Changes in viscoelastic properties of rat lung parenchymal strips with maturation

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Tanaka, R., and M. S. Ludwig. Changes in viscoelastic properties of rat lung parenchymal strips with maturation. J. Appl. Physiol. 87(6): 2081–2089, 1999.—The lung extracellular matrix changes rapidly with maturation. To further our understanding of the mechanisms underlying lung tissue mechanics, we studied age-related changes in mechanical properties in lung parenchymal strips from baby (10–15 days old), young (~3 wk old), and adult (~8 wk old) rats. Subpleural strips were cut and suspended in a fluid-filled organ bath. One end of the strip was attached to a force transducer and the other to a servo-controlled lever arm. Measurements of force (F) and length (L) were recorded during sinusoidal oscillations of various amplitudes and frequencies. Resistance modulus (R) and elastance modulus (E) were estimated by fitting the equation of motion to changes in stress (T) and stretch ratio (λ). Hysteresivity (η) was calculated as follows:  

\[ η = (R/E)2πf, \]

where f is frequency. Slow-cycling T-λ curves were measured by applying a constant slow length change. Finally, quasi-static T-λ curves were measured as stress was increased from 0 to 6 kPa and back to 0 kPa in stepwise increments. Our results showed that lung tissue from immature rats was stiffer and less hysteretic than tissue from more mature animals. In addition, tissue from baby animals behaved in a manner compatible with an increased vulnerability to plastic change.

The extracellular matrix alters rapidly during the postnatal stage. The amount of collagen and elastic fibers increases markedly during the first several weeks of life (22). The process of alveolarization occurs, which is characterized by an increase in the number and size of the alveolar walls and a decrease in alveolar thickness (5). In addition, the “ground substance” of the extracellular matrix, i.e., proteoglycans and glycoproteins, changes. For example, the concentration of hyaluronic acid and the amount of chondroitin sulfate proteoglycans have been shown to be substantially higher in neonatal rats than in more mature animals (16, 27). Hence, the maturing lung represents a naturally occurring model of altered extracellular matrix and a singular opportunity to study how changes in the extracellular matrix affect lung tissue mechanics.

In the present study we examined the viscoelastic behavior of isolated parenchymal strips from rat lungs of different ages. In this system, tissue resistance and hysteresis are most likely due to contact phenomena between stress-bearing elements and their surrounding matrix (20, 21, 29), inasmuch as the effects of the surface film, airway closure, and heterogeneous airway constriction (32) are excluded. In addition, we could characterize the specific material properties of the parenchyma without considering lung size per se. The pressure-volume curve of the lung and the length-tension curve of the parenchymal tissue are known to be highly nonlinear, and compliance increases proportionally to lung volume (14, 20, 23). Therefore, meaningful comparison of mechanical properties of lungs of different sizes or ages under a given condition, i.e., at the same pressure or at the same volume, becomes difficult. Measurements in isolated parenchymal strips allowed us to minimize this problem.

Specifically, we examined maturational differences in oscillatory behavior of isolated strips and their dependence on oscillatory frequency and amplitude of length change. Oscillatory measurements were performed at the same mean operating stress (~Tm). Because compliance increases with stretch (18, 20, 23), we reasoned that the length-tension relationship at a wide range of length change would need to be characterized to compare groups with different mechanical behavior. Therefore, we also measured slow-cycling and quasi-static tension (T)-to-stretch ratio (λ) curves. Finally, we measured changes in tension during stress relaxation and the tendency of the tissue to rupture during stretch.

MATERIALS AND METHODS

Parenchymal strip preparation. Three groups of Sprague-Dawley rats were obtained from Charles River (St. Constant,
PQ, Canada): adult (–8 wk old) male, young (–3 wk old) male, and baby (10–15 days old) male and female rats. Three separate groups of strips from different groups of rats were used for each experiment (Table 1). Each animal was anesthetized with pentobarbital sodium (30 mg/kg ip). The thorax was opened, and the animals were exsanguinated by severing the inferior vena cava. The heart, lungs, and trachea were carefully resected en bloc and rinsed in a modified Krebs solution [in mM: 118 NaCl, 4.5 KCl, 1.2 KH₂PO₄, 25.5 NaHCO₃, 2.5 CaCl₂, 1.2 MgSO₄, and 10.0 D(-)-glucose (Sigma Chemical, St. Louis, MO)] with pH 7.4. Lung parenchymal strips were cut from the subpleural edge of the lung, and the pleura was removed. The resting (unloaded) length (Lᵣ) and wet weight of each strip were measured.

