Estimating the diameter of airways susceptible for collapse using crackle sound

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Majumdar A, Hantos Z, Tolnai J, Parameswaran H, Tepper R, Suki B. Estimating the diameter of airways susceptible for collapse using crackle sound. J Appl Physiol 107: 1504–1512, 2009. First published September 3, 2009; doi:10.1152/japplphysiol.91117.2008.—Airways that collapse during deflation generate a crackle sound when they reopen during subsequent reinflation. Since each crackle is associated with the reopening of a collapsed airway, the likelihood of an airway to be a crackle source is identical to its vulnerability to collapse. To investigate this vulnerability of airways to collapse, crackles were recorded during the first inflation of six excised rabbit lungs from the collapsed state, and subsequent reinflations from 5, 2, 1, and 0 cmH2O end-expiratory pressure levels. We derived a relationship between the amplitude of a crackle sound at the trachea and the generation number (n) of the source airway where the crackle was generated. Using an asymmetrical tree model of the rabbit airways with elastic walls, airway vulnerability to collapse was also determined in terms of airway diameter D. During the reinflation from end-expiratory pressure = 0 cmH2O, the most vulnerable airways were estimated to be centered at n = 12 with a peak. Vulnerability in terms of D ranged between 0.1 and 1.3 mm, with a peak at 0.3 mm. During the inflation from the collapsed state, however, vulnerability was much less localized to a particular n or D, with maximum values of n = 8 and D = 0.75 mm. Numerical simulations using a tree model that incorporates airway opening and closing support these conclusions. Thus our results indicate that there are airways of a given range of diameters that can become unstable during deflation and vulnerable to collapse and subsequent injury.

THE ALVEOLAR GAS EXCHANGE regions deep in the lung are supplied with fresh air through the branching structure of the airways (29). In the normal lung, airways remain patent during breathing (20). In the diseased lung, airways can collapse due to excessive fluid accumulation (15), increased smooth muscle contraction (10), decreased parenchymal tethering (30), or some combination of these mechanisms. An accurate localization of closure along the airway tree is important, since the larger the size of the occluded airway, the more severely it affects gas exchange. Using histological methods, the site of closure has been estimated to be in the small airways, having diameters of ~1 mm (13). Computed tomographic imaging revealed, however, that the direct application of methacholine can result in the closure of much larger airways (4). While the first method is in vitro, the second can image only a few airways, and hence both are limited in their ability to assess the number and location of closed airways.

When a closed airway reopens during inflation, a crackle sound is generated (2, 5). Crackles are “explosive” sound energy packages, consisting of a sharp, initial, negative deflection in pressure, followed by some low-frequency ringing (11), and can be measured either on the chest wall (21) or at the trachea (2). The presence of crackle sound during inspiration signifies airway openings, which, in turn, imply that airway closure occurred during the previous expiration. Recently, we presented crackle sound data and a theory that relates the amplitude of crackle sound measured at the trachea to the attenuation of the crackle as it passes through successive bifurcations (2). This attenuation, in turn, can be expressed in terms of the diameter of the airway from which the crackle sound was originated (2). In our present study, we extended this theory to determine the number and location of collapsed airways. We measured the pressure-volume (P-V) curves and crackle sound in isolated rabbit lungs during inflation from several end-expiratory pressure (EEP) levels. From these data, we estimated the distribution of airway generations and the corresponding airway diameters vulnerable for closure as a function of EEP.

METHODS

Animal preparation. New Zealand White rabbits (N = 6, body weights: 2.5–3.0 kg) were anesthetized with pentobarbital sodium (50 mg/kg body wt) and exsanguinated by severing the abdominal artery. The intact lungs were then cannulated using a 3.5-mm tube and excised. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Indiana University.

