeneity because these parameters also increase in the tissue strip and in the IL condition, and hence it is a consequence of the stiffening behavior of the lung tissue itself at higher Ptp levels. Thus the decrease in G and H in the CC condition up to Ptp = 0.8 kPa must be related to the contribution of the chest wall tissues, including rib cage and diaphragm, and potentially the compartmental behavior of the respiratory system in the CC condition. At Ptp = 1.1 kPa, H also increases in the CC condition, most likely due to the stiffening of the lung and chest wall.

The peculiar Ptp dependence of G and H resulted in a nonmonotonic Ptp dependence of η; first showing an increase followed by a decrease with increasing Ptp in the CC and OC conditions. It is important to note that the IHL model predicted 20–30% lower η than the HL model for all Ptp levels. The η in the IL condition showed a slight but monotonic decrease with Ptp. Because the heterogeneity was the smallest in the IL condition, the Ptp dependence of η should be most relevant to the real Ptp dependence of η of the lung tissue. On the other hand, the heterogeneity increased with Ptp in the CC and OC conditions as suggested by the increase in SDR with Ptp (Fig. 6). Thus the apparent peak in η appears to be a result of increasing heterogeneity in the compartment-like behavior of the respiratory system in the CC and OC conditions. The peak in η is not seen in the IL condition and in the tissue strip. Hence η reflects more the inherent material properties of the tissues. Finally, at high mean stresses, η from the tissue strip stayed higher than η from the IL condition at high Ptp levels. This may indicate the importance of either the surface forces and/or the differences in the uniaxial stretching and the three-dimensional uniform stretching of the lung at higher lung volumes.

**Limitation of the study.** Before concluding, we note several limitations of our study. First, the order of the CC, OC, and IL conditions could not be randomized. A second limitation is related to the structural form of our models. It is well accepted that ventilation distribution in the normal lung is determined mostly by the terminal compliances in the lung (6, 33). Thus any heterogeneity in regional lung compliance would likely influence the impedance data. Our modeling approach does not include a distribution of terminal tissue properties, and it seems difficult to extract this information from impedance data. Nevertheless, one of the main findings of this study is that the heterogeneities identified are not related to lung structure but the compartment-like behavior of the respiratory system. Further investigation of these phenomena is needed to determine the effects of compliance-related heterogeneities using imaging (20, 38) or advanced computer modeling (13).

In conclusion, the present study demonstrates that inhomogeneities in the CC and OC conditions are higher than in IL, due to compartmental behavior of the respiratory system, which hinders the assessment of tissue properties from input impedance in rats. The η, as a material constant, is similar in the IL and in the tissue strip at lung volumes and stresses near functional residual capacity, indicating that η is mostly determined by the connective tissues of the lung.

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**REFERENCES**