The bimodal quasi-static and dynamic elastance of the murine lung

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1Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, Subiaco, Western Australia; 2Department of Medical Informatics and Engineering, University of Szeged, Szeged, Hungary; and 3Department of Intensive Care and Neonatology, University Children’s Hospital, Zurich, Switzerland

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Zosky GR, Janosi TZ, Adamicza Á, Bozanich EM, Cannizzaro V, Larcombe AN, Turner DJ, Sly PD, Hantos Z. The bimodal quasi-static and dynamic elastance of the murine lung. J Appl Physiol 105: 685–692, 2008. First published June 12, 2008; doi:10.1152/japplphysiol.90328.2008.—The double sigmoidal nature of the mouse pressure-volume (PV) curve is well recognized but largely ignored. This study systematically examined the effect of inflating the mouse lung to 40 cm H2O transrespiratory pressure (Pw) in vivo. Adult BALB/c mice were anesthetized, tracheostomized, and mechanically ventilated. Thoracic gas volume was calculated using plethysmography and electrical stimulation of the intercostal muscles. Lung mechanics were tracked during inflation-deflation maneuvers using a modification of the forced oscillation technique. Inflation above 20 cm H2O caused a shift in subsequent PV curves with an increase in slope of the inflation limb and an increase in lung volume at 20 cm H2O. There was an overall decrease in tissue elastance and a fundamental change in its volume dependence. This apparent “softening” of the lung could be recovered by partial degassing of the lung or applying a negative transrespiratory pressure such that lung volume decreased below functional residual capacity. Allowing the lung to spontaneously recover revealed that the lung required ~1 h of mechanical ventilation to return to the original state. We propose a number of possible mechanisms for these observations and suggest that they are most likely explained by the unfolding of alveolar septa and the subsequent redistribution of the fluid lining the alveoli at high transrespiratory pressure.

MATERIALS AND METHODS

Animals

Eight-week-old female BALB/c mice were purchased from the Animal Resource Centre (ARC; Murdoch, Western Australia) and housed in specific pathogen-free conditions with a 12-h:12-h light/dark cycle. All experiments were conducted with the approval of the Telethon Institute for Child Health Research Animal Ethics Committee and conformed to the guidelines of the National Health and Medical Research Council of Australia.

Animal Preparation

Mice were anesthetized with an intraperitoneal injection of a solution containing 40 mg/ml of ketamine (Troy Laboratories, New South Wales, Australia) and 2 mg/ml of xylazine (Troy Laboratories) at a dose of 0.1 ml/10 g body wt. Two-thirds of the dose was given initially to induce a surgical level of anesthesia. Once anesthetized, the mouse was tracheostomized and a 10-mm length polyethylene tubing (1.26 mm outer diameter: 0.86 mm inner diameter) was inserted into the trachea. The tracheal cannula was secured with surgical silk, and the mouse was placed inside a whole body plethysmograph and connected to a computer-controlled small animal ventilator (flexiVent; SCIREQ, Montreal, Quebec, Canada). The remaining third of the anesthetic dose was given, and the mouse was ventilated at 450 b/min with a tidal volume of 8 ml/kg and 2 cm H2O of positive-end expiratory pressure (PEEP). This ventilation setting allowed for the measurement of lung mechanics (see details below) without the need for paralysis.

Thoracic Gas Volume

Thoracic gas volume (TGV) was measured as described previously (7). Briefly, the trachea was occluded at end expiration (Pw = 0 cm H2O), and inspiratory efforts were induced by stimulation of the intercostal muscles with intramuscular electrodes. Six pulses of ~2–3 ms in duration were required to inflate the lungs to 0 cm H2O transrespiratory pressure.

THE RELATIONSHIP BETWEEN pressure applied to the respiratory system or the lungs and lung volume represents a simple but fundamental description of their mechanical status. The quasi-static pressure-volume (PV) relationship reflects the viscoelastic properties of the tissues of the lungs and the chest wall but is also influenced by surface forces and the progressive derecruitment and recruitment of the lung units. This classic PV curve, which has been well documented in many species including mice (15, 14, 6), is usually characterized by a flattening of the slope of the curve near what has been traditionally described as total lung capacity (TLC). The recent interest in the nature of the PV curve and, in particular, the definition of TLC in mice in vivo is associated with the biphasic pattern of the inflation limb of the PV curve (15), which is commonly ignored. When the mouse lung is inflated with pressures above some critical point, ~20 cm H2O transrespiratory pressure (Pw), lung volume continues to increase rather than reaching a plateau, which results in a double sigmoidal pattern in the PV curve and no definable TLC (15). This type of PV curve was noted in other mammalian species including the sea otter and fruit bat in the 1970s (9). However, despite knowledge of the existence of this phenomenon for some decades, little attention has been paid to the mechanisms responsible for the biphasic nature of the PV curves in these mammals.

