Dynamic properties of lung parenchyma: mechanical contributions of fiber network and interstitial cells

HUICHIN YUAN,1 EDWARD P. INGENITO,2 AND BÉLA SUKI1

1Department of Biomedical Engineering, Boston University, and
2Brigham and Women’s Hospital, Boston, Massachusetts 02215

Yuan, Huichin, Edward P. Ingenito, and Béla Suki. Dynamic properties of lung parenchyma: mechanical contributions of fiber network and interstitial cells. J. Appl. Physiol. 83(5): 1420–1431, 1997.—We investigated the contributions of the connective tissue fiber network and interstitial cells to parenchymal mechanics in a surfactant-free system. In eight strips of uniform dimension from guinea pig lung, we assessed the storage ( \( G' \)) and loss ( \( G'' \)) moduli by using pseudorandom length oscillations containing a specially designed set of seven frequencies from 0.07 to 2.4 Hz at baseline, during methacholine (MCh) challenge, and after death of the interstitial cells. Measurements were made at mean forces of 0.5 and 1 g and strain amplitudes of 5, 10, and 15% and were repeated 12 h later in the same, but nonviable samples. The results were interpreted using a linear viscoelastic model incorporating both tissue damping (G) and stiffness (H). The G′ and G″ increased linearly with the logarithm of frequency, and both G and H showed negative strain amplitude and positive mean force dependence. After MCh challenge, the G′ and G″ spectra were elevated uniformly, and G and H increased by ~15%. Tissue stiffness, strain amplitude, and mean force dependence were virtually identical in the viable and nonviable samples. The G and hence energy dissipation were ~10% smaller in the nonviable samples due to absence of actin-myosin cross-bridge cycling. We conclude that the connective tissue network may also dominate parenchymal mechanics in a surfactant-free system.

The mechanical properties of the lung parenchyma are critical determinants of the physiological functions of the lung. For example, the stress-strain relationship of the parenchyma determines how lung volume changes with respect to transpulmonary pressure. It also influences the distribution of ventilation and thus impacts significantly on gas exchange. The hysteretic properties of parenchymal tissues together with alveolar surface film are the two most important components accounting for lung tissue resistance, which appears to be a major component of total lung resistance around the breathing frequencies (17, 18, 25, 26, 38). Although various models have been proposed (9, 28, 36), the basic mechanisms at the structural level that determine tissue resistance and elastance of the lung remain largely speculative.

From a structural point of view, lung parenchyma can be considered as a mesh of connective tissue elements (40). The extracellular matrix is composed of protein fibers and amorphous matrix or ground substance. The protein fibers, thought to be the main load-bearing elements within the tissue, include elastic and collagen fibers (33, 40). The cells that synthesize and secrete individual extracellular matrix components are embedded in or lie on the connective tissue matrix (40). However, little is known about the mechanical interaction of these components (4) and how they affect lung mechanics, particularly the hysteretic properties of lung tissues. Several models have been proposed to account for how the fiber elements interact to determine the macroscopic mechanics of the lung, such as the fiber-fiber interaction model (27), the reptation theory describing fiber motion (36), or the gradual straightening of wavy fibers (23). Interstitial cells also influence the overall mechanics because lung tissues respond to various agonist challenge such as methacholine (MCh) or histamine (9, 18, 24, 26, 31, 32). Indeed, Fredberg et al. (9) found that the hysteretic properties of parenchymal tissue strips correlated with the contractile state of tissues responding to different agonists. However, Fukaya et al. (12) observed that the length-tension relationship of the lung parenchyma did not change appreciably within 36 h, over which period viability of the tissue is not maintained. To our knowledge, no direct measurement has been made to compare the contributions of cellular elements and fiber network to the macroscopic mechanical properties of parenchymal tissues. This is the primary purpose of the present study.

We measured the dynamic properties of lung parenchymal tissue strips in their viable and nonviable states. We also assessed the influence of metabolically active cells on tissue mechanics during contraction induced by MCh challenge. To simultaneously follow all parameters characterizing the mechanical status of the strip, we applied specially designed pseudorandom length oscillations that contained energy at selected discrete frequencies between 0.07 and 2.4 Hz. By minimizing the bias on the apparent complex modulus due to nonlinearities, this approach allows for mapping the frequency response as well as characterizing the nonlinearities from a single force-length recording. Our results indicate that connective tissues play an important role in determining the mechanical status of the parenchymal strip even during contraction of interstitial cells.

METHODS

Sample Preparation

Lung parenchymal strips were obtained from eight healthy male guinea pigs weighing 400–450 g killed by intraperitoneal injection of pentobarbital sodium. The fresh lungs were removed from the thoracic cavity, placed in oxygenated Krebs-Ringer organ bath perfusate (in mM: 5 KCl, 137 NaCl, 2 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, and 24 NaHCO₃) on ice, and studied within 1–2 h. The pH was controlled with bubbling
5% CO₂-95% O₂. Tissue strips of $4.5 \times 4.5 \times 10$ mm in dimension were prepared using a single-edge razor. Each end of the tissue strip was fixed by cyanoacrylate glue to small metal clips attached to straightened steel wires. The assembly was placed in a vertical glass tissue bath (Wilbur Scientific, Boston, MA), with the upper wire attached to a force transducer and the lower wire to the lever arm of a displacement generator.