Inflation maneuvers. Crackle sound and P-V curves were simultaneously measured during inflation maneuvers. The excised lungs were suspended in a 2-liter glass bottle, to maintain the humidity of the lung surface and minimize the contamination of the acoustic detection of lung sound by the environmental noise. The bottle was open to atmosphere via a thick-walled Silastic tube (length: 1 m, inner diameter: 3 mm), providing further acoustic insulation of the apparatus. The tracheal cannula was led through the lid of the bottle and attached to a Y-piece, one arm of which contained a commercial microphone (model MCE-2000, Monacor International, Bremen, Germany; outer diameter: 5 mm), and the other arm was connected to an infusion pump (model no. 55-2222, Harvard Apparatus, Boston, MA). Additionally, two 60-ml syringes were placed in parallel with the pump via a polyethylene tube (length: 1 m, inner diameter: 2 mm), which served as a pneumotachograph, while the syringe-tube system acted as a low-pass mechanical filter suppressing the unevenness of the flow delivered by the pump. The pressure drop across the tube and the tracheal pressure (P) with respect to atmospheric pressure were measured with Validyne MP-45 differential transducers (±2 and ±30 cmH2O, respectively). Flow (V) obtained as the pressure drop across the tube divided by the resistance of the tube during inflation and P were low-pass filtered at 25 Hz and sampled at a rate of 128 Hz, with a custom-made data-acquisition system run by a personal computer.
The preamplified microphone signal was fed into another computer and was recorded by using a GoldWave sound editor (version 5.12, GoldWave, St. John’s, Newfoundland, Canada) at a sampling rate of 22,050 Hz. The infusion pump drove two 60-ml syringes in parallel, and the rate was adjusted to \( V = 40 \) ml/min. Each inflation started with a 5-s baseline recording to establish \( V = 0 \) ml/min and either lasted for a maximum of 3 min or was stopped when \( P \) reached 35 cmH2O. The first inflation started from \( P = 0 \) cmH2O, i.e., the collapsed state of the lung. Following the inflation, the lungs were kept at \( \sim 30 \) cmH2O for 1 min and slowly deflated (\( \sim 1 \) min) to an EEP of 5 cmH2O. After another 1-min period of equilibration, the inflation-deflation maneuver was repeated with successive EEP levels of 2, 1, and 0 cmH2O. Inflation volume was obtained by numerical integration of \( V' \).

Detection of crackle sounds. The sound recordings were first high-pass filtered at 2 kHz with the GoldWave sound editor to improve the temporal resolution of crackles by enhancing the sharp initial transients of the crackles and suppressing the lower frequency ringing (11, 23). After the high-pass filtering, the maximum level of background noise was estimated in each recording from the baseline ringing (11, 23). After the high-pass filtering, the maximum level of background noise was estimated in each recording from the baseline and the end-inflation segments that contained no crackles, and a threshold was determined for the minimum discernable crackle amplitude. The recordings were then divided into intervals of length \( \Delta T \).

For each interval \( i \), the sound energy \( (\Delta E_i) \) was computed as the sum of the squared amplitude. If the ratio \( \Delta E_{i+1}/\Delta E_i \) computed from two successive intervals exceeded a preset ratio \( \lambda \), a crackle was registered. The \( \Delta E \) values were computed from subsequent intervals, while \( \Delta E \) increased monotonically (i.e., \( \Delta E_i < \Delta E_{i+1}, \ldots \) ). The amplitude \( s \) of the crackle was then defined as the maximum sound amplitude within the last interval. Based on preliminary investigation, whereby the identified crackles were confirmed by listening to segments of the original unfiltered recordings, the parameters \( \Delta T = 0.35 \) ms and \( \lambda = 3 \) provided the most reliable crackle identification results with the minimum number of missed or falsely identified acoustic events.

