Maturational changes in extracellular matrix and lung tissue mechanics

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Tanaka, R., R. Al-Jamal, and M. S. Ludwig. Maturational changes in extracellular matrix and lung tissue mechanics. J Appl Physiol 91: 2314–2321, 2001.—The viscoelastic properties of the pulmonary parenchyma change rapidly postparturition. We compared changes in mechanical properties with changes in tissue composition of rat lung parenchymal strips in three groups of Sprague-Dawley rats: baby (B; 10–14 days), young (Y; ~3 wk), and adult (A; ~8 wk). Strips were suspended in an organ bath, and resistance (R), elastance (E), and hysteresivity (η) were calculated during sinusoidal oscillations before and after the addition of acetylcholine (ACh) (10⁻³ M). Strips were then fixed in formalin, and sections were stained with hematoxylin and eosin, Verhoff’s elastic stain, or Van Gieson’s picric acid-fuchsin stain for collagen. The volume proportion of collagen (%Col), the length density of elastic fibers (Lₑ/Prₑᵥₑ), and the arithmetic mean thickness of alveolar septae (Tₐ) were calculated by morphometry. Tissue was also stained for α-smooth muscle actin (ASMA), and the volume proportion of ASMA (%ASMA) was calculated. Hyaluronic acid (HA) was quantitated by radioimmunoassay in separate strips. R and E in B strips were significantly higher, whereas %ASMA, and HA were greater in B strips. Changes in these parameters with ACh were greater in B strips. Ta, %ASMA, and HA were smaller than in Y or A strips. Changes in these parameters with maturation to the thickened alveolar wall and the relatively greater percent of contractile cells.

The anatomic elements that potentially determine tissue viscoelastic behavior include the network of stress-bearing collagen and elastic fibers, the molecules that comprise the “ground substance” of the extracellular matrix, i.e., PGs and glycosaminoglycans (GAGs), as well as the contractile elements present in the parenchymal tissues (10, 11, 21). Previous authors (30, 31, 33) have suggested that the pulmonary parenchyma can be modeled as an interconnected network of elastic elements, which are presumed to be composed of collagen and elastic fibers and which determine the mechanical behavior of the system. However, Nardell and Brody (24) were unable to show a correlation between lung compliance and elastin or collagen concentration in saline-filled rat lungs between days 4 and 40 postpartum. We questioned, therefore, whether the mechanical behavior would be correlated with collagen or elastin content, especially in view of the described pattern of change in mechanics and biochemical composition of the tissues or whether other of the above-mentioned structural components might also be important in determining changes in tissue viscoelastic properties with maturation.

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To address this hypothesis, we performed experiments in parenchymal strips isolated from Sprague-Dawley rats of different ages. We measured tissue mechanics in the organ bath and then made measurements of collagen, elastin, alveolar thickness, α-smooth muscle actin (ASMA), and HA using histochemistry, morphometry, and radioimmunoassay. In addition, we exposed the parenchymal tissues to ACh to determine whether changes in the mechanical parameters with stimulation would be correlated with age-related differences in the anatomic or structural makeup of the pulmonary parenchyma.

MATERIALS AND METHODS

Strip Preparation and Experimental Setup

Three groups of Sprague-Dawley rats were obtained from Charles River (St. Constant, PQ): baby, 10–15 days, 19–26 g, male and female; young, ~3 wk, 40–85 g, male; and adult, ~8 wk, 230–320 g, male. Each animal was anesthetized with a peritoneal injection of pentobarbital sodium (30 mg/kg). The thorax was opened, and the animals were exsanguinated prior to 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 a pH of 7.4. Lung parenchymal strips were cut from the subpleural edge of the lung, and the pleura was removed. Strips were 1.0–1.5 mm in width and 5–10 mm in length (strips from baby lungs were shorter). One strip per animal was examined. The initial unstressed length (L0) and wet weight (W) of each strip were measured. The unstressed cross-sectional area, A0, was calculated with the formula

\[
A_0 = \frac{W}{\rho L_0} \tag{1}
\]

where \( \rho \) is the mass density of the tissue taken as 1.06 g/cm³.

Metal clips were glued to either end of the tissue strip with cyanoacrylate. Steel music wires (diameter of 0.5 mm) were attached to the clips, and the strip was suspended vertically attached to the clips, and the strip was suspended vertically to pass through the bath but preventing the Krebs solution from leaking out. One end of the strip was attached to a force transducer. Length and force output signals were recorded on an AT-compatible computer, using LABDAT data acquisition software (RHT-InfoDat, Montreal, PQ).