### Experimental Setup

The apparatus included a servo-controlled lever arm (model 300H, Cambridge Technologies) providing the prescribed cyclic elongations. This actuator system has a linearity range of 0.5%, resolution of 1 µm, and 99% response time of 5 ms. The force developed by the tissue strip was measured by a force transducer (model 400A, Cambridge Technologies), which has a resolution of 100 µg, 95% response time of 1 ms, hysteresis magnitude of 0.5%, and compliance of 1 µm/g. Calibrations were performed between 0 and 2 g of force by using standard weights. The transducer system was shown to be linear and accurate to within 1% over the force range (0–2 g) of interest. 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, Cambridge) 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 50 Hz by the computer.

Linearity and hysteresis of the measurement system itself were tested with a steel spring of known stiffness (0.2 N/cm²) similar to that of the tissue strip. The spring was attached to the apparatus in the same way as the lung tissue strip, and the same experimental protocol was carried out. The measured spring stiffness showed neither frequency nor amplitude dependence and thereby appeared to be ideally Hookean. The hysteresis area showed very weak frequency and amplitude dependence and it was very small, more than an order of magnitude smaller than that of the tissue strip. In other words, the apparatus was able to measure tissue elastic and dissipative properties with very small hysteresis area, an order of magnitude smaller than that expected for the parenchymal strip.

### Protocol

The experiments were performed at room temperature. Before the protocol was started, the system had to be aligned. Proper alignment was crucial and was ensured as follows. The force-length relationship of the strip was displayed on an oscilloscope during sinusoidal oscillations, and the hysteresis area between force and displacement was minimized by adjusting the horizontal position of the actuator. The strip was then preconditioned by performing single slow stretch to 2 g of mean force. The mean force was reset to 0.5 g, and the strip was oscillated sinusoidally at 2 Hz for ~5 min until steady state was reached. The dynamic properties of the strip (see below) were measured at 5, 10, and 15% peak-to-peak strain amplitudes of the length oscillations. A similar preconditioning procedure was applied again, and the measurements were repeated at a mean force of ~1 g. After control measurements, MCh (10⁻⁵ M) was added to the tissue bath, and the dynamic response was measured continuously for 20–25 min. In one strip, the MCh response was obtained at 0.5 g of mean force and 5% strain amplitude, whereas for the other seven strips, the mechanical properties were measured at a mean force of 1 g and 10% strain amplitude. After completion of the MCh challenge, the MCh was washed out, and the strip was left at room temperature in the tissue bath for at least 12 h, a period over which cellular components become nonviable. Another strip was also prepared from a nearby region of the same lung and stored at 4°C during this period. The control protocol was then repeated for both strips. The loss of viability of these two strips was confirmed by observing no response to MCh. To identify the various conditions, the control tissue strips will be denoted by V to indicate a viable strip. The same samples after 12 h of storage in the tissue bath will be denoted by N (nonviable strip), and the strips prepared from the same lung and kept at 4°C for 12 h will be called preserved, denoted by P.

### Measurement Approach

Instead of the traditional sinusoidal oscillation approach, we used a broad-band pseudorandom displacement input signal. The signal was a sum of seven sinusoids chosen according to the nonsum-nondifference (NSND) frequency composition introduced by Suki and Lutchen (37). The NSND signal includes frequency components that are not integer multiples of each other, and the input frequencies cannot be obtained as a linear combination of two, three, or four different input frequencies corresponding to NSND orders of two, three, or four, respectively. The essence of the NSND signal is to avoid harmonic distortion and minimize the influence of cross talk in the output at the input frequencies. For not strongly nonlinear systems, the interactions among the components are reduced to a level that the response can be considered as if it were measured with independent sine waves of an equivalent amplitude (37). In this study, we chose a fourth-order NSND signal with flat power spectrum and random phases as the displacement input signal. The length of the NSND sequence was 2,048 points, so that using a sampling rate of 50 Hz corresponded to a time period of 41 s. The frequency components and their corresponding phase angles are given in Table 1. For each condition, a total of three NSND cycles was delivered, and only the last two cycles were collected to avoid transients.

### Data Analysis

Characterization of strip mechanics. The displacement input and force output were normalized to obtain strain $\epsilon$ and stress $T$ as

$$\epsilon(t) = \frac{l(t) - l_0}{l_0}; \quad T(t) = F(t)/A_0$$

where $l$ is displacement, $l_0$ is reference length of the strip at the given mean force, $F$ is force, $A_0$ is cross-sectional area of the strip corresponding to $l_0$, and $t$ is time. The mechanical properties of the tissue strips are characterized by the complex modulus defined in the frequency domain as

$$G^*(\omega) = T(\omega)/\epsilon(\omega) = G'(\omega) + jG''(\omega)$$

where \(j\) is the imaginary unit, and \(\omega\) is the circular frequency. In Eq. 2, \(G'\) is the storage modulus or elastance defining the

