Crackle sounds and lung recruitment

PhD Thesis

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List of papers included in this thesis

I. Hantos, Z., J. Tolnai, T. Asztalos, F. Peták, Á. Adamicza, A. M. Alencar, A. Majumdar, B. Suki: Acoustic evidence of airway opening during recruitment in excised dog lungs. *J. Appl. Physiol.* 97: 592-598 (2004)

II. Peták, F., W. Habre, B. Babik, J. Tolnai, Z. Hantos: Crackle sound recording to monitor airway closure and recruitment in ventilated pigs. *Eur. Respir. J.* 27: 808-816 (2006)

III. Hantos, Z., Á. Adamicza, T. Janosi, M. V. Szabari, J. Tolnai, B. Suki: Lung volumes and respiratory mechanics in elastase-induced emphysema in mice. *J. Appl. Physiol.* 105:1864-1872 (2008)

IV. Tolnai, J., M. V. Szabari, G. Albu, B. A. Maár, H. Parameswaran, E. Bartolák-Suki, B. Suki and Z. Hantos: Functional and morphological assessment of early impairment of airway function in a rat model of emphysema. *J. Appl. Physiol*. 22. Mar 2012 [Epub ahead of print] PMID: 22442024

List of papers related to the subject of this thesis

V. Alencar, A. M., Z. Hantos, F. Peták, J. Tolnai, T. Asztalos, S. Zapperi, J. S. Andrade, Jr., H. E. Stanley, B. Suki: Scaling behavior in crackle sound during lung inflation. *Phys. Rev. E* 60: 4659-4663 (1999)

VI. Suki, B., A. M. Alencar, J. Tolnai, T. Asztalos, F. Peták, M. K. Sujeer, K. Patel, J. Patel, H. E. Stanley, Z. Hantos: Size distribution of recruited alveolar volumes in airway reopening. *J. Appl. Physiol.* 89: 2030-2040 (2000)

VII. Majumdar, A., Z. Hantos, J. Tolnai, H. Parameswaran, R. S. Tepper, B. Suki: Estimating the diameter of airways susceptible for collapse using crackle sound. *J. Appl. Physiol.* 107: 1504-1512 (2009)

INTRODUCTION

In various respiratory diseases, but also in the normal lung, respiration can be associated with acoustic phenomena. Depending on their dominant acoustic properties, the lung sounds are conventionally classified into the main categories of discontinuous (rales or crackles) and continuous sounds (wheezes and rhonchi); depending on the anatomical locus of the sound generation, tracheal, bronchial, bronchovesicular and vesicular sounds are distinguished. In clinical practice, the expression “breath sounds” usually means the respiratory noise heard through the stethoscope on the chest wall. These sounds therefore relate to the specific peripheral area, in contrast with the sounds that can be recorded at the airway opening and are transmitted from the source through a gaseous medium to the entrance of the airway tree.

The present thesis includes measurements made in experimental animals with sound recordings at the airway opening, and concentrates on a single type of acoustic event, crackles. Special emphasis is placed on the association of crackles with the development of the pressure–volume (P–V) characteristics of the lungs.

Crackles

Investigations on crackling sounds date back to the invention of the stethoscope by Laënnec[1] in 1819. Forgacs[2] proposed that crackles are associated with the sudden opening of closed airways. Crackles are observed in a number of pulmonary diseases, such as fibrosis, asbestosis, bronchiectasis, chronic obstructive pulmonary disease (COPD), pneumonia and sarcoidosis, and their acoustic properties can be widely different and qualitatively characteristic of the disease.

When the lungs deflate to low *V*, many peripheral airways may be closed by a liquid bridge forming between the collapsed airway walls. During slow inflation of the lungs, closed airways suddenly reopen to the accompaniment of short transient explosive sounds. A crackle recorded in the central airway consists of a sharp initial (negative) deflection in *P*, followed by an acoustic after-ring. The intensity of the initial transient is determined by the size of the reopening bronchial segment and is also influenced by the number of attenuating bifurcations it travels across. Although crackle sounds have been studied extensively *in vitro* in collapsed lungs, the potential of acoustic measurements for the detection of airway recruitment *in vivo* has not yet been exploited.