Experimental apparatus. Metal clips were glued to either end of the tissue strip with cyanoacrylate. Steel music wires (0.5 mm diameter) were attached to the clips, and the strip was suspended vertically in an organ bath filled with Krebs solution that was maintained at 37°C and continuously bubbled with 95% O₂-5% CO₂. A mercury bead was placed in the bottom of the organ bath, allowing the wire to pass through the bath but preventing the Krebs solution from leaking out. One end of the strip was attached to a force transducer (model 400A, Cambridge Technologies, Water-town, MA) that had an operating range of ±10 g, resolution of 1 µg, and compliance of 1 µm/g, and the other end was attached to a servo-controlled lever system (model 300B, Cambridge Technologies). The lever arm was capable of peak-to-peak length excursions of 8 mm and a length resolution of 1 µm. The lever system was connected to a function generator (model 3030, BK Precision, Chicago, IL), which controlled the frequency, amplitude, and waveform of the oscillation. The resting tension was set by means of a thumbscrew system that effected changes in the vertical displacement of the force transducer. Length and force output signals were digitized with an analog-to-digital converter (model DT2801-A, Data Translation, Marlborough, MA) and recorded on an AT-compatible computer with use of LABDAT data acquisition software (RHT-InfoDat, Montreal, PQ, Canada).

The linearity and hysteresis of the system were tested by measuring the stiffness of a steel spring of stiffness comparable to that of the tissue strip. The spring was suspended in the bath by music wire in the same manner as the strip. The frequency and amplitude dependence of the system were assessed over a range of frequencies (0.1–10 Hz). The spring stiffness did not show any dependence on oscillation frequency below 10 Hz. The hysteresivity (n) of the system was independent of frequency and had a value of <0.01.

Measurement of oscillatory mechanics. Parenchymal strips were preconditioned by slowly cycling the tissue from zero stress to a maximum of 6 kPa Lagrangian stress over a cycling period of 10 s. Lagrangian tensile stress (T) was calculated from the following formula

\[ T = F/A_0 \]  

where

\[ A_0 = W/pL_r \]  

In Eqs. 1 and 2, F is force, A₀ is unstressed cross-sectional area, W is wet weight of muscle strip, and p is mass density of the tissue taken as 1.06 g/cm³; (λ) was defined as L/Lᵣ, where L is the operating length. After three cycles of preconditioning were performed, tension was adjusted to a value ±10–20% larger than 3 kPa, and stress relaxation was allowed to occur for 45 min.

After stress adaptation, T was adjusted again to 3 kPa and left to stabilize for 6 min, during which time we considered that a plateau tension had been reached. Sinusoidal length oscillations with an amplitude (ΔL) of 1% Lᵣ at different frequencies (0.3, 1, 3, and 10 Hz) were applied. Thirty-second recordings of force and length were collected at each frequency. The frequency was varied in random order. The oscillatory amplitude was changed to 3% and then 10% Lᵣ, and recordings at each frequency were repeated. During these measurements, mean length was not changed.

Elastance modulus (E) and resistance modulus (R) were estimated by fitting the equation of motion

\[ T = \eta (\lambda - 1) + Rd\lambda/dt + T_0 \]  

where t is time (in s) and T₀ is a constant. The tissue η, a dimensionless variable coupling the dissipative and elastic behaviors, was calculated with the following equation

\[ \eta = (R/E)2\pi f \]  

Measurement of slow-cycling T-λ curve, stress relaxation, and failure curve. Four to five cycles of 0.02-Hz constant-rate length perturbations were applied to each strip as preconditioning. After the strip was held at Lᵣ for 6 min, tension was adjusted to a value slightly larger than 3 kPa. During the subsequent 45-min stress relaxation, force was recorded. We fit the following two equations to the recorded data

\[ T = At^{-k} \]  

where A and k are constant, and

\[ T = A\exp(-t/\tau_1) + B\exp(-t/\tau_2) + T_c \]  

where A, B, and Tc are constants and τ₁ and τ₂ are time constants.