### Table 1. Frequency components and corresponding phase angles of displacement input

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Phase angle, rad</th>
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<tbody>
<tr>
<td>3</td>
<td>-3.02 1.229</td>
</tr>
<tr>
<td>7</td>
<td>-0.028 -2.512</td>
</tr>
<tr>
<td>19</td>
<td>3.047 -2.914</td>
</tr>
<tr>
<td>37</td>
<td>2.512 3.047</td>
</tr>
<tr>
<td>61</td>
<td>2.914 1.555</td>
</tr>
<tr>
<td>89</td>
<td>1.229 -2.512</td>
</tr>
<tr>
<td>97</td>
<td>-0.028 3.047</td>
</tr>
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</table>
component of the stress that is in phase with the displacement. $G^*$ is the loss modulus or component of the stress that is in phase with strain rate. The calculation of $G^*$ was carried out as follows. The data records of $F(t)$ and $(t)$ were first transformed to $T(t)$ and $e(t)$, respectively, which were then divided into four blocks (2,048 points/cycle) with an overlap percentage of 25%. The complex spectra for each cycle was obtained by taking the fast Fourier transforms of the blocks. The complex modulus $G^*$ was then estimated in the frequency domain by taking the ratio of the average $T$ and $e$ spectra, which had been corrected for the frequency response of the measuring apparatus. The hysteretic properties of the tissue were characterized by the tissue hysteresivity, $\eta$, introduced by Fredberg and Stamenovic (11) and defined as the ratio of dissipated to stored energy over a force-length cycle, which is simply $G^*/G$. This allows the calculation of $\eta$ as a function of frequency.

Viscoelastic modeling. The special design of the NSND input signal allows a robust estimation of the apparent linear transfer function of the system at the NSND frequencies in the absence of very strong nonlinearities. Therefore, to evaluate the dynamic properties of the tissue strips, we fitted a linear viscoelastic model to the complex modulus spectra at the seven input frequencies at both mean force levels and at all input amplitudes. Several viscoelastic tissue models have been proposed and examined in the literature (3, 13, 17–21, 29, 30, 36). We chose the constant-phase model that originated from the power law type of stress relaxation of a rubber balloon (20)

$$T(t) = t^{-\beta}$$

where $\beta$ is the relaxation exponent. The Fourier transform of Eq. 3 was later applied to lung impedance spectra in the frequency domain by Hantos et al. (18). The tissue impedance $Z(\omega)$ is described by

$$Z(\omega) = G^*(\omega) + jH = \frac{G}{\omega^\alpha} - j\frac{H}{\omega^\beta},$$

with

$$\alpha = \frac{2}{\pi} \tan^{-1}\left(\frac{H}{G}\right) = 1 - \beta$$

where the parameters $G$ and $H$ are the tissue damping and elastance coefficients, respectively. Note that $\alpha$ is not an independent parameter and governs the frequency dependence of the real and imaginary parts of $Z(\omega)$. With only two parameters, $G$ and $H$, Hantos et al. (17, 18) showed that this model can fit the tissue impedance in cat and dog lungs better than other viscoelastic models. Additionally, a mathematical framework and a possible molecular basis of Eqs. 3 and 4 have also been offered (36). According to our preliminary modeling, we observed that the tissues behaved as if they had a purely viscous component $R$, which was also added to the complex modulus $G^*(\omega)$ such that

$$G^*(\omega) = H\omega^\beta + j(G\omega^\beta + R\omega)$$

Note that the first term is the storage modulus, which increases slowly and quasilogarithmically with $\omega$, since $\beta$ is between 0.04 and 0.1 (3, 17, 36). The second term is the loss modulus, which also increases quasilogarithmically and with the same exponent as the storage modulus, since $R\omega$ is negligible at low frequencies. The tissue hysteresivity, $\eta$, in this model is the ratio of the imaginary and real parts of $G^*$. However, because the term $R\omega$ is small compared with $G\omega^\beta$ and to remain consistent with previous analyses of whole lung mechanics (17, 25, 36, 38), we define $\eta$ as the ratio $G/H$ except when we examine the frequency dependence of $\eta$ directly from $G^*$ and $G$. By use of a global optimization algorithm (7), the model parameters were estimated by minimizing the following root-mean-square error (RMSE)

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left| G^*(\omega_i)_{\text{data}} - G^*(\omega_i)_{\text{model}} \right|^2}$$

where $\omega_i$ refers to the NSND frequencies and $N = 7$ is the number of NSND frequencies.

Harmonic distortion. When a NSND broad-band input is applied, the degree of system nonlinearity can be characterized by the so-called extended harmonic distortion index, $k_d$, which quantifies the influence of both harmonic distortion and cross talk (42). The coefficient $k_d$ is defined as

$$k_d = \sqrt{\frac{P_{NI}}{PTOT}} \times 100\% \tag{7}$$

where $PTOT$ is the total power in the output and $P_{NI}$ is the output power due to system nonlinearities only, i.e., the power at noninput or non-NSND frequencies.

Statistical Analysis

By use of paired t-test and analysis of variance, statistical analysis was carried out to compare the mechanical properties of the parenchymal strips corresponding to the different mean forces, input levels, and conditions (V, N, P).

RESULTS

Basic Tissue Mechanics

Examples of $G^*$ and $G$ as a function of frequency in one of the tissue strips are shown in Fig. 1, A and B, respectively, corresponding to the two mean forces and the three strain amplitudes. As evident from Fig. 1, the most important feature of the mechanical behavior of the parenchymal strip is that both $G^*$ and $G$ increased steadily and approximately linearly with the logarithm of frequency regardless of the mean force and strain amplitude. $G^*$ increased by ~20 and 30% with increases in frequency from 0.07 to 2 Hz for mean forces of 0.5 and 1 g, respectively. The magnitudes of both $G^*$ and $G$ were mean force dependent, i.e., the tissue was stiffer and more dissipative at 1 than at 0.5 g. In addition, at both mean forces, $G^*$ and $G$ showed a negative strain amplitude dependence. These findings are consistent with data found in other species (27, 29) and in whole lungs measured in situ (17, 18, 25, 26, 30, 38).