Estimating the generation number of collapsed airways. To analyze the crackle time series, we invoked a previously developed model (2). We assume that each crackle is generated with a constant amplitude \( s_0 \) in an opening airway segment, called the source. At each bifurcation between the source and the trachea, the crackle amplitude is attenuated by a factor \( b \), which can be expressed in terms of the geometry of the bifurcation (Fig. 1) as follows:

\[
b = \frac{2A_1}{A_0 + A_1 + A_2}\]

(1)

where \( A_0 \) is the cross-sectional area of the parent airway into which the attenuated crackle propagates; \( A_1 \) is the cross-sectional area of the daughter airway from which the crackle arrives; and \( A_2 \) is the cross-sectional area of the other daughter airway. We further assume that the airway tree is symmetric with \( A_1 = A_2 \) and that the factor \( b \) is the same for all bifurcations. Using literature values, we obtain an average value of \( b = 0.52 \) for the rabbit lung (18, 24). After passing \( n \) bifurcations, the crackle reaches the trachea, and its amplitude is attenuated by \( b^n \). We can thus calculate the generation number \( n \) from the amplitude \( s \) at the trachea of each identified crackle as,

\[
n = \frac{\log s - \log s_0}{\log b}\]

(2)

Since we assume that each crackle has the same amplitude \( s_0 \) at the source, we use the largest recorded crackle amplitude as a first estimate of \( s_0 \). Due to the attenuation of the sound (Eq. 1), the largest recorded crackles are generated at the generations closest to the trachea. However, only one or two distinct crackles per animal could be attributed to the lowest generation, and this varied from animal to animal. On the other hand, the smallest discernable crackles from all inflations should have similar amplitudes in all animals, as these amplitudes are determined by the threshold of the detection algorithm. In addition, there are thousands of crackles generated from the deeper generations. Hence, the average of these small crackles provides a more reliable estimation of \( s_0 \), and we adjusted the value of \( s_0 \) such that the maximum computed generation numbers from all inflations coincided.

Analysis of crackle statistics. Using Eq. 2, we calculated the histogram of generation numbers of crackle sources for each EEP, combining the identified crackles from the inflation data of six rabbit lungs. We note that each bin of the histogram corresponded to a different integer generation number, which is obtained by rounding the value of \( n \) calculated using Eq. 2. Since, during inflation, a crackle was generated only if the airway collapsed during the preceding deflation, the number of crackles detected also signifies the number of airways that were closed. We can thus convert each histogram to a probability curve by dividing the number of crackles associated with each generation \( n \) by the total number of branches at that generation, \( 2^n \). This probability curve represents the likelihood that a branch at generation \( n \) collapses during deflation to a given EEP. Preliminary calculations, however, resulted in probabilities larger than unity for certain \( n \) values. The likely reason for this is that the central airways with significant cartilage content do not collapse during a passive deflation (13) and thus do not produce crackles. To take this effect into account, we increased all generation numbers in a stepwise manner until all closure probabilities became smaller than unity. Finally, the fraction of collapsed airways in the lung was calculated by dividing the total number of recorded crackles for a particular inflation by the total number of airways, after accounting for the shift in \( n \) due to the central airways.

Effects of generation dependence of threshold pressures on crackles. We investigated the effects of generation dependence of opening (\( P_c \)) and closing threshold pressures (\( P_c \)) on the pattern of airway collapse and reopening. Both the generational trend of the threshold pressures, as well as the amount of overlap among threshold pressures at consecutive generations, have an impact on crackle production. When the \( P_c \) of adjacent generations have sufficient overlap in their distributions, a parent airway can collapse earlier than its daughter airways. In such a case, the daughter airways are “protected” from collapse, as they are not subject to further pressure changes experienced by the open region of the lung. The collapsed parent thus traps air in the subtended region, reducing the fraction of collapsed seg-
ments and subsequent crackle production at higher generation numbers. We also address our assumption that the amplitude of every crackle sound at the source is identical, irrespective of the generation number of the source airway and its opening pressure. We study the case where the source amplitude of the crackle sound is proportional to the opening pressure of the airway. In the case of generation-dependent threshold pressure distributions, this also makes the crackle sound amplitude generation dependent.