This study aimed to explore the mechanisms responsible for the double sigmoid PV curve observed in mice. We systematically examined the changes in lung mechanics associated with inflation over 20 cm H2O. The effect of derecruitment of lung units by degassing or forcing lung volume below functional residual capacity (FRC) on the changes in lung mechanics following inflation beyond a Pw of 20 cm H2O was also examined.

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at 20 V were delivered over a 6-s period while recording changes in tracheal pressure (P\(_{\text{tr}}\)) and plethysmograph box pressure (P\(_{\text{b}}\)). TGV was calculated by applying Boyle’s law to the relationship between P\(_{\text{b}}\) and P\(_{\text{tr}}\) after correcting for the box impedance (7).

**Lung Mechanics**

The volume dependence of lung mechanics was assessed during a slow (~40 s) inflation-deflation (ID) maneuver between 0 and 20 cm H\(_2\)O \(P_{rs}\). Inspiration was induced by applying a controlled negative pressure to the plethysmograph, and expiration was achieved by the slow equilibration of the plethysmograph to atmospheric pressure through a resistor. During the maneuver, an oscillatory signal was applied to the lung with a loudspeaker in-box setup via a wave tube, with the respiratory input impedance of the mouse measured as a load impedance on the wave tube as described previously (4). Briefly, the oscillatory signal contained 9 noninteger-multiple frequencies ranging from 4 to 38 Hz. The respiratory system impedance spectrum was calculated for these frequencies with 0.5-s data segments throughout the ID maneuver. The four-parameter model with a constant-phase tissue impedance (5) was fitted to the data obtained for each 0.5-s data segment to allow calculation of airway resistance (R\(_{aw}\)) and inertance (I\(_{aw}\)) and coefficients of tissue damping (G) and elastance (H). The resistance and inertance of the tracheal cannula were subtracted from R\(_{aw}\) and I\(_{aw}\), respectively. The values of the small and physiologically insignificant I\(_{aw}\) are not reported. This system allowed simultaneous calculation of the corresponding PV curves, whereby pressure changes were tracked and the commensurate volume measurements were calculated by integrating the wave tube flow during the maneuver. Starting TGV was calculated before all ID maneuvers.

**Experimental Protocol**

**Effect of inflation to \(P_{rs} = 40\) cm H\(_2\)O.** Mice were prepared as described above (n = 4). A series of ID maneuvers were performed as follows; 0-20-0 (ID\(_{20}\)), 0-40-0 (ID\(_{40}\)), and 0-20-0 cm H\(_2\)O (ID\(_{20}\)). Each maneuver was separated by ~5 min of regular ventilation. PV curves were constructed for each of the IDs, and respiratory mechanics were tracked throughout.

**Exploring a critical maximum \(P_{rs}\).** Serial ID maneuvers were performed (n = 4) with increasing peak \(P_{rs}\), as follows; 0-15-0, 0-20-0, 0-25-0, 0-30-0, 0-40-0, 0-20-0 cm H\(_2\)O.

**Response of lung mechanics after inflation to \(P_{rs} = 40\) cm H\(_2\)O to degassing or negative transrespiratory pressure.** A series of IDs were performed per Effect of inflation to \(P_{rs} = 40\) cm H\(_2\)O. The mice were then either degassed (n = 3) or exposed to negative \(P_{rs}\) (n = 3) (to force lung volume down to levels approaching residual volume) before a third inflation to 20 cm H\(_2\)O. Partial degassing of the lungs was achieved by ventilating the mouse with 100% O\(_2\) for 10 min and occluding the tracheal cannula for 1 min. A \(P_{rs}\) of approximately ~7 cm H\(_2\)O was achieved by slowly injecting 2 ml of air into the plethysmograph over a 2-s period. The positive plethysmograph pressure was held for 4 s before inflating the mouse to 20 cm H\(_2\)O and allowing passive deflation per a regular ID maneuver. After a short period of regular ventilation, the mouse was then subject to a regular ID maneuver to 20 cm H\(_2\)O (ID\(_{20}\)).