After tension was held at zero for 6 min, another five cycles of 0.02-Hz constant-rate length perturbations were applied (Fig. 1). Amplitude of length change was 60% Lᵣ for adult

<table>
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<th>Table 1. Characteristics of animals and parenchymal strips</th>
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Values are means ± SE for unstressed cross-sectional area (A₀) and resting length (Lᵣ). T, tension; λ, stretch ratio. *P < 0.05 vs. adult and baby; †P < 0.05 vs. adult.
strips and 20–40% L_r for baby strips. The latter modification was necessary to prevent rupture of the tissue. Therefore, the rates of length change were $\sim 2.4\% \text{L}_r/\text{s}$ for adult strips and $0.8–1.6\% \text{L}_r/\text{s}$ for baby strips. The T-$\lambda$ loops for a given strip were almost identical, with the exception of the first loop. The last unloading limbs were used for analysis. We calculated elastance modulus ($dT/d\lambda$) as a function of T. We also calculated $\lambda$ at a stress of 3 kPa (Fig. 1).

Finally, each strip was stretched from $L_r$ at a rate of $\sim 2\% \text{L}_r/\text{s}$ until the strip ruptured or $\lambda$ reached $\sim 2.6$ (failure test). From this measurement, we constructed T-$\lambda$ curves and defined the yielding point as the maximum stress before an abrupt decrease in stress with further stretch.

Measurement of quasi-static T-$\lambda$ curve. Parenchymal strips were preconditioned, and 45-min stress adaptation was allowed to occur as described above. Quasi-static T-$\lambda$ curves were measured as stress was increased from 0 to 6 kPa and back to 0 kPa in stepwise increments of $\sim 0.8 \text{ mm}$. At each step, tension was allowed to decay for 3 min, at which point $\lambda$ and T were measured. From the quasi-static T-$\lambda$ loop, we calculated quasi-static $dT/d\lambda$ at stresses of 1.5, 3, and 4.5 kPa on the unloading limb. We also calculated the hysteresis ratio (HR) as follows:

$$HR = A_r/(\Delta \lambda \times \Delta T)$$  \hspace{1cm} (7)

where $A_r$ is the area of the T-$\lambda$ loop and $\Delta \lambda \times \Delta T$ is the area bordered by the change in $\lambda$ and the change in T (22). HR was first described as the shape factor $K$ by Bachofen and Hildebrandt (1).

Data analysis. To linearize and normalize the data, we transformed values of R, E, and $\eta$ into their common logarithms. Three-way ANOVA (amplitude, frequency, and age) was performed for the three oscillatory variables. Age-frequency interactions were not significant; age-amplitude interactions were significant. Therefore, we proceeded to do a series of two-way ANOVAs (frequency and age) at each amplitude, then we performed Bonferroni tests for multiple comparisons. To test differences in frequency dependence of the variables, a two-way ANOVA (frequency and strip) and a test of linearity for each age group and amplitude were performed. Inasmuch as linear relations between log(R) or log(E) and log(frequency) were highly significant, linear regression analysis was done to determine whether there were age-related differences in frequency dependence. In other experimental data, one-way ANOVA was used to compare the three age groups, and the Bonferroni test was performed for multiple comparisons. In instances where data were collected in only two groups, i.e., measurements of stress relaxation and slow-cycling T-$\lambda$ curves, Student’s t-test was used for comparison. Means were considered significantly different at a probability level of 5% ($P < 0.05$). Values are means $\pm$ SE.

RESULTS

Oscillatory mechanics. Dynamic properties (R, E, and $\eta$) are shown in Figs. 2, 3, and 4, respectively. R and E of parenchymal strips decreased with maturation (except at $\Delta \varepsilon = 5\%$, at which point R and E in baby strips were not significantly different from those in young animals but were significantly different from those in adults). Conversely, the values of $\eta$ in adult strips were larger than those in the two other groups.