The linearity and \( \eta \) of the system were tested by measuring the stiffness of a steel spring comparable with 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 (0.3–3 Hz). The spring stiffness did not show any dependence on oscillation frequency below 3 Hz. The \( \eta \) of the system was independent of frequency and had a value of <0.003.

Oscillatory Measurements at Baseline

Parenchymal strips were preconditioned by slowly cycling the tissue from zero stress to a maximum of 6 kPa Lagrangian stress. This preconditioning was manually controlled and had a cycling period of ~10 s. Lagrangian tensile stress (T) was calculated from the formula

\[
T = F/A_0 \tag{2}
\]

where \( F \) is the force. Strain (\( \varepsilon \)) was defined as \( (L - L_0)/L_0 \), where \( L \) is the operating length. After three cycles of preconditioning were performed, \( T \) 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 a strain amplitude (\( \Delta \varepsilon \)) of 1% at a frequency (\( f \)) of 1 Hz were applied. Thirty-second recordings of force and length were collected.

\( E \) and \( R \) were estimated by fitting the equation of motion to \( T \) as

\[
T = E\dot{\varepsilon} + R\ddot{\varepsilon} + T_0 \tag{3}
\]

where \( t \) is time (s) and \( T_0 \) is a constant. The tissue \( \eta \), a dimensionless number coupling the dissipative and elastic behaviors (11), was calculated with the equation

\[
\eta = (R/E)2\pi f \tag{4}
\]

Oscillatory Measurements After Induced Constriction

After the baseline measurement, the data were collected continuously for 6 min at \( f = 0.3 \) Hz. One minute after recording had begun, acetylcholine chloride (ACh; BDH, Toronto, ON) was added to the organ bath to yield a final concentration of \( 10^{-5} \) M. (Preliminary experiments showed that this concentration of ACh resulted in a maximal response.) Responses are shown as the percentage increase in \( R \), \( E \), \( \eta \), and mean stress (\( T_m \)) compared with the initial values at \( f = 0.3 \) Hz immediately before ACh challenge. On completion of the experiment, the parenchymal strips were removed from the organ bath and fixed in 10% formalin for histology and immunohistochemistry.

Histology and Morphometry

Volume proportion of tissue constituents. Formalin-fixed strips were embedded in paraffin, and serial longitudinal 5-μm-thick sections were cut on a microtome. Every 10th section was stained with hematoxylin and eosin (HE). One slide from each strip was stained with Verhoeff’s elastic stain and another slide stained with Van Gieson’s picric acid-fuchsin stain for collagen. HE-stained sections were examined by light microscopy at ×100 magnification, and an ocular equilateral triangular grid (Weibel type 2, 10 × 10 mm, 42 points) was applied to measure the volume proportions of tissue constituents using point counting. Although this approach has traditionally been applied to fixed, inflated lungs (7), we have adapted this approach to making measurements in parenchymal strips (19). Volume fractions were measured for AW, blood vessel wall (BVW), and bronchial wall (BW). All points falling on these components were
counted from consecutive nonoverlapping fields of view until all sections from each strip were counted. BVW and BW were counted when a point fell on either the smooth muscle, the epithelial layer, the endothelial layer, or its associated connective tissue. Approximately 1,200 points were counted per strip (after the points falling on alveolar airspace, blood vessel lumen, and bronchial lumen were excluded). Volume fractions (%AW, %BVW, and %BW) were defined as the number of points falling on AW, BVW, and BW, divided by the total number of test points.

Alveolar septal thickness. One HE-stained slide per strip was used for measurement of linear intercept. The field was viewed at ×400 magnification through a superimposed grid of test lines. The point at which an airspace septum intersected a test line was identified (between alveolar lumen to the left and alveolar tissue to the right), and the shortest distance across the alveolar septum in any direction was measured. This yielded “orthogonal intercepts” of the AW, and the arithmetic mean (Lₐ) of the intercepts was calculated. Arithmetic mean thickness (Tₐ) of alveolar septae was calculated by using the following equation (13)

\[ Tₐ = (\pi/4) Lₐ \]  

Approximately 400 intercepts were measured, which required ~30 fields per strip. In a previous study (13), as few as 50 intercept measurements provided acceptable results.