Lung recruitment and the *P–V* relationship

The lower and upper knees in the *P–V* curve of the respiratory system have been used to optimize the positive end-expiratory pressure (PEEP) and tidal *V* during mechanical ventilation. With the "open lung" approach, the PEEP is set slightly above the *P* at the lower knee of the *P–V* curve to minimize alveolar collapse at end expiration. In the normal lung, the lower knee is present in the inspiratory *P–V* curve exists only if the inflation proceeds from the collapsed state. However, in human subjects with the ARDS and in animal models of the ARDS, the *P–V* curve often exhibits a lower knee, even though the lung is inflated from functional residual capacity (FRC). It is likely that multiple mechanisms contribute to the lower and upper knees in the *P–V* curve, including the recruitment and the mechanics of fluid-filled alveoli. However, it is commonly assumed in most studies that all mechanisms governing the recruitment process occur at the alveolar level, whereas airway closure and reopening can also affect the process of recruitment. Suki *et al* [3] put forward the idea that airways open in cascades or avalanches.

If recruitment occurs via avalanche-like airway reopenings, then it may be reasoned that the lung volume (V_L) recruited along the *P–V* curve should consist of a highly irregular sequence of discrete volume increments (ΔV). The distribution of these ΔVs, which correspond to avalanches

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[1] *Laennëc, De l'Auscultation Me´diate ou Traite´ du Diagnostic des Maladies des Poumons et du Coeur. Brosson & Chaude´, Paris (1819)*
[2] *Forgacs, P., Crackles and wheezes. Lancet, 1967. 2(7508): p. 203-5.*
[3] *Suki et al. Nature 368: 615-618 (1994)*

reaching the alveoli, should follow a power law. Additionally, as the airway opening is also associated with crackle sound generation it may be expected that the density of crackles is highest near the lower knee in the *P–V* curve.

Airway and parenchymal determinants of recruitment

In lung diseases, different degrees of bronchoconstriction, airway collapse and parenchymal dysfunction are observed. The resulting lung function impairment in diseases primarily affects the lung periphery. It is therefore essential to employ a method that provides an objective assessment of the patency of the peripheral airways, and this can be assessed by analysing the lower knee in the static *P–V* curve, using lung-imaging techniques or with low-frequency forced oscillation (LFOT). The closure of peripheral airways at low transpulmonary pressures (P_L) is exaggerated in the presence of bronchoconstrictor stimuli, and we argue that the recruitment during a subsequent reinflation would be accompanied by an increased crackle activity.

The patency of the lower airways is fundamentally maintained by the parenchymal tethering forces, and loss of elasticity in the parenchymal network would lead to increased collapsibility of the bronchi. In emphysema, which is characterized clinically by a loss of elastic recoil and significant hyperexpansion of the lungs, permanent destruction of the parenchymal structure takes place. In clinical studies, absolute V_Ls [such as the residual volume (RV), FRC and total lung capacity (TLC)] and their ratios are used to characterize emphysematous changes.

The animal models of emphysema commonly concentrate on the parenchymal destruction and less attention has been paid to the possibility of airway impairment. Indeed, the airway resistance (R_{aw}) has been reported to be similar in normal and emphysematous mice, and pulmonary resistance has been reported to return to the control level after 3 weeks of porcine pancreatic elastase (PPE) treatment in rats. However, remodelling processes following the elastolytic intervention alter the bronchial wall structure, and presumably also the airway function. The net effect of these structural changes on airway mechanics is basically unclear, but it may be anticipated that both the overall resistance of the airway tree and the stability of the bronchial wall, as reflected by the reopening processes, are affected by the emphysematous changes.

AIMS AND HYPOTHESES OF THE PRESENT THESIS

The overall aims of this thesis were to measure and analyse the acoustic events of lung reopening as indicators of the pre-existing derecruitment state, and to interpret the crackles as markers of the discrete events of the development of the pulmonary *P–V* during slow inflations. The specific aims and hypotheses were as follows.