**Fig. 1.** Representative measurements of slow-cycling stress (T)-stretch ratio ($\lambda$) curve. A: changes in length and force as a function of time. B: T-$\lambda$ loops after stress adaptation.
Frequency dependence of R, E, and η at different amplitudes was observed for all groups. Linear relations between log(R), log(η), or log(E) and log(frequency) were highly significant (data not shown). There were no significant differences in the slope of the regression line between log(R) and log(frequency) among the three age groups. For the relationship between log(E) and log(frequency), only the slope of the regression line in strips from young animals at Δε = 1% was statistically different from the others.

Amplitude dependence of R, E, and η at the different frequencies was also observed (data not shown). There were, however, significant differences in the age-amplitude interaction between strips from baby animals and strips from the other two groups: in R between strips from young and baby animals, in E between strips from adult or young and baby animals, and in η between strips from adult and baby animals. This difference may relate to changes in Tm during the experimental protocol (Fig. 5). Although there was some decline in Tm within all groups as amplitude of oscillation was increased from 1 to 3 to 10%, the decrease in Tm was significantly greater in baby strips than in strips from the other two groups.

Slow-cycling T-λ curve, stress relaxation, and failure test. The unloading limb of the T-λ curve is shown in Fig. 6. At λ > 1.5, values of stress were larger in babies than in adults. At λ < 1.5, T was similar in the two groups. E as a function of T is shown in Fig. 7. E increased almost linearly with T. Table 3 shows values of λ and E at a stress of 3 kPa on the unloading limb before (λ₁) and after (λ₂) stress adaptation. λ₁ and λ₂ were lower in baby than in adult strips; conversely, E₁ and E₂ were lower in adult than in baby strips.

Stress-relaxation curves for each strip are shown in Fig. 8. T₀ is the initial maximum stress, which we defined at t = 1 s. (We considered that the initial length change required 1 s.) Log(T) decreased almost linearly with log(t), which means that Eq. 5 fits these data well [correlation coefficient of the regression fit was 0.983 ± 0.005 (SE)]. The stress relaxation component (k) was significantly larger in babies (0.063 ± 0.008) than in adults (0.034 ± 0.003; P < 0.01). The biexponential
Table 2. Significance of difference in mechanical parameters among the three groups

<table>
<thead>
<tr>
<th>Δε, %</th>
<th>R</th>
<th>E</th>
<th>η</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as probability (P); NS, not significant. Δε, amplitude; R, resistance modulus; E, elastance modulus; η, hysteresivity; A, adult; Y, young; B, baby. Common logarithms of R, E, and η were used for statistical analysis.

Eq. 6 fitted the results less accurately, although Eq. 6 has as many as five parameters [correlation coefficient of the regression fit was 0.965 ± 0.005 (SE)].

Table 4 shows λ and T at the yielding point in the failure test. Both were significantly lower in baby than in adult strips. All four baby strips showed yielding at λ < 2.11. Only two of five adult strips demonstrated yielding behavior at the highest λ imposed.

Quasi-static T-λ curve. Quasi-static E at various stresses calculated from the unloading limb of the curve is shown in Fig. 9. Quasi-static E was significantly higher in baby strips than in strips from young and adult animals at T = 1.5 and 3 kPa. Quasi-static E at T = 4.5 kPa was not different among the three groups. The HR of the quasi-static T-λ loop was significantly higher in baby strips than in young or adult strips (0.33 ± 0.04, 0.17 ± 0.02, and 0.13 ± 0.02, in baby, young, and adult strips, respectively, P < 0.001).

DISCUSSION

The major findings of this experiment include the following. The lung parenchymal strip of immature rats was stiffer than that of more mature animals as a function of λ and T. Dynamic and static elastance and resistance were higher in immature rat strips than in strips from more mature rats. In addition, plastic change or tissue nonlinearities were greatest in parenchymal strips from immature rats. Conversely, η, which reflects the ratio of energy dissipated to that conserved (9), was less in parenchymal strips from baby rats.