**Time taken to reverse the effect of inflation to \(P_{rs} = 40\) cm H\(_2\)O.** In another group of mice (n = 5), an ID\(_{20}\), ID\(_{40}\), and ID\(_{20}\) series of maneuvers were conducted as described above. This series was then followed by ID maneuvers from 0-20-0 cm H\(_2\)O after 30 and 60 min of regular ventilation. In additional groups of mice (n = 5 in each), a series of 0-20-0 cm H\(_2\)O or 0-40-0 cm H\(_2\)O were performed with measurements of TGV and lung mechanics taken at baseline, after 30 min, and after 60 min of ventilation.

**RESULTS**

**Effect of Inflation to \(P_{rs} = 40\) cm H\(_2\)O**

**PV curves.** The PV curve for the initial inflation to 20 cm H\(_2\)O (ID\(_{20}\)) had the shape of a classical PV curve with an apparent plateau in volume as the \(P_{rs}\) approached 20 cm H\(_2\)O (Fig. 1). For the subsequent inflation to 40 cm H\(_2\)O (ID\(_{40}\)), the slope of the PV curve increased again beyond 20 cm H\(_2\)O such that the PV curve developed a double sigmoidal pattern. Following this maneuver, inflation to 20 cm H\(_2\)O (ID\(_{20}\)) demonstrated a significant elevation in the slope of the PV curve with a significant increase in the volume reached at 20 cm H\(_2\)O (TGV\(_{20}\)) [ID\(_{20}\), 1.07 ml (SD 0.07) vs. ID\(_{20}\), 0.74 ml (SD 0.08); \(P = 0.001\)] but not in the starting lung volume (TGV\(_{0}\)) [ID\(_{20}\), 0.32 ml (SD 0.03) vs. ID\(_{20}\), 0.28 ml (SD 0.06); \(P = 0.23\)] compared with the ID\(_{20}\).

**Oscillatory mechanics.** R\(_{aw}\) decreased with increasing TGV during inflation in the ID\(_{20}\), ID\(_{40}\), and ID\(_{20}\) maneuvers. There was little hysteresis in the R\(_{aw}\) vs. volume curves for the ID\(_{20}\) and ID\(_{20}\) with the inflation and deflation limbs of the curve overlapping (Fig. 2). In contrast, the values of R\(_{aw}\) were systematically higher at the same TGV during deflation compared with inflation. There was no significant difference between R\(_{aw}\) at 0 cm H\(_2\)O (R\(_{0}\)) [ID\(_{20}\), 0.38 cm H\(_2\)O s\(^{-1}\) ml\(^{-1}\) (SD 0.05) vs. ID\(_{20}\), 0.39 cm H\(_2\)O s\(^{-1}\) ml\(^{-1}\) (SD 0.04); \(P = 0.88\)] for the ID\(_{20}\) and ID\(_{20}\) or the R\(_{aw}\) at 20 cm H\(_2\)O (R\(_{20}\)) [ID\(_{20}\), 0.04 cm H\(_2\)O s\(^{-1}\) ml\(^{-1}\) (SD 0.04) vs. ID\(_{20}\), 0.07 cm H\(_2\)O s\(^{-1}\) ml\(^{-1}\) (SD 0.04); \(P = 0.14\) (Fig. 2).

The coefficient of dynamic elastance, H, demonstrated a curvilinear relationship with increasing volume, as previously described (4, 13) (Fig. 2). For the ID\(_{20}\), H decreased initially before reaching a plateau and then steadily increased for the remainder of the inflation (Fig. 2). The first section of the ID\(_{40}\) H vs. TGV curve was superimposed on the ID\(_{20}\) curve; however, in contrast to the volume dependence of R\(_{aw}\) at TGV\(_{20}\), there was a shift in the relationship between H and TGV with little change in H for the bulk of the inflation from 20 to 40 cm H\(_2\)O before H began to increase again as \(P_{rs}\) approached 40 cm H\(_2\)O (Fig. 2). The deflation limb of H vs. TGV for the ID\(_{40}\) ran
parallel to the ID$_{20}$ curve. The tissue elastance at 0 cm H$_2$O transrespiratory pressure (P$_{rs}$) up to 20 cm H$_2$O/ml (SD 4.5) vs. ID$_{20}$, 43.0 cm H$_2$O/ml (SD 4.9); P < 0.001]. Similarly the minimum H (H$_{min}$, minimum H during plateau on inflation limb) [ID$_{20}$, 14.7 cm H$_2$O/ml (SD 2.1) vs. ID$_{20}$, 26.9 cm H$_2$O/ml (SD 0.7); P = 0.02] and H at 20 cm H$_2$O (H$_{20}$) [ID$_{20}$, 82.4 cm H$_2$O/ml (SD 12.2) vs. ID$_{20}$, 102.1 cm H$_2$O/ml (SD 10.3); P = 0.01] were significantly lower in the ID$_{20}$ compared with the ID$_{20}$. Superficially the volume dependence of H for the ID$_{20}$ had been shifted down and the “characteristic” u-shaped response had been widened.