We construct a symmetric airway tree of $N = 11$ generations. Each airway at generation $n$ is assigned a $P_0$ drawn from a distribution $\Pi_n$ dependent on the generation number $n$. The distributions $\Pi_n$ are uniform between an upper and a lower limit given by the following interval:

$$\left[ \frac{g^2 - g_{\text{min}}}{g_{\text{max}} - g_{\text{min}}}, \frac{g_{\text{max}} - g_{\text{min}}}{g_{\text{max}} - g_{\text{min}}} \right]$$

These distributions are constructed such that for $n = 11$, the upper bound is always 1, and the lower bound is 0, i.e., for the last generation of the tree, $P_0$ is distributed between 0 and 1. As $g$ increases from 0.95 to 1.15, the lower and upper bounds of $\Pi_n$ become increasingly more generation dependent (see Fig. 6, symbols). Note that it is possible that $P_0$ takes negative values. Once an airway is assigned a $P_0$, it is also assigned a $P_C$ drawn from an uniform distribution in the range $[0, P_0]$, only if $P_0 > 0$ (see Fig. 6, dashed lines). If $P_0$ were negative, then the airway was not allowed to collapse. This ensures that, for each collapsed airway, $P_C < P_0$, and thus, once an airway opens during inflation, it does not collapse until the subsequent deflation.

To compare the crackles generated in the above model to a model with closures at preferred generations, we incorporated preferential closures at three consecutive generations $n^* = 6, 7, 8$. For $n^* = 7$, $PC$ was distributed between $[0.75P_0, P_0]$, while, for $n^* = 6$ and 8, $PC$ was distributed between $[0.5P_0, P_0]$. For all other generations, $n \neq n^*$, $PC$ was distributed between 0 and $P_0$. This procedure resulted in a peak in the mean closing pressures as a function of $n$ (see Fig. 6, thick shaded line).

To assess the error caused by the assumption that all crackles have unit amplitude at the source, we used a model, where the amplitude of a crackle was proportional to the opening pressure of the source airway. The attenuation of the crackle amplitude at the trachea was unity, and the difference between the actual generation number of the source airway and the parent are held constant throughout the tree. Airway branching ceases when the diameters ($D$) reach a cut-off value $D_0$. The difference between the two diameter ratios leads to different amounts of attenuation for crackles entering the parent branch from the major or the minor daughter. These attenuations can be obtained by inserting the diameter ratios into Eq. 1:

$$b_{\text{maj}} = \frac{2k_{\text{maj}}^2}{1 + k_{\text{maj}}^2 + k_{\text{min}}^2} \quad \text{and} \quad b_{\text{min}} = \frac{2k_{\text{min}}^2}{1 + k_{\text{maj}}^2 + k_{\text{min}}^2}$$

Using a computer-generated tree structure and assuming $s_0 = 1$ throughout the tree, we calculated the crackle amplitudes at the trachea corresponding to each airway segment serving as a source. The relation between $s$ and $D$ can then be used to estimate the diameter of previously collapsed airway.

### RESULTS

With the elevation of EEP, the number of crackles significantly decreased (Table 1). However, during the second inflation from EEP of 0 cmH$_2$O, the number of crackles was even higher than that obtained during the inflation from the collapsed state. A set of representative P-V curves during inflations from different EEP levels is shown in Fig. 2. Since the absolute lung volumes were not known, the P-V curves of the successive maneuvers were adjusted at ~30 cmH$_2$O by assuming that the end-inflation segments were identical. For inflations from the collapsed state (first inflation), as well as the inflation from 0-cmH$_2$O EEP (fifth inflation), the P-V curves show a prominent inflection point, indicating significant recruitment by avalanches of airway openings (27).

Figure 3 shows the time series of crackle amplitudes recorded during the same inflations as in Fig. 1. The generation numbers corresponding to the crackle amplitudes were calculated using $Eq. 2$. For simplicity, we first assume that the largest crackle in each inflation came from the trachea. Consequently, the maximum generation number was found to be 11. As described in METHODS, the generation numbers $n$ in each inflation were then shifted such that the smallest crackles were

<table>
<thead>
<tr>
<th>End-expiratory Pressure, cmH$_2$O</th>
<th>No. of crackles</th>
<th>4.553±559</th>
<th>105±108</th>
<th>179±135</th>
<th>2.944±2.732</th>
<th>17.004±6.579</th>
</tr>
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<tr>
<td>0</td>
<td>5</td>
<td>10</td>
<td>0</td>
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<tr>
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<tr>
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Values are means ± SD.