Before discussing the results, we should consider some potential problems in the experimental approach. First, we think it is important to consider the appropriate strain or stress to apply to the tissues. In previously published reports in which oscillatory mechanics of parenchymal strips were measured, Fredberg et al. (8) applied a Tm of 1.1 and 2 kPa, Ludwig and Dallaire (18) used 2.2 and 3.7 kPa, and Mijailovich et al. (20) chose 0.7 and 2.2 kPa. The actual T to which the tissue is exposed during physiological tidal breathing may be somewhat less. Resting lung volume, when transpulmonary pressure equals zero, is thought to be ~15% of total lung capacity (TLC) (2). Functional residual capacity and tidal volume are roughly calculated as 0.41 and 0.14 TLC, respectively, for a 250-g adult rat and 0.35 and 0.15 TLC, respectively, for a 27-g baby rat (17). If we assume that parenchymal strip λ scales as the cube root of lung volume ratio, then normal tidal lung deflation to functional residual capacity would correspond roughly to λ of 1.54 to 1.40 for the adult rat and 1.49 to 1.32 for the baby rat, and TLC would correspond roughly to λ of 1.88. Therefore, according to our data, the physiological range of tidal volume should correspond to T < 1 kPa, at least in adult rats (Fig. 6). It is possible that the ratio of resting volume to TLC is somewhat higher in baby rats than in adult rats, such that the λ corresponding to tidal breathing may be less in baby rats than in adult rats. Because the T-λ curve of the parenchymal tissue is nearly flat when the stress is small, resting λ is difficult to determine and to measure. Therefore, we chose to use a fixed T, rather than a fixed λ, around which to perform oscillations. As stated above, this Tm may be higher than under physiological tidal breathing. However, applying a lower stress was technically very difficult in our experimental setup.

![Fig. 5. Changes in mean operating stress (Tm) during oscillatory experiment. *P < 0.05 vs. adult and young.](image)

![Fig. 6. Unloading limb of slow-cycling T-λ curve (after stress adaptation).](image)
especially with strips as small as those obtained from baby rats.

Although the conditions during oscillation measurements may not have reflected those during tidal breathing, they give important information regarding dynamic properties of the tissues, as well as their dependence on frequency and amplitude. Moreover, with this approach, it is not necessary to take into account effects of lung size, differences in breathing regimens related to age differences, or the effects of surface tension and atelectasis (32). All these variables need to be considered in an in vivo experiment when age-related differences are evaluated. Airway closure, for example, has been shown to occur more readily in newborn animals, and this may result in increased values of lung resistance and elastance (30). Therefore, it is difficult to be certain from intact lung experiments that differences in tissue properties are related to true differences in parenchymal structure.

It is possible that differences in strip size could affect the mechanical data. Although $L_1$ of baby strips was significantly smaller than that of adult strips (Table 1), $A_0$ was not different. In those strips used for oscillation experiments, $A_0$ of young strips was smaller than that of adult strips. Nonetheless, the data from young strips seem to be consistent, inasmuch as the values of $R$, $E$, and $h$ were intermediate in value between those in adult and baby strips.

Whether the strips of different age groups were taken from an equivalent part of the lung is also an important question. Because the immature lung is much smaller than the mature lung, immature strips might sample a more proximal portion of the lung. Salerno et al. (26) reported that, under baseline conditions, oscillatory mechanics of parenchymal strips were not dependent on anatomic makeup. According to their data, volume fractions of blood vessel wall and bronchial wall in strips obtained from a more proximal location were significantly higher than those in strips obtained directly from the subpleural location. However, no correlation was found between the baseline values of the oscillatory parameters, $E$, $R$, and $h$, and the relative proportion of these anatomic constituents.

The results of this study clearly show that immature rat lung tissue was stiffer than more mature tissue, whether stiffness was assessed during tissue oscillation at a wide range of frequencies and amplitudes, during slow cycling at various $T$ and $\lambda$, or during quasi-static unloading at $T \leq 3 \text{kPa}$. Tissue $R$ was also shown to be larger in immature rat tissue than in tissue from more mature animals. Finally, $h$ was less in strips from immature animals than in strips from more mature animals. Previous investigators have examined maturational changes in tissue resistance and elastance in vivo and found that they decreased with age (6, 22).