Qualitatively, the pattern seen in the volume dependence of tissue damping (G), was similar to that observed in H. When P$_{rs}$ was increased beyond 20 cm H$_2$O, there was little change in the viscous properties of the lung as volume increased. As with H, there was a significant decrease in G$_{0}$ [ID$_{20}$, 6.2 cm H$_2$O/ml (SD 0.6) vs. ID$_{20}$, 10.2 cm H$_2$O/ml (SD 1.0); P = 0.004], G$_{min}$ [ID$_{20}$, 5.2 cm H$_2$O/ml (SD 0.1) vs. ID$_{20}$, 8.8 cm H$_2$O/ml (SD 1.2); P = 0.01], and G$_{20}$ [ID$_{20}$, 15.9 cm H$_2$O/ml (SD 1.4) vs. ID$_{20}$, 21.5 cm H$_2$O/ml (SD 1.8); P = 0.001] for the ID$_{20}$ compared with the ID$_{20}$. These “shifts” in the volume dependence of G and H during the ID$_{20}$ maneuver, compared with the ID$_{20}$, were mirrored when they were plotted against pressure (Fig. 2). Similarly, the pressure dependence, particularly of H, clearly changed trajectory when P$_{rs}$ reached 20 cm H$_2$O during the ID$_{20}$.

Critical inflation pressure. Average PV curves for the inflations to 15 and 20 cm H$_2$O had the shape of typical PV curves. When inflation continued beyond 20 cm H$_2$O to 25 cm H$_2$O, there was a shift in trajectory of the PV curve in subsequent maneuvers (Fig. 3). Accordingly, the inflation limbs of the 0–30 cm H$_2$O and 0–40 cm H$_2$O maneuvers departed from the

Fig. 2. Volume dependence of airway resistance (R$_{aw}$) (A), tissue elastance (H) (B), and tissue damping (G) (C) for mice subjected to 3 ID maneuvers starting at 0 cm H$_2$O transrespiratory pressure (P$_{rs}$) up to 20 (open circle), 40 (gray circle), and 20 (solid circle) cm H$_2$O. Also shown are the pressure dependence of tissue elastance (D) and tissue damping (E). Data presented are the group means (SD).
common paths of the initial inflations. The TGV at $P_{rs}/H_{11005}$ 20 cm $H_2O$ (TGV20) was significantly higher in the 0–20 cm $H_2O$ inflation following inflation to 40 cm $H_2O$ (ID$_{/H11032}$ 20) [ID$_{/H11032}$ 20, 0.78 ml (SD 0.17); $P = 0.01$], whereas the starting volume (TGV0) was not significantly elevated [ID$_{/H11032}$ 20, 0.40 ml (SD 0.07) vs. ID20, 0.35 ml (SD 0.16); $P = 0.39$] (Fig. 3).

Response to Degassing or Negative $P_{rs}$

**PV curves.** The change in the standard PV curve following inflation to 40 cm $H_2O$ was as seen in the above experiments with a significant increase in TGV20 in the ID$_{/H11032}$ 20 compared with the ID20 for both groups of mice (Fig. 4). Partial degassing of the lung, following an inflation to 40 cm $H_2O$, reversed the increase in TGV20 observed in the ID$_{/H11032}$ 20 PV curve [ID20 postdegas vs. ID$_{/H11032}$ 20, 0.76 ml (SD 0.14); 0.82 ml (SD 0.12); $P = 0.59$] and did not cause any change in TGV0 ($P = 0.12$). Applying a negative $P_{rs}$ decreased TGV20; however, TGV20 was still significantly higher than the TGV20 for the ID20 maneuver [ID$_{/H11032}$ 20 postnegative $P_{rs}$ vs. ID$_{/H11032}$ 20, 0.76 ml (SD 0.11); 0.88 ml (SD 0.16); $P = 0.04$].