The values of $G^*$ are close to those obtained in guinea pigs by Ingenito et al. (21) using sinusoidal oscillations corresponding to a similar prestress level. The magnitude of $G^*$ obtained by others are somewhat higher (9, 24, 27, 32), but their data are not directly comparable to ours due to differences in species or protocol (mean force was much higher and/or stretch history was different). Because $G^*$ shows only slight frequency dependence, the corresponding tissue resistance ($G^*/\omega^\alpha$) decreases nearly hyperbolically with frequency, which is consistent with whole animal studies (2, 17, 18, 25, 26, 36, 38). The hysteresivity $\eta$, calculated as $G^*/G$, showed virtually no dependence on frequency, mean force, or strain amplitude except for the slight ampli-
Mechanics of Viable and Nonviable Tissues

Comparison of the mean force and frequency dependence of $G'$ and $G''$ in a strip in the V and N conditions is shown in Fig. 2. The tissue strip in both conditions demonstrated a very similar mechanical behavior. Also, the model provided good quality fits to all data. The RMSE values obtained from fitting the model to all strip data under all conditions are summarized in Table 2. Paired $t$-test indicated that the RMSE values corresponding to different conditions were not significantly different from each other. We therefore concluded that the constant-phase model provided equal quality fits to the data under all conditions. The good correspondence between model and data suggests that our oscillatory data are consistent with the slow power law type of stress relaxation (Eq. 3 with exponent $0.075 \pm 0.012$) observed by Bates et al. (3) in tissue strips and by others in isolated lungs (19, 30).

We evaluated and compared tissue mechanical properties under different conditions (V, N, P) by examining the parameters tissue damping $G$, tissue elastance $H$, pure viscous resistance $R$, and harmonic distortion index $k_d$. The population mean of $G$ for the three strain amplitudes is shown in Fig. 3, A and B, at the two mean forces. The $G$ showed a mild negative strain amplitude and a stronger and positive mean force dependence. For example, when the mean force was 0.5 g for condition V, the mean $G$ at 5% strain amplitude decreased by 5.2 and 10.8% when strain amplitude increased to 10 and 15%, respectively. As the mean force increased to 1 g, $G$ increased by 40, 35, and 34% for strain amplitudes of 5, 10, and 15%, respectively. Analysis of variance indicated that, although the strain amplitude and mean force dependence of $G$ were statistically significant ($P < 0.002$ and $P < 0.004$, respectively), $G$ did not depend on the conditions V, N, or P. The $H$ displayed a very similar behavior (Fig. 4). The values of $H$ showed statistically significant strain amplitude and mean force dependence ($P < 0.002$ and $P < 0.02$, respectively) but no dependence on the condition V, N, or P regardless of strain amplitude or mean force. Nevertheless, across all strain amplitudes and mean forces, the population means of $G$ and $H$ in the V condition tended to be higher than in the N condition by $10.9 \pm 2.1$ and $4.4 \pm 3.7\%$, respectively.

In contrast to $G$ and $H$, $R$ displayed a positive strain amplitude dependence (Fig. 5) that was also statistically significant ($P < 0.0003$). Interestingly, $R$ depends on neither the mean force nor the condition (V, N, or P). $R$ is negligible at low frequencies, but it can amount to...
up to 20–25% of \( G \) at the highest frequency (~2.5 Hz). Notably, in the viable tissue at 0.5 g and with 5% strain amplitude [which is in the range of normal breathing near functional residual capacity (FRC)], purely viscous behavior was not observed, since the population mean of \( R \) was not statistically different from zero. Although the \( R \) is thought to be related to the viscosity of ground substance (36), its physiological significance is not clear. Lutchen et al. (26) found a small, purely viscous component of lung tissue resistance in open-chest dogs but concluded that it had no physiological relevance to breathing.

Hysteresivity \( \eta \) calculated as \( G/H \) decreased by \(~10\% \) (Fig. 6) when mean force increased to 1 g. This change was statistically significant (\( P < 0.04 \)). The \( \eta \) also showed a slight strain amplitude dependence, i.e., it dropped by \(~5\% \) when amplitude increased from 5 to 15\% (\( P < 0.02 \)), but only at 0.5 g of mean force. Nevertheless, similarly to all other mechanical indexes, the values of \( \eta \) did not depend on the conditions (V, N, P) of the strips. The negative amplitude and mean force dependence of \( \eta \) are in good agreement with the findings of Navajas et al. (29). The negative mean force dependence of \( \eta \) is also consistent with the results of Mijailovich et al. (27); however, they observed a positive amplitude dependence of \( \eta \). At around a fixed lung volume, Suki et al. (38) found a small negative volume-amplitude dependence of \( \eta \) in intact dog lungs. Again, across all strain amplitudes and mean forces, the means of \( \eta \) in the V condition tended to be higher than in the N condition by \( 6.3 \pm 3.1\% \).