Table 3. Stretch ratio and elastance on unloading limb of the slow-cycling $T$-$\lambda$ curve

<table>
<thead>
<tr>
<th>Strip</th>
<th>$\lambda_1$</th>
<th>$\lambda_2$</th>
<th>$E_1 \times 10^4 \text{Pa}$</th>
<th>$E_2 \times 10^4 \text{Pa}$</th>
</tr>
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<tbody>
<tr>
<td>Adult</td>
<td>1.651 ± 0.025</td>
<td>1.788 ± 0.040</td>
<td>2.552 ± 0.165</td>
<td>1.974 ± 0.100</td>
</tr>
<tr>
<td>Baby</td>
<td>1.464 ± 0.037*</td>
<td>1.550 ± 0.045*</td>
<td>5.853 ± 0.279†</td>
<td>5.174 ± 0.243†</td>
</tr>
</tbody>
</table>

Values are means ± SE. $\lambda_1$ and $\lambda_2$, stretch ratio at a stress of 3 kPa on unloading limb before and after stress adaptation, respectively; $E_1$ and $E_2$, elastance at a stress of 3 kPa on unloading limb before and after stress adaptation, respectively. *$P < 0.01$ vs. adult strips; †$P < 0.001$ vs. adult strips.

Table 4. Failure curve

<table>
<thead>
<tr>
<th>Strip No.</th>
<th>$\lambda$</th>
<th>$T$, $\times 10^9 \text{Pa}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&gt;2.85</td>
<td>&gt;2.41</td>
</tr>
<tr>
<td>2</td>
<td>2.33</td>
<td>2.51</td>
</tr>
<tr>
<td>3</td>
<td>2.42</td>
<td>2.33</td>
</tr>
<tr>
<td>4</td>
<td>&gt;2.42</td>
<td>&gt;3.57</td>
</tr>
<tr>
<td>5</td>
<td>&gt;2.56</td>
<td>&gt;3.24</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>&gt;2.52 ± 0.09</td>
<td>&gt;2.81 ± 0.25</td>
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</table>

| Baby      |           |                           |
| 1         | 1.67      | 2.03                      |
| 2         | 2.11      | 1.75                      |
| 3         | 1.98      | 2.19                      |
| 4         | 1.70      | 1.27                      |
| Mean ± SE | 1.87 ± 0.11* | 1.81 ± 0.20* |

* $P < 0.01$ vs. adult strips.
Because the absolute value of resistance and elastance decreases with lung volume and airway closure occurs more readily in newborn animals (30), it is difficult to be certain from in vivo experiments that differences in tissue properties are related to true differences in parenchymal structure. The data from the present experiment suggest that these in vivo changes reflect actual alterations in parenchymal makeup.

We also found that R, E, and $\eta$ showed frequency and amplitude dependence. These results are in agreement with previous studies in adult lungs from different species. A number of investigators have shown in parenchymal strips that $R$ decreases hyperbolically with frequency (15, 20, 23), $E$ increases linearly with log(frequency) (15, 20), and $\eta$ is frequency invariant (9) or decreases modestly with frequency (20). Amplitude dependence of $R$, $E$ and $\eta$ has been shown in guinea pig lung strips (32). Finally, $T_m$ dependence of $R$, $E$ and $\eta$ has also been demonstrated in several previous studies (18, 20, 23, 32).

We found no systematic difference in the frequency dependence of these parameters in parenchymal strips from the different age groups. However, there were differences in amplitude dependence in the baby strips. $R$ and $E$ decreased with $\Delta e$, and $\eta$ increased with $\Delta e$ much more markedly in baby strips than in mature strips. One possible explanation relates to the relatively greater fall in $T_m$ in baby strips, as amplitude was increased from 1 to 3 to 10% L. Amplitude dependence could include a component of $T_m$ dependence. A second explanation relates to a greater plastic change or nonlinearities in strips from baby animals. Plastic change refers to a residual deformation in the tissue that does not reverse when the distorting force is removed (28). Measurements of the failure curve showed an increased vulnerability to yielding in baby strips (Table 4), and quasi-static measurements showed a higher HR in baby strips. Both of these results are consistent with an important plastic change. This large plastic change in baby strips may also explain the somewhat curious observation that $\eta$ calculated from the HR is quite different from that measured during oscillation. The shape factor $K$ is related to $\eta$ as follows:

$$\eta = [(\pi/4K)^2 - 1]^{-1/2}$$ (1).