**Dynamic elastance.** The characteristic widening of the volume dependence of H curve, decrease in $H_0$, $H_{min}$, and decrease in $H_{20}$ were again observed in these experiments in the ID$_{/H11032}$ 20 (Fig. 5). Degassing the lung was sufficient to restore $H_0$ [ID$_{/H11032}$ 20 postdegas, 36.6 cm $H_2O/ml$ (SD 12.4) vs. ID$_{/H11032}$ 20, 49.5 cm $H_2O/ml$ (SD 3.6); $P = 0.12$], $H_{20}$ [ID$_{/H11032}$ 20 postdegas, 96.4 cm $H_2O/ml$ (SD 9.6) vs. ID$_{/H11032}$ 20, 110.5 cm $H_2O/ml$ (SD 16.3); $P = 0.22$], and $H_{min}$ [ID$_{/H11032}$ 20 postdegas, 20.8 cm $H_2O/ml$ (SD 5.0) vs. ID$_{/H11032}$ 20, 28.4 cm $H_2O/ml$ (SD 3.6); $P = 0.09$] to ID20 levels (Fig. 5). In contrast, following the negative $P_{rs}$ maneuver, there was still evidence that $H_0$ [ID$_{/H11032}$ 20 postnegative $P_{rs}$, 36.1 cm $H_2O/ml$ (SD 2.2); $P = 0.03$], $H_{20}$ [ID$_{/H11032}$ 20 postnegative $P_{rs}$, 94.2 cm $H_2O/ml$ (SD 8.7); $P = 0.06$], and $H_{min}$ [ID$_{/H11032}$ 20 postnegative $P_{rs}$, 22.6 cm $H_2O/ml$ (SD 2.4); $P = 0.01$] were lower than the initial ID$_{/H11032}$ 20 levels. However, the magnitude of all these parameters had shifted toward the ID20 values, and a large portion of the widened H vs. TGV curve had been recovered (Fig. 5).

**Time Course of Recovery From Effects of Inflation to 40 cm $H_2O$**

**PV curves.** For this series, the TGV$_{20}$ was elevated significantly in the ID$_{/H11032}$ 20 compared with the ID20 (Fig. 6). After 30 min of ventilation, TGV$_{20}$ had decreased but was still significantly higher than TGV$_{20}$ for the ID$_{/H11032}$ 20 [ID$_{/H11032}$ 20 + 30 min, 0.94 ml (SD 0.09) vs. ID$_{/H11032}$ 20, 0.81 ml (SD 0.06); $P = 0.005$]. After 60 min of regular ventilation, TGV$_{20}$ had returned to baseline levels [ID$_{/H11032}$ 20 + 60 min, 0.83 ml (SD 0.06); $P = 0.51$] (Fig. 6).
Dynamic elastance. The pattern of “recovery” observed in the PV curves was also seen in the volume dependence of H. Both $H_0$ [ID$_{20}$ + 30 min, 29.2 cm H$_2$O/ml (SD 6.3)] vs. ID$_{20}$, 44.1 cm H$_2$O/ml (SD 5.8); $P = 0.05$] and $H_{min}$ [ID$_{20}$ + 30 min, 18.6 cm H$_2$O/ml (SD 1.6)] vs. ID$_{20}$, 22.1 cm H$_2$O/ml (SD 1.7); $P < 0.001$] were significantly higher than baseline after 30 min, whereas $H_20$ [ID$_{20}$ + 30 min, 98.2 cm H$_2$O/ml (SD 6.3)] vs. ID$_{20}$, 101.2 cm H$_2$O/ml (SD 7.0); $P = 0.44$] and $H_{min}$ [ID$_{20}$ + 60 min, 22.0 cm H$_2$O/ml (SD 2.2); $P = 0.68$] had returned to baseline levels (Fig. 6).

Repeated Inflations to 40 cm H$_2$O

PV curves. The first part of the PV curve (up to ~30 cm H$_2$O) for the ID$_{40}$’ + 30 min was higher than the initial PV curve obtained from the ID$_{40}$. Above this point the curves coincided. The PV curve for the inflation to 40 cm H$_2$O 60 min after the initial inflation coincided almost perfectly with the PV curve from the ID$_{40}$’ + 30 min (Fig. 7).

Dynamic elastance. As with the PV curves, inflation to 40 cm H$_2$O caused a shift in the volume dependence of H that was still evident upon inflation to 40 cm H$_2$O after 30 min of ventilation. The H vs. volume curve for the inflation to 40 cm H$_2$O after 60 min of ventilation overlaid the curve obtained from the inflation after 30 min (Fig. 7).