The values of \( k_d \) calculated for different mean forces, strain amplitudes and conditions are shown in Fig. 7. Statistical analysis showed that \( k_d \) did not depend on the conditions V, N, or P of the tissue. However, \( k_d \) showed strong positive strain amplitude as well as mean force dependence (\( P < 0.0005 \) and \( P < 0.002 \), respectively). For example, for condition V and at 0.5 g of mean force, \( k_d \) increased by 58 and 120\% for strain amplitudes of 10 and 15\%, respectively, from its value at 5\% strain amplitude. As mean force increased to 1 g, \( k_d \) increased by 25, 21, and 19\% for strain amplitudes of 5, 10, and 15\%, respectively. This indicated the presence of nonlinearities in the tissue strip. The strain amplitude-dependent characteristics of \( k_d \) in the lung tissue strip are very similar to the volume-dependent behavior of \( k_d \) in intact lungs (42), implying that

<table>
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<tr>
<th>Strain Amplitude</th>
<th>RMSE, ( \times 10^{-3} ) N·s·cm(^{-2} )</th>
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<tr>
<td></td>
<td>V</td>
</tr>
<tr>
<td>5%</td>
<td>4.2±4.9</td>
</tr>
<tr>
<td>10%</td>
<td>3.2±3.7</td>
</tr>
<tr>
<td>15%</td>
<td>2.7±1.4</td>
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Values of root-mean-square error (RMSE) are means ± SD for viable (V), nonviable (N), and preserved (P) strips under 0.5 or 1 g of mean force.

Fig. 3. Mean ± SD of tissue damping coefficient G in viable (V), nonviable (N), and preserved (P) samples measured at 3 different strain amplitudes at mean forces of 0.5 g (A) and 1 g (B). See text for comparison of groups.

Fig. 4. Mean ± SD of tissue elastance coefficient H. A: 0.5 g mean force; B: 1 g mean force. For definitions see Fig. 3.
nonlinearities of the lung are mostly contributed by lung tissues.

MCh Response

Figure 8 shows an example of the model parameters $G$, $H$, $\eta$, and $k_d$ normalized by their control values (denoted by subscript $c$) as a function of time measured at 1 g mean force with 10% strain amplitude during MCh challenge. $G$, $H$, $\eta$, and $k_d$ displayed a peak response with increases of 9, 5, 4, and 5%, respectively, 5 min after MCh was added. In six of the eight strips, $G$ and $H$ showed a peak response, whereas in the other two strips they displayed a plateau response. The time-to-peak response also varied from 5 to 10 min among the strips. The variability of time course response to MCh challenge among strips could be partly attributed to different elapsed times between excision and the start of the experiments. Moreover, the differences in the response to MCh challenge may possibly be due to the fact that the strips were excised from different locations. Ludwig and Dallaire (24) have shown that the volume proportions of alveolar, blood vessel, and bronchial walls do not correlate with the tissue elastance in subpleural parenchymal strips under baseline conditions. However, in a subsequent study, they found that, after acetylcholine-induced constriction, the increases in elastance and resistance and time-to-peak response were greater in strips from more proximal locations containing greater amounts of bronchial and blood vessel walls than in subpleural strips (32).

Next we applied statistical analysis to examine the influence of MCh on the mechanical properties of the parenchyma. The data portions during control and peak response (or plateau in strips without a distinct peak response) were evaluated for all strips and the mean values of $G$, $H$, $R$, and $k_d$ are compared in Fig. 9. During MCh challenge, $G$ increased by a small amount (6%) from $0.058 \pm 0.011$ N/cm² to $0.062 \pm 0.009$ N/cm², which, however, was statistically significant ($P < 0.02$). Similarly, $H$ also increased significantly by 7% from $0.57 \pm 0.13$ to $0.62 \pm 0.13$ N/cm² ($P < 0.004$), and $k_d$ increased slightly and nonsignificantly by 6% from $11.6 \pm 3.7$ to $12.3 \pm 3.1$. $R$ showed a tendency to increase (from $0.61 \pm 0.21 \times 10^{-3}$ to $0.68 \pm 0.17 \times 10^{-3}$ N·s·cm⁻²), but it did not reach a statistically significant level. These changes are comparable to those found by others in strips (9, 24, 31, 32) but smaller than the values obtained in intact animals (18, 25, 26).
DISCUSSION

The major stress-bearing elements of the lung parenchyma are the extracellular fiber matrix, the surface lining layer, and the contractile apparatus. The micro-mechanical basis of parenchymal elasticity is relatively well understood (34, 41). However, the fundamental mechanisms responsible for the dissipative and nonlinear properties of lung tissues are still speculative. In this study, we address one important aspect of this question: what are the separate contributions of the collagen-elastin fiber network and interstitial cells to the mechanical properties of the parenchyma as an integrated tissue system? We achieve this by eliminating the confounding influence of the air-liquid interface in the tissue bath and comparing the mechanics in samples under viable and nonviable conditions. Our findings suggest that tissue mechanics at the macroscopic level are dominated by the connective tissue fiber network, whereas interstitial cells play a less significant role. Before discussing the underlying mechanisms and physiological implications, we first address some issues related to the new methodology that we introduced to assess the dynamic properties of the tissue strip.