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assigned \( n = 11 \). The individually shifted generation numbers were combined to calculate the total histogram of the generation numbers for each EEP (Fig. 4).

To calculate the probability of closure for airways at a given generation, the histograms in Fig. 4 were further shifted by five generations and normalized as described in METHODS. The resulting probabilities \( p(n) \) are shown in Fig. 5A for three different EEP levels. The probabilities for inflations from higher EEP levels were nearly zero and are not shown. Note that the curves in Fig. 5A are not probability distributions, and hence the area under them is not equal to unity. The inset demonstrates how the peak of \( p(n) \), the airway generation with the highest likelihood of closure, varies with EEP. With decreasing EEP, smaller airways in the tree become more vulnerable to collapse. Since the small airways make up a large fraction of the airway tree, the total number of collapsed airways, and hence the number of crackles recorded during subsequent inflations, increases as EEP decreases (Fig. 5B).

Figure 6 shows the distribution of opening pressures \( P_O \) as a function of \( n \) for \( g = 0.95 \) (Fig. 6A) and \( g = 1.15 \) (Fig. 6B). The solid symbols show the mean opening pressures \( \langle P_O \rangle_n \), while the error bars show the range. The dashed lines show the mean closing pressures \( \langle P_C \rangle_n \) as a function of \( n \), while the thick shaded line corresponds to \( \langle P_C \rangle_n \) for the case of preferential closures at \( n^* = 6, 7, \) and 8. For every \( g \), preferential collapse creates a local peak in \( \langle P_C \rangle_n \) around \( n = n^* \). Thus airways at these generations tend to collapse at higher inflation pressures than airways in the generations directly above or below \( n^* \).

Figure 7 shows the mean error in the estimated generation number, assuming unit crackle amplitudes in their source airways compared with the model where the crackle amplitudes are proportional to the opening pressures of their source airways. The standard deviations of errors over the entire

Fig. 3. Examples of crackle amplitude time series during inflations from the collapsed state and 5-, 2-, 1-, and 0-cmH\(_2\)O EEP (PEEP). The time series correspond to the same inflations as in Fig 1. The amplitudes are normalized to maximum value of crackle amplitudes and displayed on a logarithmic scale.

Fig. 2. A representative example of pressure-volume (P-V) curves of an excised rabbit lung from the collapsed state, and subsequent inflations from end-expiratory pressure (EEP) levels of 5, 2, 1, and 0 cmH\(_2\)O. The inflection point is prominent along the 1st and 5th inflations.
airway tree for 1,000 independent simulations are shown as error bars. We note that, for $0.95 < g < 1.15$, the mean error is $< 2$, which allows us to claim reasonable confidence in our estimation of generation numbers from measured crackles in Fig. 5A.

Figure 8 shows the fraction of airways that were collapsed after deflation to 0 EEP for different values of $s$ in models without (A) and with (B) a preferential generation for closure. Note that, for $g = 0.95$, the probability of closure slightly decreases above $n = 7$. This is due to air trapping, i.e., protective effect of closures of airways with $n < 6$. As $g$ increases, the generation dependence of opening pressures is enhanced, and, consequently, there is less air trapping. Thus a larger fraction of airways with $n > 7$ is collapsed. When airway tree for 1,000 independent simulations are shown as error bars. We note that, for $0.95 < g < 1.15$, the mean error is $< 2$, which allows us to claim reasonable confidence in our estimation of generation numbers from measured crackles in Fig. 5A.

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preferential closure was built into the model, we observe a prominent peak in the estimated probability of closure (Fig. 8B). The height of this peak is larger at smaller values of \( g \). This leads us to believe that the observed peak in measured data is indeed a consequence of a higher probability of closure of airways at the corresponding generation.