Repeated Inflations to 20 cm H$_2$O

PV curves. There was no difference between TGV$_0$ after 30 [ID$_{20}$ + 30 min, 0.20 ml (SD 0.02)] or 60 min [ID$_{20}$ + 60 min, 0.19 ml (SD 0.02)] of ventilation compared with baseline [ID$_{20}$, 0.23 ml (SD 0.07)] ($P = 0.21$) (Fig. 8). However, after
30 min of ventilation, TGV[20, 30 min, 0.67 ml (SD 0.04); \( P = 0.003 \)] had decreased below baseline levels [ID[20, 0.76 ml (SD 0.08)] and even further after 60 min [ID[20, 60 min, 0.61 ml (SD 0.08); \( P = 0.013 \)] of ventilation (Fig. 8).

**Dynamic elastance.** The changes observed in the PV curve over time corresponded with an increase in \( H_{20} \) [ID[20, 30 min, 113.7 cm H\(_2\)O/ml (SD 20.7) vs. ID[20, 102.6 cm H\(_2\)O/ml (SD 13.5); \( P = 0.05 \)] and \( H_{min} \) [ID[20, 30 min, 28.3 cm H\(_2\)O/ml (SD 4.2) vs. ID[20, 23.3 cm H\(_2\)O/ml (SD 1.7); \( P = 0.02 \)] after 30 min of ventilation and a further increase in \( H_{min} \) after 60 min of ventilation [ID[20, 60 min, 35.8 cm H\(_2\)O/ml (SD 5.6); \( P = 0.005 \)] (Fig. 8). There was no change in \( H_0 \) (\( P = 0.07 \)).

**DISCUSSION**

Inflating mouse lungs beyond 20 cm H\(_2\)O resulted in fundamental changes in the baseline PV curve and the volume dependence of tissue mechanics.

In the absence of being able to measure starting lung volume to construct PV curves, previous studies designed to examine this phenomenon have involved complete degassing of the lungs before inflation (15). Consequently, large pressures were required to reopen the lungs (>20 cm H\(_2\)O) for the first inflation maneuver. Since such a maneuver is not used in studies where lung volume history is standardized, it is unclear whether inflation to these pressures has an impact on subsequent lung function measurements. In the present study, we were able to measure TGV, which allowed construction of absolute PV curves without prior degassing of the lung. Serial ID maneuvers demonstrated a classic sigmoidal PV curve when the peak pressure applied to the lung was 20 cm H\(_2\)O or below. However, once 20 cm H\(_2\)O was exceeded, the horizontal asymptote in the PV curve was no longer apparent with a second knee appearing in the PV curve, as shown previously (9, 15). As the maximum \( P_{rs} \) was increased, the volume excursion increased with no apparent limit to total lung volume. The deflation limbs of these PV curves ran parallel to each other, resulting in increased hysteresis in the PV curve with increasing maximum pressure.

Our study has clearly shown that a \( P_{rs} \) of about 20 cm H\(_2\)O represents a “critical point” in the inflation of the mouse lung whereby something fundamental changes in the processes or structures that are involved. This observation may have implica-

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**Fig. 7.** PV (Left) and H vs. TGV (Right) curves for mice subjected to an ID maneuver starting at 0 cm H\(_2\)O transrespiratory pressure up to 40 (open circles) cm H\(_2\)O. Mice were then exposed to ID maneuvers up to 40 cm H\(_2\)O transrespiratory pressure after 30 (gray triangles) and 60 (gray diamonds) min of ventilation. Data are the mean curves.

**Fig. 8.** PV (Left) and H vs. TGV (Right) curves for mice subjected to an ID maneuver starting at 0 cm H\(_2\)O transrespiratory pressure up to 20 (open circles) cm H\(_2\)O. Mice were then exposed to ID maneuvers up to 20 cm H\(_2\)O transrespiratory pressure after 30 (gray triangles) and 60 (gray diamonds) min of ventilation. Data are the mean curves.
tions for studies in mice where lung volume history is standard-
ized. Evidence for the changes that occur at 20 cm H2O arises
from a number of results including the significant decrease in
baseline tissue elastance we observed. In the absence of a signif-
icant change in TGV at 0 cm H2O Prs, this suggested that the lung
parenchyma was more compliant after this maneuver. As a result,
a larger volume of air was inhaled for the same pressure excursion
in subsequent maneuvers. It was also clear that this phenomenon
could be largely attributed to the response of the lung paren-
chyma. Although there was some hysteresis in the volume depen-
dence of Req in the ID maneuver, this can be explained by the fact
that a much lower Prs was acting on the airways for the equivalent
TGV during deflation compared with inflation. There was evi-
dence from our data that something fundamental was changing in
the way the lung elastance responded to volume excursion at
pressures beyond 20 cm H2O with a sharp change in the trajectory
of the H vs. volume curves. At Prs approaching 20 cm H2O, there
was a relatively constant increase in H, which we have described
previously and which may be attributed partly to the progressive
 stiffening of the lung (13, 4, 1). However, beyond 20 cm H2O H
and G stopped increasing abruptly and remained relatively con-
stant with no change in dynamic elasticity or viscosity of the lung
despite a relatively large change in lung volume.