Measurement Approach

All previous studies have used sinusoidal length oscillations as input to the tissue strip (9, 21, 24, 27, 29, 31, 32). The advantage of the sinusoidal oscillation approach is that it is simple and easy to analyze the data. However, it has several drawbacks with regard to both linear and nonlinear system analysis. First, to assess the frequency and amplitude-dependent characteristics of tissue mechanical properties at a fixed mean force, measurements need to be repeated at each frequency and amplitude of interest. Because stress relaxation in lung tissue is a long-lasting process (3, 19, 30, 36) and strip viability may vary with time, the mean force may change from one measurement to the other, and hence the data at different frequencies and amplitudes may not correspond to the same condition. Second, a single sinusoid is the least suitable input signal to detect, analyze, and characterize possible nonlinearities in a system (37). Because the main purpose of this study was to carefully compare all aspects including nonlinearities of the mechanical characteristics of the tissue strip, we introduced a new method to investigate these properties. Our NSND pseudorandom input waveform overcomes most of the above difficulties because it allows for an efficient and simultaneous assessment of the frequency-dependent properties as well as a characterization of tissue nonlinearities. Because the response to the NSND input can be considered as if it were obtained by applying individual sinusoids of some equivalent amplitude, the detailed mechanical properties of the strip can be evaluated from a single measurement record, so that the frequency-dependent and nonlinear features correspond to the same mean force. This is indeed very important because, as Fig. 1 demonstrates, mean force has a significant influence on the mechanics. Furthermore, the NSND signal is espe-

![Graphs and figures from the document]

Fig. 8. Model parameters G (A), H (B), \( \eta \) (C), and \( k_d \) (D), normalized by their control values (denoted by subscript c) evaluated at every 40 s after methacholine (MCh) challenge in a typical tissue strip. Arrows indicate time at which MCh was added to organ bath.

**Fig. 9.** Means of parameters G, H, R, and \( k_d \) evaluated in control and under MCh challenge. * \( P < 0.02 \); ** \( P < 0.004 \). Units: G, N/cm²; H, N/cm²; R, \( 10^{-3} \) N·s·cm⁻².

Fig. 1426 MECHANICS OF PARENCHYMAL STRIPS
cially useful in following the dynamic response of the mechanical parameters of the tissue during MCh challenge. By use of single sinusoids, mapping the frequency- and amplitude-dependent characteristics of the tissues is prohibited during the transient response of the system if the period of the sinusoids is comparable to the transients.

Comparison of Viable and Nonviable States

The primary result of this study is that the mechanical status of the parenchymal strip is nearly identical in the viable (V) and the two nonviable (N and P) states of the samples. This paradigm gives rise to the following two hypotheses: 1) the interstitial cells do not contribute to the mechanical properties of the parenchyma at all, or 2) the contribution of the cells to the macroscopic mechanics is independent of whether or not the cells are metabolically active.

With regard to the first possibility, the interstitial cells embedded in and anchored to the fiber network may remain relaxed or the degree of stretch may not be high enough to bring alteration in the sample's overall mechanical properties. Another possibility is that, because the stiffness of the interstitial cells is much smaller than that of the elastin-collagen fiber network, the mechanical contribution of the cells would be completely negligible. However, during stimulation of the contractile cells both mean force and stiffness increase. Due to the strong coupling between mean force and elasticity for a wide spectrum of agents (9), it appears that the increase in stiffness is a direct consequence of the increase in mean force caused by the contraction of interstitial cells. One may speculate that, during contraction, the activation of contractile cells results in increased local tensions throughout the extracellular fiber network, which in turn produce a corresponding change in the mechanical properties of parenchyma. Furthermore, Fredberg et al. (9) also found that, when the strip was exposed to isoproterenol, an agent that reduces the smooth muscle tone, both mean force and tissue stiffness decreased in a correlated manner. Thus it then seems unlikely that in control the cells do not contribute to the macroscopic mechanics.

To resolve the apparent contradiction that the mechanics are very similar in the V, N, and P conditions, we first note that the elasticity of the contractile cells is provided by the number of myosin heads of the attached cross bridges (9). After death of interstitial cells, a state of rigor develops (39) in which the cross bridges freeze and the contribution of the cells to the sample's elasticity would become passive depending on the number of attached cross bridges. If the average number of frozen cross bridges within the contractile cell population is similar to the mean number of attached cross bridges over a force-length cycle in the viable cells under control conditions, then we would observe a macroscopically similar stiffness or H in the viable and nonviable samples. The average relative difference between the mean H values in the V and N conditions was 4.4 ± 3.7%, with H being larger in viable samples.

With regard to energy dissipation, Fredberg et al. (10) recently showed that mechanical friction in smooth muscle cells is associated with the rate of cross-bridge cycling. They also argued that, in the steady state, rapidly cycling cross bridges convert to slowly cycling latch bridges, a state characterized by low mechanical energy dissipation. Accordingly, in the nonviable samples, in which cross-bridge cycling rate is zero due to the lack of metabolic activity, one may expect less energy dissipation and hence smaller G. This is in good agreement with our data, since the mean G and η values corresponding to different strain rates and mean forces were on average 10.9 ± 2.1 and 6.3 ± 3.1% larger in the viable samples, respectively, but these differences did not reach a significant level due to interindividual variability. Although our data do not allow us to estimate the contribution of cells to parenchymal elasticity, the above arguments suggest that metabolic activity of cells may provide ~10% of lung tissue resistance during physiological tidal stretching near FRC. We therefore favor the second hypothesis that the mechanical contribution of the cells to the macroscopic mechanics are about the same in viable and nonviable tissues, but their contribution remains small both during control and MCh challenge for the range of strain amplitudes, mean forces, and agonist concentration studied here.

Finally, it may also be possible that the mechanics of parenchyma are contributed to by different mechanisms in the viable and nonviable samples. In this case, however, the apparent mechanical behavior due to the separate mechanisms must be well matched. The existence of a “plastic matching” at low frequencies as a general biomechanical principle has indeed been proposed both at a much larger scale for the components of the respiratory system (2) and at the level of parenchyma between the various constituents of lung tissue (11). Below, we expand on several possible mechanisms that apparently have matched hysteretic properties and can influence the mechanical behavior of the parenchymal tissue strip.