Whereas HR was measured during a quasi-static maneuver over a large range of $\lambda$, $\eta$ during the dynamic maneuver was measured over a relatively small length amplitude. The plastic change or nonlinearities would more likely contribute in an important way to the $\eta$ calculated from the quasi-static curve compared with that measured during the dynamic oscillation. Previous investigators have also shown that the hysteresis or stress-strain behavior measured during dynamic and quasi-static maneuvers can be markedly different (23, 25).

However, this does not explain why $k$ gives a different result from $\eta$ derived during dynamic oscillation. A larger $k$ means the tissue is more viscous and $\eta$ should be greater. Although $k$ values were highest in baby tissue, $\eta$ was lowest in baby tissue. One explanation may be that certain nonlinearities in the tissue are evident during stress relaxation that are not apparent during small length oscillations. Alternately, the difference may lie in the fact that during stress relaxation the tissue is moving in only one direction, as opposed to during oscillation, when the tissue is forced in two directions.

Conventionally, the Kelvin body has been used to model viscoelastic material such as lung tissue (3). This model can account for much of the observed mechanical behavior. Recently, Hantos et al. (10–12) described the “constant phase model” first introduced by Hildebrandt (13). In this model, frequency dependence of $R$ and $E$ are more accurately formulated than in the Kelvin body, and the time domain expression $[p(t) = At^n$, where $p(t)$ is pressure and $A$ is a constant] predicts stress relaxation nearly perfectly (4). The applicability of this model was confirmed by our results.

The amount of collagen and elastin in the rat lung increases with maturation (22). Turino (31) reported that elastic fibers contribute as a stress-bearing element over the physiological range of the pressure-volume curve, whereas the mechanical properties of collagen become more prominent at higher lung volumes. That the collagen content is relatively small in the immature parenchymal strip has been proposed as the reason immature strips are more vulnerable to yielding (31). Inasmuch as collagen and elastin are thought to be largely responsible for the elastic behavior of the tissues, it seems counterintuitive for elastance modulus to decrease with maturation. Nonetheless, we found a significant decrease in $E$ and quasi-static elastance with increasing age. The alveolarization process occurs throughout early postnatal life, i.e., the number, size, and thickness of alveoli change markedly (5). Hence, differences in fiber structure, orientation, or alveolar geometry, rather than the absolute amount of collagen and elastin per se, may be more important in determining tissue viscoelastic behavior. The decrease in $R$ with maturation is also difficult to reconcile. Again, changes in the alignment or structure of matrix fibrils may be important.

The one dynamic measure that did increase with age was $\eta$. $\eta$ is a measure of the mechanical friction in the tissue and reflects the energy dissipated to that conserved in the system during dynamic oscillation. Mijai-
lovich et al. (21) postulated that energy dissipation in the tissues is related to fiber-fiber interactions. Suki and co-authors (29) invoked the concept of reptation, a process whereby fibers disengage from the surrounding matrix, to explain mechanical friction in tissues. One could speculate that $\gamma$ is increased in lungs of more mature animals, because the absolute number of fibers interacting with each other increases. Alternately, the increase in $\gamma$ may reflect changes in the ground substance. The concentration of hyaluronic acid and the amount of chondroitin sulfate proteoglycans have been shown to be higher in neonatal rats than in more mature animals (16, 27). Proteoglycans are macromolecules containing many hydrophilic glycosaminoglycan side chains; the latter have the capacity to attract ions into the tissue and thereby alter tissue turgor. In addition, they have been shown to coat individual collagen and elastic fibers (7, 27); as proteoglycans and glycosaminoglycans become less plentiful with maturation, the time course of those changes, and how precisely which matrix components are altered with maturation, the time course of those changes, and how those changes modify parenchymal tissue mechanics.

Finally, plastic changes or tissue nonlinearities were greatest in parenchymal strips from babies. Again, this may reflect changes due to “immature” collagen and elastic fibers, immature alveolar structure, or the intrinsic mechanical properties of excess proteoglycans. Further studies need to be directed at determining precisely which matrix components are altered with maturation, the timecourse of those changes, and how those changes modify parenchymal tissue mechanics.

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