We investigated three separate strategies for “recovering” the
changes in lung mechanics observed following inflation above
20 cm H2O: 1) partially degassing the lung, 2) applying a
negative transrespiratory pressure such that lung volume fell
below FRC, and 3) allowing spontaneous recovery. All of these
strategies, to varying degrees, were able to recover the shift in
the PV curve and volume dependence of tissue elastance. The
most effective method was degassing the lung, which resulted
in recovery of all the characteristic points on the H vs. TGV
curve back to baseline levels. Although a negative transrespi-
atory pressure maneuver was not able to recover the H vs.
TGV curve and V20 completely, all of these parameters had
shifted in the direction of the original (ID20) values. Similarly,
after 60 min of regular ventilation, although not all parameters
had returned to baseline levels, given enough time, the changes
in lung mechanics observed following inflation to 40 cm H2O
spontaneously revert to baseline conditions.

There are a number of possible explanations for the appear-
ance of a second knee in the mouse PV curve. First, one
obvious explanation could be the contribution of the chest wall
since we measured Prs rather than transpulmonary pressure
throughout this study. However, studies in open-chested mice
(Z. Hantos, unpublished observations) have demonstrated that
the second knee is still present, which suggests that the con-
tribution of the chest wall is unlikely to explain this phenom-
emon. Alternatively, the double sigmoid PV curve may be a
result of the appearance of a “second” lung. By this we mean
a portion of the lung that is not recruited until the critical
pressure (20 cm H2O) is reached. This could be in the form of
collapsed lung units that are not recruited until 20 cm H2O
(recruitment of atelectic regions of the lung) or a second
population of alveoli, a possibility raised by Soutiere and
Mitzner (15), that are present but do not expand until this
pressure is reached. These possibilities are consistent with the
observations about the behavior of lung mechanics in this study
following inflation to 40 cm H2O. The upward shift in the PV
curve and the widening of the H vs. TGV curve, if this
explanation is true, could be a result of the stability of these
lung units/alveoli once they have been opened by inflation
above 20 cm H2O. Upon subsequent inflations they then open
uniformly with the other regions of the lung that are usually
inflated when Prs is below 20 cm H2O. As a result, the stable
part of the H vs. TGV curve, which is roughly associated with
the central part of the PV curve where the bulk of the volume
excursion occurs, is stretched because the previously opened
lung units inflate smoothly during subsequent inflations, result-
ing in a more compliant lung and a larger volume of inflated
air.

This hypothesis is also consistent with the response to the
recovery maneuvers. By degassing the lungs or forcing the
lung below FRC, the reacquired lung units/alveoli would
completely collapse, thus resetting the system to its original
condition with 20 cm H2O needed to reopen these “lost”
units. However, this explanation also requires that mice
have an unused lung reserve that is not accessible under
baseline conditions unless Prs exceeds 20 cm H2O. This
seems like an unusual biological system, and one would
have to wonder under what circumstance a mouse would be
required to access this reserve. If there were a second
population of alveoli or previously atelectic regions of the
lung were being reopened, one would expect morphological
evidence of an increase in alveolar number or evidence of a
new population of alveoli (subset with a smaller size than the
others) at pressures beyond 20 cm H2O. A very recent
study by Namati and colleagues (10) using confocal imaging
of isolated lung preparations found that, at transrespiratory
pressures above 20 cm H2O, the alveoli appeared to become
smaller and more numerous, suggesting that a “secondary”
population of alveoli is recruited at high Prs.