Tissue Mechanics: Possible Mechanisms

The basic characteristics of the viscoelastic properties of lung tissues are that both the storage and loss moduli increase almost linearly with the logarithm of frequency at all strain amplitudes and at both mean forces (Fig. 1). This frequency dependence is consistent with the constant-phase model of Eq. 4, which also implies that the stress relaxation of the tissue would follow a slowly decaying power law (Eq. 3) over many time decades (3, 36). The negative amplitude and positive mean force dependence of the tissue parameters G and H are phenomenologically consistent with either plasticity (19, 35) or nonlinear viscoelasticity (13, 21, 29, 37). Although the fundamental mechanism that gives rise to these particular viscoelastic properties of lung tissue has not been unequivocally identified, several potential mechanisms have been offered.
Recently, Suki et al. (36) argued that a mechanistic basis for the constant-phase-type tissue viscoelasticity or power law stress-relaxation behavior is a consequence of the so-called "reptation" motion of the collagen-elastin fibers, whereby the fibers rearrange through a series of highly constrained "wormlike" displacements or undulations under the influence of external stresses. In particular, the reptation of branching fibers as originally described by de Gennes (14, 15) and the distribution of fiber width and length (6) have been identified as candidate mechanisms at the level of the fiber network responsible for the macroscopic viscoelasticity. It was concluded (36) that, based on the architecture of the microstructure of the lung, slow reptation of branching fibers can contribute to the viscoelastic properties of lung tissue, whereby G and H would depend on the concentration of fibers and their average distance. Our data are indeed consistent with this behavior. Additionally, as argued by Suki et al. (36), on the basis of polymer viscoelasticity, the parameter R may reflect the viscous properties of the ground substance. The fact that R depends on strain amplitude (Fig. 5) suggests that the ground substance behaves as a non-Newtonian viscous fluid. However, the reptation model has several deficiencies. First, when the fibers are in close proximity, they could attach to each other through some charged groups, which would then give rise to static friction. Indeed, dry friction seems to provide a contribution to reptation in gel electrophoresis (5). Second, the reptation does not yet explain the mean force dependence of tissue elasticity.

Another possibility is the fiber-fiber interaction described by Mijailovich et al. (27). This model also identifies the connective tissue network as the primary source of the macroscopic behavior. In this picture, the fibers are in close contact, and a stick-and-slip motion transfers the load between the fibers. In the linear regime, this model provides predictions that are similar to measured stiffness and hysteresivity. Although the predictions of the model were in reasonable agreement with measured data, this model does not take into account the statistical nature of the orientation, length, width, and branching of the fibers within the tissue, and it predicts at high frequencies a convergence of the elastic moduli corresponding to different amplitudes, which our data do not support.

Fredberg et al. (9) have demonstrated the existence of distinct mechanical states due to contractile cells in the parenchyma, i.e. the changes in hysteresivity differed according to the concentration and type of agonist by which parenchymal tissue was stimulated. More recently, studying airway smooth muscle mechanics, Fredberg et al. (10) provided experimental evidence that \( \eta \) is directly associated with the cross-bridge cycling rate and hence the metabolic state of the cells. Because \( \eta \) has been shown to be invariant of frequency, such a mechanical behavior is also consistent with a slow, power law-like stress relaxation. Indeed, the hysteresivity calculated from our data in Fig. 1 is fairly constant with frequency. Thus cell mechanical properties could, in principle, account for our data. However, we found that the tissue samples in their viable and nonviable states had nearly identical mechanical parameters, implying that the extracellular matrix may also play an important role in the mechanical properties of normal intact lung tissues. Nevertheless, this does not mean that the cellular components do not influence the mechanics. The increase of tissue stiffness during MCh challenge (though small, i.e., <15%) can only be due to contraction of cells. Additionally, for higher concentrations and different contractile agents, cells may play a much more important role. Indeed, Fredberg et al. (9) found increases in tissue stiffness as high as 50% when the tissue was challenged with histamine.

It is possible that all of the above mechanisms contribute to some extent to the observed tissue mechanics. If the contributions of the different mechanisms occur at overlapping time scales, then the macroscopic relaxation could be a result of many interacting mechanisms. The lung tissues, being composed of innumerable protein molecules, cells, and fibers interacting in a complicated manner, have been postulated by Bates et al. (3) to exhibit a rheological behavior that is a reflection of the complexity of the system per se. Accordingly, a "nonmechanistic mechanism" could be the so-called self-organized criticality that has been proposed as the common underlying basis for the ubiquitous occurrence of fractals and 1/f noise in nature (1). In this picture, strong nonlinearities exist in the system at the stress-bearing level. During stretching, strain energy would accumulate, and when a threshold is reached, part of the energy is spilled onto its neighbors. When the neighbors take up the energy, they may themselves reach their own thresholds and transfer the extra energy to further neighbors. This can lead to cascades of energy spillover with their energy magnitudes and spatial extension covering orders of magnitudes. This mechanism would then manifest itself at the macroscopic level as a very long power law-like stress relaxation. The strong elementary nonlinearities could then be static friction (5) or the type of fiber-fiber interaction described by Mijailovich et al. (27). Although this idea is attractive, no direct experimental evidence supports it. Nevertheless, it seems quite plausible that the mechanisms (reptation, fiber-fiber interaction, contractility of cells) discussed above are not mutually exclusive.