Volume proportion of collagen-positive parenchyma. %Col was determined using light microscopy at ×400 magnification by point counting as described above. Volume proportion was measured as the number of points falling on collagen-positive AW, divided by the total number of test points falling on AW. Approximately 1,000 points were counted per strip. We defined %Col as percentage of collagen-positive points in AW.

Length of elastic fibers per unit volume. Length density of elastic fibers (LV/Pr added) was determined by using light microscopy at ×400 magnification (4). We chose 20 nonoverlapping fields of view in each slide. In each field, the number of intersections of elastic fibers with a test line was determined. Elastic fibers that completely transected the test line and were located in the alveolar parenchyma were counted. Elastic fibers observed in blood vessels, bronchus, or pleura were excluded. We also determined the volume proportion of AW (Pr added = counts falling on alveolar septum divided by the counts falling on either alveolar septum or lumen) in each field using point counting. Approximately 2,500 points were counted per strip. Length of elastic fibers per unit volume (LV) was calculated as follows

\[ Lₐ = 2N(Lₐtest \times d) \]  

where N is total number of intersections, Lₐtest is total length of test line, and d is thickness of section (5 μm). We compared LV/Pr added in the three age groups.

Immunohistochemistry for ASMA

Tissues were incubated with a mouse primary monoclonal antibody to ASMA (Sigma Chemical, Mississauga, ON), washed, and treated with biotinylated rabbit anti-mouse antibody. The antibody-antigen complex was developed with alkaline phosphatase-conjugated avidin complex and fast red. The volume proportion of ASMA-positive parenchyma was examined by light microscopy at ×400 magnification by using point counting. Volume proportion (%ASMA) was measured as the number of ASMA-positive points divided by the total number of test points falling on tissue, including airways or blood vessels (points on lumen or alveolar airspace were excluded). Approximately 3,000 points were counted per strip. Control experiments were performed with secondary antibody alone, and no positive staining was observed.

Measurement of Hyaluronic Acid

Lung parenchymal tissue separate from those used for study of mechanics was cut from the subpleural edge of the lung (n = 7 per group). Each strip was placed in 300 μl of sodium acetate solution (100 mM, pH 7.4) and frozen at 20°C; 300 μl of PBS (pH 7.4) were added. The strip was then digested with alkaline protease from Streptomyces griseus at 0.6 U/tissue for 20 h at 60°C. This digestion was performed to remove any HA binding protein in the tissue. Samples were boiled for 5 min to stop the reaction and then centrifuged for 1 h at 10,000 rpm and 4°C. The resulting supernatant was used to estimate the amount of HA by using a HA radiometric assay kit (Pharmacia, Montreal, PQ). HA from the sample was allowed to bind 125I-labeled HA binding protein isolated from bovine cartilage. The unbound 125I-labeled HA binding protein was then quantitated after interaction with HA covalently bound to HA-sepharose beads. The amount of HA per gram wet tissue was calculated for each sample.

Data Analysis

One-way ANOVA followed by Bonferroni tests for multiple comparisons was used to compare the three age groups. We tested whether the baseline mechanical parameters (R, E, and η) were significantly correlated with the histological data (Ta, %Col, LV/Pr added, and %ASMA) and whether Ach responsiveness (%R, %E, %η, and %Tm) was significantly correlated with the histological data (%BW, %Col, LV/Pr added, and %ASMA). Means were considered significantly different at a probability level of 5% (P < 0.05). Results are reported as means ± SE.

RESULTS

Baseline values of the mechanical parameters, R, E, and η are shown in Table 1. Both R and E of parenchymal strips decreased with maturation. R and E in baby strips were significantly greater than in those of adults; R and E in strips from young animals were intermediate in value between those of strips from adult and from baby animals. Conversely, the value of η increased with maturation: η in adult strips was significantly larger than that in the two other groups.

Photomicrographs of parenchymal strips stained for collagen, elastic fibers, and ASMA for baby and adult strips are shown in Fig. 1. The AW was relatively thick in the parenchymal strips from baby animals compared with those of adults. In addition, there was a markedly