The relationship between the amplitude \( s \) of a crackle at the trachea as a function of the diameter \( D \) of the source obtained from the simulations using an asymmetrical model of the rabbit airway tree is shown in Fig. 9. The approximate linear relation between \( s \) and \( D \) on a log-log graph suggests the following power law form:

\[
D(s) \propto s^{1.469}
\]

with an exponent \( \xi = 0.469 \). This suggests that, once a crackle is measured to have amplitude \( s \) at the trachea, Eq. 4 can be used to associate it with a source airway having a diameter \( D \). However, Eq. 4 cannot directly be applied to measured crackles, since \( s \) and \( D \) in the model were normalized to unity at the trachea. As pointed out in relation to Fig. 5A, the largest crackles were assumed to come from generation 5. The mean diameter of rabbit airways at generation 5 (i.e., 1.32 mm) from Ramchandani et al. (24) was used in Eq. 4 to scale \( D \) to absolute values. We thus estimated the probability of closure \( p(D) \) as a function of real diameter (Fig. 10), which suggest that \( p(D) \) is qualitatively similar to \( p(n) \) in Fig. 5A, with the
probability of closure being the highest during the fifth inflation. However, the $p(D)$ is significantly skewed toward the smaller diameters, whereas $p(n)$ is nearly symmetric.

**DISCUSSION**

Airway closure is known to occur in many lung diseases (17, 26) and thought to play a critical role in the development of abnormal lung function by introducing heterogeneity and hindering gas exchange (9). The localization of closure along the airway tree has been difficult and limited to the larger airways due to insufficient resolution of current imaging technologies. In this study, we estimated the serial distribution of airway closure as a function of EEP, utilizing the acoustic properties of crackles. The main findings of the study are that 1) the number of crackles observed depend both on EEP and lung volume history; 2) the probability of closure of an airway displays a maximum at a given generation number or diameter; 3) the probability of closure as a function of EEP and lung volume history; and 4) the fraction of collapsed airways is also a function of lung volume history and decreases with increasing EEP.

With regard to the effect of lung volume history on the number of crackles detected (Table 1), we can compare the inflation from the collapsed state and the fifth inflation, both of which were initiated from EEP = 0 cmH$_2$O. According to Fig. 3, crackles cease to arrive before reaching the end of full inflation to 30 cmH$_2$O, except during the inflation from the collapsed state. In this case, the recruitment may not have been completed during the inflation, and hence not all of the crackles were recorded. A more likely explanation is, however, related to amount of trapped gas in the lung (6). Figure 2 demonstrates that there was significantly more trapped air in the Lung before inflation from EEP = 0 cmH$_2$O than from the collapsed state. The mechanism of crackle production likely requires the rupture of a liquid bridge with trapped air behind it (16). Therefore, the small amount of trapped air in the collapsed lung could limit the number of detectable crackles generated during inflation.

The results regarding the vulnerability of airways to collapse rely on several important assumptions about the generation of crackles and the propagation of the crackle sound through the airway tree. The primary assumption necessary for the calculations is that all crackles have identical amplitudes $s_0$ when generated at the source. In reality, crackles likely have a distribution of amplitudes at the source, depending on the airway size and surfactant properties, as well as the amount of air trapped behind them. It has been shown that the distribution of crackle amplitudes at the trachea follows a power law and the exponent of this power law is primarily determined by the geometric factor $b$ in Eq. 1 (2). Furthermore, it was shown that including a distribution of crackle amplitudes at the source does not alter the exponent of the power law. Hence, it is expected that random fluctuations in $s_0$ would have a minor effect on the estimated generation numbers or diameters of airways based on crackles measured at the trachea.