Critical to this argument is that Prs a 20-g mouse is capable
of generating. Equations for allometric scaling of TLC for
mammals (16) would suggest that the TLC for a mammal of
this size should be ~0.85 ml, which is similar to the TGV20
observed in our study for the ID20 maneuver and suggests that,
by inflating beyond this pressure, we may be inducing a
response that is a result of a process beyond the normal
operating range for a mouse lung in vivo. If we are operating
in a pressure range that may not be physiological for a mouse,
then it is entirely possible that we are simply exceeding the
structural limit of the lung and it is possible to inflate mice to
this extent due to their highly compliant chest wall (8). There
is some data reporting transpulmonary pressures up to 30 cm
H2O in spontaneously breathing mice in response to inhaled
irritants (17) although these pressures only seemed to result in
volume excursions of 0.2 ml; data from Leith’s studies from
the 1970s (9) suggest that small animals are quite capable of
producing large transrespiratory pressure with a fruit bat pro-
ducing a spontaneous breath with a Prs up to 60 cm H2O. If 20
cm H2O did represent a critical pressure limit, then one would
predict that applying a pressure twice this amount would result
in severe structural damage that would alter the mechanical
properties of the lung. However, we had no evidence of irrever-
sible damage to the system in our data with simple maneuvers,
such as degassing the lung and recovering the lung to its baseline
condition from a lung mechanics point of view. There was also a
high level of consistency in the repeated inflations to a Prs of 40
cm H2O. Similarly, previous studies that have fixed the lung at Prs
values up to 60 cm H2O showed no evidence of gross structural
damage upon examination of histology (15).
An alternative explanation for this phenomenon can be found by considering surface tension at the alveolar surface. A large portion of the mechanical properties of the lung can be explained by the surface tension at the air-liquid interface (2). As a result, it is entirely possible that by inflating the mice to such high lung volumes we are altering the surface acting forces in the lung in such a way as to alter the compliance of the lung. This could occur in a number of ways, either by stimulating the release of additional surfactant by mechanical stretch of alveolar type II cells (19, 11, 3) or by other physical mechanisms, such as redistribution of the available surfactant pool or a change in the surface tension of the air-liquid interface, resulting from thinning of this layer at high lung volumes (20). There is some evidence to support this mechanism as a potential explanation in other murine species. Nicolas et al. (12) have shown that exercising rats will increase their tidal volume by up to 30%, resulting in a significant increase in their surfactant pool. It was proposed that a large portion of this increased surfactant pool originated from direct stimulation of alveolar type II cells. This hypothesis is consistent with the primary observations in this study that changes in surface acting forces could increase the compliance of the lung, resulting in increased TGV20 and decreased tissue elastance. The recovery of these parameters by degassing and negative trans-respiratory pressure requires that the reverse process occurs during these maneuvers such that compliance is decreased back to baseline levels. Similarly, the spontaneous recovery of lung mechanics back to baseline levels could be attributed to the progressive uptake of surfactant. However, although this hypothesis provides an explanation for the effect of inflation above a P50 of 20 cm H2O on the subsequent changes in lung mechanics, it does not provide an explanation for the appearance of the second sigmoid in the PV curve, which is more consistent with a recruitment phenomenon.

As suggested above, it is possible that this inflection point in the PV curve is a result of the recruitment of a second population of alveoli by way of a mother/daughter alveolar structure, whereby the daughter (secondary) population of alveoli are recruited at high pressure via the pores of Kohn (10). However, this assertion is based on data using techniques that are only able to visualize the peripheral surface of the lung, which may not be able to take into account changes in alveolar shape and, as such, may not be representative of the changes occurring in the lung as a whole. Another alternative is that, rather than recruiting additional alveolar “units,” the recruitment that occurs at the start of the second sigmoid in the mouse PV curve is a result of a change in the configuration of the existing alveoli. The configuration of each individual alveolus, once they begin to inflate, changes considerably during a single ID event. The change in configuration of the septa in an individual alveolus is dependent on the counteracting forces from the tethering of the septa to adjacent alveoli and the surface tension as a result of the fluid lining the alveolar surface. The interplay between these forces, in conjunction with the irregular shape of the alveolus, is thought to cause the septal folding observed in corners of the alveoli at moderate distension pressures (18). In light of this observation, one potential explanation for the appearance of the second sigmoid in the mouse PV curve is that 20 cm H2O is the point where these regions begin to unfold. This hypothesis is consistent with a number of the observations in this study. First, it provides an explanation for the basic phenomenon in which different elements of the lung are recruited at 20 cm H2O. It may also explain the subsequent changes in lung mechanics observed following inflation beyond 20 cm H2O. If these regions become unfolded during an inflation, then the fluid in these folds is likely to be distributed more evenly throughout the alveolus. Under these circumstances these regions are likely to unfold at lower P50 in subsequent maneuvers, resulting in a more compliant lung.

Although it is possible that a number of mechanisms could be responsible for these observations, it is clear that fundamental changes in the mechanical properties of the mouse lung occur at a transrespiratory pressure around 20 cm H2O. Further structural and functional studies are warranted to clarify the mechanisms involved.

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