Table 1. Baseline value of mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Baby (n = 10)</th>
<th>Young (n = 8)</th>
<th>Adult (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R, Pa·s</td>
<td>615 ± 19a</td>
<td>445 ± 40</td>
<td>346 ± 15</td>
</tr>
<tr>
<td>E, 10⁴ Pa</td>
<td>5.76 ± 0.19w</td>
<td>4.06 ± 0.40†</td>
<td>2.68 ± 0.14</td>
</tr>
<tr>
<td>η</td>
<td>0.069 ± 0.001†</td>
<td>0.072 ± 0.004‡</td>
<td>0.084 ± 0.003‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. R, resistance modulus; E, elastance modulus; η, hysteresivity. Baby, 10–14 days; young, −3 wk; adult, −8 wk. *P < 0.001 vs. young and adult; †P < 0.005 vs. adult; ‡P < 0.05 vs. adult.
increase in collagen and elastic fibers in mature animals. On the other hand, there was less actin staining observed in lungs from mature animals. Table 2 documents the results of the morphometric analysis. Although the volume proportion of the AW, BW, and BVW was similar in tissue from the three age groups, $T_a$ was greater in baby strips as opposed to young and adult strips. The $\%$Col and the $L_v/Pr_{alv}$ were significantly lower in baby strips than in those from more mature animals. Finally, the $\%$ASMA in baby strips was higher than that in strips from young rats ($P < 0.05$); the difference did not reach statistical difference when baby strips were compared with those of adult rats ($P = 0.057$). Figure 2 shows the HA content per gram wet tissue. HA concentration in baby strips was significantly higher than that in other groups.
The correlations between the physiological parameters at baseline and the morphometric findings are reported in Table 3. There were significant positive correlations between R and E vs. Ta. The correlations between R and E vs. %ASMA were suggestive but did not achieve statistical significance (0.054 and 0.066, respectively). On the other hand, these physiological parameters were inversely correlated with %Col and LV/Pralv.

Addition of ACh to the organ bath caused increases in Tm, R, E, and hysteresivity in parenchymal strips from adult, young, and baby rats. The magnitude of the increase was significantly greater in baby strips compared with the two other groups (Fig. 3). Correlations between the percent increase in the mechanical and morphometric parameters are shown in Table 4. Changes in mechanics in response to ACh were positively correlated with the amount of ASMA in the parenchymal strips. They were also correlated with Ta but not with the volume proportion of BW. Percent increases in Tm, R, E, and η were negatively correlated with the %Col.

DISCUSSION

In this study, maturational changes in tissue viscoelastic behavior were accompanied by changes in the composition and configuration of the AW. With increasing time postparturition, collagen and elastic fibers were increased, and HA and ASMA-positive cells were decreased. In addition, parenchymal tissue from immature animals showed a greater responsiveness to contractile stimulation. Changes in mechanics were, in general, positively correlated with the thickness of the AW and with the percent of ASMA-positive cells and negatively correlated with the absolute amount of collagen and elastic fibers.

The finding that parenchymal mechanics are modified with maturation has been previously described in the literature. We recently reported on the viscoelastic properties of parenchymal strips obtained from rats at 10–15 days, 3 wk, and 8 wk postpartum (32). R and E in parenchymal strips from baby rats were greater and hysteresivity was less than in strips from older animals. In addition, lung tissue from baby animals behaved in a manner compatible with an increased vulnerability to plas...
tic change. Nardell and Brody (24) studied quasi-static pressure-volume curves of saline-filled rat lungs between 4 and 40 days of age and reported that lung E and $\eta$ ratio fell with age, whereas the pressure required to rupture the lung increased. Mansell and colleagues (20) measured bulk and shear moduli of excised piglet lungs and showed that bulk modulus initially increased between immediately postpartum and 3–5 days and then subsequently fell. Shear modulus fell between newborn and 3- to 5-day-old lungs. Other laboratories have also published data on the dynamic mechanical properties of the parenchymal tissue. Polgar and String (25) measured viscous R of the lung indirectly in newborn infants and reported that tissue R (Rti) values were higher than those observed in adults. Dreshaj and colleagues (9) measured Rti directly in newborn piglets using the alveolar capsule to obtain alveolar pressure. At 2–3 days postpartum, Rti values were approximately three times that measured in 10-wk-old animals. However, as opposed to our studies in which the operating stress was controlled, these latter studies did not address potential volume-related effects on Rti.

Thus, whereas there is some information in the literature regarding changes in mechanics with maturation, relatively little is known about the mechanism of that change. Moreover, much of the data has been collected in intact lungs, where maturation-related changes in surfactant may also contribute to viscoelastic behavior. In the present study, we attempted to address this question by performing careful morphometric analyses of parenchymal tissue on which physiological measurements had also been made. We determined that collagen and elastic fibers increased significantly over the 6-wk period sampled in the present experiment. These findings are consistent with those previously reported by Nardell and Brody (24), who measured levels of lung collagen and elastin in rats from days 4–40 of age. They documented that collagen concentration increased linearly. Absolute elastin content increased 10-fold over the first 20 days and continued to rise thereafter. Similar results have been reported in rats and other species by other investigators (9). We also measured a decrease in the $T_a$ of the AW with maturation. Previous authors have also described that the thick-walled alveoli become thinner and less cellular as alveolarization occurs with maturation (6, 7). Mansell and colleagues (20) have reported data on the $T_a$ in newborn piglets; $T_a$ decreased markedly from day 0 to days 25–30.