When a liquid bridge breaks, the system comprising of the airway lining liquid and the elastic airway wall assumes a configuration with lower free energy. The excess free energy is primarily released as sound. The amplitude of the generated sound would thus reflect the sudden free energy drop during the rupture of the bridge. Alencar et al. (3) found that the free energy drop on rupture of a liquid bridge was only weakly related to the bridge dimensions, although the geometry considered was different from the geometry of a liquid bridge in an airway. This is consistent with our assumption that the source amplitude of a crackle sound is insensitive to the properties of the airway in which it is generated. In addition, previous publications related to crackle sounds during airway opening have successfully modeled several key experimental results using the assumption that the sound amplitudes were either constant or distributed without a generation dependent bias (1, 2).

The crackle amplitude at the source has been associated with the critical opening pressure of a liquid bridge within a closed airway (2). The critical opening pressure was shown to depend on surfactant composition and airway diameter (8) and airway wall stiffness (22). These factors can vary significantly within airways of the same generation number (18). Assumptions about the generation independence of the threshold pressure distributions have been used in several studies (2, 19). However, generation dependence of $P_c$ distributions could significantly influence the amplitude of crackle sounds. We thus
examined the effect of generation-dependent threshold pressure distribution on the recorded crackle amplitudes when the initial amplitude of a crackle was proportional to the opening pressure of the source airway. Figure 7 shows that incorporating generation-dependent threshold pressure distributions causes an average error of about two generation numbers in estimating \( n \) using our method.

Generation dependence is also the key factor that can affect our interpretation of airway vulnerability via a different phenomenon. When an airway collapses, all open airways in the subtree subtended by it trap air and do not collapse during subsequent deflation. Thus the closure of an airway at small \( n \) protects airways from closure at larger \( n \). The protected airways do not generate crackles during the subsequent inflation phase. We assessed the effect of such protective effect on crackle production for different generation dependence of threshold pressures. Figure 8A shows the fraction of airways at generation \( n \) that collapse at end expiration for different values of \( g \). As \( g \) increases, the generation dependence of the \( P_C \) increases (dashed line in Fig. 6A), and airways at larger \( n \) tend to close earlier than airways with smaller \( n \). This leads to less trapping and less airways protected from collapse. Interestingly, for \( g = 0.95 \), there is a mild enhancement of the probability of closure at \( n = 7 \) (Fig. 8A). However, the peak in the estimated probability of closure derived from the experimental data is significantly more pronounced.

We thus examined the case in which the generation-dependent threshold pressure distribution was superimposed on vulnerable generations at \( n^* = 6, 7, \) and 8, where the average \( P_C \) was higher than that of its neighbors (thick shaded lines in Fig. 6). The corresponding probability of closure at end-expiration indeed shows a discernable peak near \( n^* \) (Fig. 8B). Since this peak is similar to those in Fig. 5A, these simulation results support the notion of preferential collapse deduced from the experimental data.

An additional factor that may have introduced errors in the estimation of \( n \) is due to the fact that airways corresponding to different generations have different airway wall stiffness leading to different pressure-diameter curves (25). We have investigated this possibility by incorporating the pressure-diameter curves directly into our numerical airway model at each generation. In this case, the attenuation factor \( b \) in Eq. 1 depends on the inflation pressure \( P \), as well as generation number \( n \). The results of the simulations (not shown) demonstrated that airway wall elasticity had negligible influence on the estimated \( n \) corresponding to a given crackle. Airway wall stiffness is a function of lung volume history due to the viscoelastic nature of the wall tissue (7). Additionally, since air trapping influences the pressure difference across the liquid bridge, lung volume history also has an effect on the critical opening pressure and hence the crackle amplitude. Indeed, significant differences were found in the number of crackles (Fig. 5B), as well as the maximum probability of closures (Fig. 5A, inset) between the first and fifth inflations from the same EEP.