Regarding the nonlinear behavior of the tissue, we first note that both the strain amplitude dependence of G and H and the values of \( k_w \) were identical in the V, N, or P conditions. Thus similar mechanical nonlinearities must be present in the viable and nonviable tissue. The above three mechanisms (reptation, fiber-fiber interactions, and cell mechanical properties) display apparently similar negative amplitude dependence of the mechanical moduli. In the reptation model, the stress-relaxation modulus is reduced when strain amplitude is increased (8). The fiber-fiber interaction model (27) appears at the macroscopic level as plasticity with negative amplitude dependence of the stiffness.
and damping (or G and H). Also, trachealis smooth muscle shows a very strong nonlinear behavior, which would again predict a negative amplitude dependence of H (16). This has been attributed to the number of cross bridges attached (9, 10). Taken together, because amplitude dependence of G and H is the same in V, N, and P conditions, we suspect that the mechanism for the nonlinear behavior in the intact lung is most likely related to the characteristics of the collagen-elastin fiber network. We need to point out that this type of nonlinearity is quite different from the nonlinear quasistatic pressure-volume curve of the lung. The quasistatic pressure-volume curve predicts a positive dependence of the incremental moduli on the applied input amplitude. Therefore the mechanism behind the negative amplitude dependence during dynamic stretching is not likely to be related to the exponential stress-strain curve of the strip. Instead, it is related to the mechanism responsible for the hysteretic or viscoelastic nature of the tissue. During MCh challenge, \( k_d \) also increased slightly but systematically by 4–10% from control. This may indicate that the contraction of cells results in an increased mean force and, hence, slight increases in tissue nonlinearities via a stiffening of the fiber network. We thus conclude that the basic tissue mechanics are mainly due to extracellular fiber network, which can be modified by the tone or contraction of interstitial cells.

Mechanics of Parenchymal Strips During MCh Challenge

After MCh challenge, the time course response of the mechanical parameters is consistent with the findings in the literature (9). The slight increase in \( \eta \) at the peak response is in agreement with the simple molecular interpretation of \( \eta \) in contractile cells associated with the increase in cross-bridge cycling rate (9, 10). We also observed that the mean force developed by the tissue strip at a fixed length increased by 4–10% from control, which is less than the 28% reported by Fredberg et al. (9). However, the MCh concentration they used was 10 times higher (10^{-4} vs. 10^{-5} M). Because the mean force appears to be a key factor in determining the mechanics, we tested if the \( G' \) and \( G'' \) spectra would be the same at identical mean forces independent of whether the increase in mean force was achieved by passive stretching or active contraction of cells. We thus compared the \( G' \) and \( G'' \) spectra when the mean force increased from 0.5 to 0.53 g due to cell contraction and when the mean force was adjusted with passive stretching to 0.53 g. As Fig. 10 demonstrates, the increase in tissue stiffness from its value in control at 0.5 g of mean force is the same regardless of whether the increased force was produced by passive stretch or active cell contraction. This is in accord with the data of Fredberg et al. (9), who found that increases in tissue stiffness are always closely associated with increases in active force over a wide spectrum of contractile agonists. Additionally, because after both constriction and passive stretching the \( G' \) spectrum is shifted in a parallel fashion, this behavior is invariant of frequency.

This appears to be a consequence of the anatomical arrangement of the interstitial cells in the neighborhood of the collagen-elastin fiber network within the alveolar septa. Recently, Salerno et al. (31) argued that there are contractile cells in the alveolar walls responding separately from smooth muscles in the small airways. Thus, besides the smooth muscle cells in the alveolar entrance rings, the myofibroblast cells or Kapanci cells (22), for example, which produce and maintain collagen fibers, are also contractile cells and run close and almost parallel to these fibers (40). Therefore, by their contraction, they may induce local tension or shear in the neighborhood of the fibers and hence influence the macroscopic mechanics indirectly, through the fiber network. An interesting implication of this is that, conceptually, the interstitial cells are mechanically in parallel with the fiber system rather than in series. Accordingly, the parenchyma can be conceptualized as a hexagonal mesh of line elements (41). Each line element would be an in-parallel connection of two springs, one with a low elastic constant to represent the cells and the other with a high elastic constant for the elastin-collagen fibers. An increase in the stiffness of the soft springs due to active cell contraction would simply lead to an equivalent increase in elastic modulus of the whole system, importantly, however, independent of the frequency.

In contrast to \( G' \), the \( G'' \) spectra do not fall exactly on the same curve after MCh response and after passive
stretching. This suggests that contraction and passive stretching correspond to different hysteretic states of the tissue as suggested by Fredberg et al. (9). One possibility is that passive stretching involves more connective tissue response than active contraction.

In summary, this study indicates that interstitial cells within the normal parenchyma do not directly influence tissue stiffness at the macroscopic level and may contribute to tissue resistance by <12%. During contraction of interstitial cells, the response is almost equivalent to a shift in passive mean force, which implies that cells and fibers are mechanically coupled in parallel. These findings suggest that the connective tissue network is the dominant factor in determining the mechanical properties of the parenchyma, but the basic tone of the fiber network and hence the corresponding macroscopic mechanical parameters of the tissue can be modulated by the contraction of interstitial cells. As a consequence, the extracellular fiber network plays an important role in the lung at the level of parenchymal mechanics after agonist-induced contraction. Finally, our results also support the notion that the dramatic increases in pulmonary resistance and elastance during MCh challenge are probably not due to alterations at the parenchymal tissue level (26).

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