We also examined changes in HA, one of the molecules which make up the ground substance of the lung. HA is a nonsulfated GAG, which has the ability to form aggregates with chondroitin sulfate PG. GAGs are very hydrophilic and hence have the capacity to attract water and ions into the tissues and thereby affect tissue turgor and viscoelasticity (2, 27). We found that HA was significantly increased in lung tissue from baby rats compared with that from young and adult rats. This finding has also been reported by Juul and colleagues (16) in newborn rat pups examined between days 3 and 13 of age. These investigators (17) also examined the profile of HA and PG in the developing lung in nonhuman primates. They reported that HA metabolism is maximal at term and falls abruptly thereafter and that a large chondroitin sulfate PG predominates in fetal lung tissue, which then declines in relative importance with developmental age. Horwitz and Crystal (14) showed marked variations in the profile of GAGs among late-gestation, neonate, and weanling rabbit lungs. Also in rabbits, Radhakrishnamurthy et al. (26) reported a decline in total GAGs from 1 to 12 wk of life.

Finally, we demonstrated that the %ASMA-positive cells was increased in immature rat lungs. Similar findings were reported by Mitchell et al. (22), who showed that the number of ASMA-positive cells was increased in newborn rat lung during the saccular stage of development (the first 2 wk of life) and then fell dramatically. Authors (19) have also reported on changes in smooth muscle myosin isoforms during development.

As stated above, the primary purpose of this study was to attempt to correlate changes in the dynamic mechanical properties with changes in the structural makeup of the pulmonary parenchyma. Nardell and Brody (24) in their previous experiments were unable to correlate changes in the elastic behavior of the lung tissues with changes in the amount of collagen and elastic fibers. In previous modeling work (30, 31, 33), it has been presumed that the elastic line elements of the parenchyma that determine elastic behavior are composed of collagen and elastic fibers and hence these molecules account for elastic behavior. Pulmonary parenchyma is relatively hysteretic, especially after induced constriction (9, 10, 19), and collagen and elastin are molecules with minimal viscoelastic properties (12). One possible way in which these largely elastic fibers may account for hysteretic behavior is that fibers arranged in a network may behave differently from the

Table 4. Correlation coefficients between ACh responsiveness and morphometric parameters

<table>
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<tr>
<th>%BW</th>
<th>%E</th>
<th>%η</th>
<th>%T_m</th>
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<tr>
<td>%BW</td>
<td>$-0.210$ (0.40)</td>
<td>$-0.156$ (0.54)</td>
<td>$-0.200$ (0.43)</td>
</tr>
<tr>
<td>$T_a$</td>
<td>$0.654$ (0.015)</td>
<td>$0.123$ (0.69)</td>
<td>$0.780$ (0.0016)</td>
</tr>
<tr>
<td>%Col</td>
<td>$-0.844$ (0.0003)</td>
<td>$-0.647$ (0.017)</td>
<td>$-0.742$ (0.0037)</td>
</tr>
<tr>
<td>$L_v$Pr</td>
<td>$-0.478$ (0.999)</td>
<td>$-0.121$ (0.69)</td>
<td>$0.583$ (0.036)</td>
</tr>
<tr>
<td>%ASMA</td>
<td>$0.610$ (0.027)</td>
<td>$0.283$ (0.35)</td>
<td>$0.687$ (0.0095)</td>
</tr>
</tbody>
</table>

Values are means. $T_m$, mean tensile stress. $P$ values are in parentheses.
individual components (5). However, in the present study, we observed that collagen and elastin were inversely correlated with mechanical behavior, both under baseline conditions and after induced constriction. In other words, the more collagen and elastin in the system, the less stiff and viscous the tissue. These data suggest that collagen and elastin are not entirely responsible for the mechanical behavior of the line elements. A further consideration is that it is not the absolute amount of fibers that is important in affecting mechanical behavior, but rather the organization and/or interaction of these fibers. Changes in fiber subtype, topology, or cross-linking with maturation could contribute. Although few data are available on maturation-related changes in fiber subtype or organization, there are some reports of alterations in collagen cross-linking with aging (28).