With regard to the propagation of crackles, we assumed that attenuation due to energy dissipation in the viscoelastic airway walls is not important. This is reasonable, because the spikes that we included in the calculations carry very high-frequency energy. Indeed, the spikes were obtained following high-pass filtering of the crackle sound at 2,000 Hz, for which the airways behave essentially as rigid pipes (28). Furthermore, for the high frequencies of the spikes, the airways also behave as nearly perfect wave guides. Consequently, the attenuation of the spikes as they propagate through the bifurcating structure is simply the ratio of cross-sectional areas given in Eq. 1. It has been estimated that multiple reflections do not affect the measured crackle amplitudes (2). Numerical simulations in the same study also suggested that it is the average value of \( b \) that determines the distribution of crackle amplitudes. Hence small local variations in \( b \) due to variability in structure or a closed side branch are not expected to have a major influence on the results reported here.

Another important assumption was that we used a simple mechanism of shifting the histograms to obtain the probability values \( p(n) \) in Fig. 5A. Furthermore, shifting \( n \) also results in a corresponding scaling of the diameters \( p(D) \) in Fig. 7. Although such a shift does not alter the relative probabilities of closure, we can only obtain an upper bound for the values of \( p(n) \) and a lower bound for the peak generation numbers (Fig. 5A, inset). A smaller shift is not possible, because the probabilities have to be smaller than unity. Larger shifts are possible; however, shifts that are too large would result in unrealistically large generation numbers, and the corresponding amplitudes at the source would have to be unrealistically large in order for such crackles to be detectable with our measurement system. This assumption remains somewhat arbitrary, and future studies should use simultaneous imaging and acoustic measurements to resolve the actual source of the largest crackles.

For the calculation of \( p(n) \), we assumed that a single geometric factor \( b \) in Eq. 1 is sufficient to account for the attenuation of crackles as they pass through bifurcations. This calculation does not account for the asymmetry of the airway tree. To address this issue, we also calculated the mean and standard deviation of \( n \) as a function of crackle amplitude using our numerical model (data not shown). These simulations suggested that estimating \( n \) is considerably less accurate than estimating \( D \) from crackle amplitudes. When \( p(D) \) was estimated in Fig. 7, we used directly Eq. 4, which was obtained from numerical simulations of crackle sound attenuation along an asymmetric tree. Thus we believe estimating diameters from crackles is more feasible and practical than obtaining generation numbers.

To explain the fact that the probability of closure, in terms of both \( n \) and \( D \), showed a maximum (Figs. 5A and 7, respectively), we note the following. The likelihood of an airway to collapse at any pressure is proportional to the surface tension in the liquid lining and inversely proportional to the diameter of the airway (8). Surface tension in the liquid lining of the airways increases for smaller generation numbers. On the other hand, airway diameter increases from the alveoli toward the trachea (14), so surface tension in the liquid lining of the airways increases for smaller generation numbers. The critical combination of diameter and surface tension at which the probability for an airway to collapse is at maximum. During deflation, airway diameters continuously decrease. Once the diameter of an airway reaches this critical condition, the airway is likely to collapse. However, if the EEP is high, the critical condition for collapse may not be reached (Fig. 5B).

In this study, we promoted airway closure by collapsing excised normal lungs. The distribution of mechanical stresses in the excised lung is likely different from those in intact lungs.
The deflation rate may also play a role here, because it affects wall viscoelasticity, liquid viscosity, as well as the dynamics of the air-liquid interface. All of these factors could alter the location of vulnerable airways, and hence extrapolation of our results to the lung in vivo needs further investigations. Nevertheless, we note that, in diseased lungs, the distribution of surfactant concentration in acute respiratory distress syndrome or airway diameter due to smooth muscle contraction can be abnormal. Consequently, the probability of closure and hence the vulnerability of airways to subsequent shear stresses during reopening are likely different from the curves in Figs. 5A and 7.

In conclusion, in this study, we have developed an “acoustic” imaging technique to estimate the probability of collapse along the airway tree. The technique does not involve any formal fitting of a model to measured cracks, nor does it rely on the knowledge of the exact mechanism of crackle generation. Instead, we use a model-based transformation to infer the location of closures. Our approach may prove useful to identify airways vulnerable to repeated closure and opening in various lung diseases.

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