A second possibility is that alterations in other components of the extracellular matrix drive the mechanical changes. In this regard, we did observe some positive correlations. Under baseline conditions, a positive correlation between $R$ and $E$ and $T_{\alpha}$ of the AW was found. Hence, the thicker AW may be important. A positive relation between $R$ and $E$ and $\%$ASMA was suggested but did not attain statistical significance. When the tissues were stimulated with a contractile agonist, correlations between percent increase in $R$ and $\eta$ and $T_{\alpha}$ were observed, and correlations with $\%$ASMA became significant.

The thickened AW in immature animals may be accounted for by cellular components, edema fluid, and extracellular matrix molecules (6, 7). We found that HA was increased in immature animals. We could not examine for direct correlations between mechanical behavior and HA, because it was necessary to perform RIA measurements on separate strips. Extracellular matrix molecules such as HA, PGs, and other GAGs may contribute to viscoelastic behavior because they are large hydrodynamic molecules that coat the stress-bearing fibers; they can affect tissue turgor and thereby alter tissue mechanics (15). We have shown in parenchymal strips obtained from adult rat lungs that degradation of GAG side chains results in alterations in tissue viscoelasticity (1). These molecules may act as a “lubricant” to modulate the energy dissipated as fibers slide past each other (21). As the amount of lubricant changes with maturation, the dissipation of energy as the fibers move across each other may be altered. Furthermore, the ratio of energy dissipated to that conserved, or $\eta$, may be affected. This ratio may change in a manner distinct from that of $R$ and $E$, although changes in response to contractile stimulation tend to be of a similar nature (10). Hence, changes in these ground substance molecules may be responsible for the altered viscoelastic behavior with maturation.

Another possibility to account for the thickened AW in immature lungs is tissue edema, and it is the water content of the tissue per se that accounts for the increased $R$ and $E$. HA is known to be important in interstitial fluid dynamics. Bhattacharya et al. (2) showed that lung hyaluronan correlated with extravascular lung water in unanesthetized rabbits. Some data are available regarding lung water in immature animals. Nardell and Brody (24) showed in their study that lung water content as assessed by wet-to-dry ratios was stable between days 4 and 40. Cherukupalli and colleagues (8) also reported on lung wet-to-dry ratios in rats, showing a modest decline in the first 2 wk of life but a plateauing thereafter. Hence, our data showing an important decrease in $R$ and $E$ between 10–15 days and 3 wk is not likely explained by changes in lung water.

Significant correlations between $\%$ASMA and changes in lung mechanics were found after induced constriction, and correlations were close to significant under baseline conditions. In addition, lung tissue from baby rats showed an enhanced response to contractile stimulation compared with older animals. Dreshaj and co-workers (9), in a study in maturing piglets in which tissue responses were partitioned using the alveolar capsule technique, showed an enhanced tissue response at 2–3 wk compared with 10 wk of age. Murphy and colleagues (23) showed that contractile responses were greater in airway smooth muscle from 2-wk-old farm swine compared with that of airways from 10-wk-old animals. Increased tone in contractile cells may contribute to increased $R$ and $E$ in the baby animals. Recently, Schittny and co-workers (29) described enhanced spontaneous peristaltic activity in the airways of fetal lungs. It is possible that this enhanced contractile activity persists through the first few postpartum weeks and that this increased tone contributes to the elevated $R$ and $E$ observed in immature lungs. We observed a negative correlation between the contractile response and the $\%$Col. Increased collagen in the lung tissues could provide an impedance to constriction by contractile cells and thereby modulate the response to constrictor agonists.

In summary, we have shown that the viscoelastic behavior of the lung parenchyma changes with maturation. $R$ and $E$ decrease; $\eta$ increases. These mechanical changes appear coincident with changes in the thickness of the AW, in the amount of collagen and elastic fibers, and in tissue HA. Mechanical changes were positively correlated with changes in AW thickness and in the percentage of ASMA-positive cells and negatively correlated with the absolute amount of collagen and elastic fibers. The precise components contributing to the thickened AW in immature animals are not known but likely include extracellular matrix molecules such as PGs and GAGs, which, themselves, are known to influence tissue viscoelastic behavior. Further studies to specifically identify which extracellular matrix molecules are responsible for the changes in tissue viscoelastic properties with maturation are warranted.

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