First, we hypothesized that the process of recruitment and the lower knee in the *P–V* curve in the normal lung are mainly determined by avalanche-like airway reopenings rather than simple alveolar recruitments. We expected that the density of crackles is highest around the lower knee in the *P–V* curve, because the opening of the airways is also associated with crackle sound generation. We further assumed that reinflation crackles would accompany the discrete ΔV changes. To this end, *ex vivo* studies were conducted on dog lung lobes (Study 1), which ensured the highest sensitivities both in crackle recording and in reinflation airflow measurement.

Second, we assumed that reinflation crackles sensitively characterize the preceding derecruitment state of the lungs which is determined both by the end-expiratory lung *V* and the constriction of the airways to be reopened. In order to validate this assumption, experiments were designed in mechanically ventilated open-chest pigs, with different levels of end-expiratory P_L and methacholine (Mch) doses applied prior to the slow reinflations (Study 2).

Third, we hypothesized that alterations in the parenchymal support of the airways are manifested in the airway wall stability, and hence the reinflation dynamics from very low lung *V* differ in health and experimental emphysema. We therefore set out to investigate the crackle intensity as a marker of airway reopening during slow inflations in normal and PPE-treated animals: in mice (Study 3) and in rats (Study 4). We were also interested in whether PPE treatment causes pure parenchymal destruction or whether it also involves airway abnormalities.

METHODS

Impedance measurements

The input mechanical impedance (*Z*) of the lungs (Z_L) or the respiratory system (Z_{rs}) was measured with the LFOT. The airway and parenchymal mechanical properties were separated by fitting the constant-phase model to the *Z* spectra. The model consists of an airway compartment containing R_{aw} and I_{aw} (airway inertance), and a constant-phase tissue unit characterized by damping (*G*) and elastance (*H*)[4].

Recording of tracheal sounds

A miniature microphone was suspended in the lobar bronchus in dogs and in the tracheal tube in pigs (Study 1 and Study 2). In mice and rats, a metal tube was attached to the microphone and positioned in front of the tracheal cannula (Study 3 and Study 4).

The electrical signal of the microphone was preamplified and digitized. The recordings were made during slow inflations from the collapsed state in excised dog lung lobes (Study 1), from

[4] *Hantos et al. J. Appl .Physiol. 72: 123-132 (1992)*

PEEP levels of 1 and 4 hPa in pigs (Study 2), from the RV in mice (Study 3) and from the *in vivo* degassed state in rats (Study 4) to the TLC (30-35 hPa). In order to collect more acoustic events and assess the reproducibility of the reopenings, the manoeuvres were repeated usually 2 or more times in the same condition in each animal. The duration of inflation was selected so that the temporal resolution was sufficiently high for the identification of the individual crackles. The signals of the transrespiratory (or translobar) P and central flow ($\dot{V}$) were recorded, and the inflation volume [V(t)] was obtained by integration of $\dot{V}$.

Identification of crackles

The *in vivo* raw sound recordings were first high-pass-filtered at 2 kHz, in order to suppress the strong cardiogenic sound and enhance the sharp initial transients of the crackles (the *ex vivo* recordings were processed differently). The high-frequency (HF) waveforms provided an increased temporal resolution for identification of the successive crackles, which often overlapped and hence were inseparable in the unfiltered signals.

After this pre-processing, the maximum levels of background noise were estimated in each recording from the baseline and the end-inflation segments that were free of crackles, and a threshold was determined for the minimum crackle amplitude. The recordings were then divided into short intervals (ΔT), and the sound energy (ΔE_i) was computed for each interval. A crackle was defined as occurring when the increase in ΔE in two successive intervals exceeded a preset value $\Lambda = \Delta E_{i+1}/\Delta E_i$; any further increase in ΔE was then checked ($\Delta E_{i+2}>\Delta E_{i+1}$, *etc.*) and the crackle amplitude was identified as the highest-magnitude sample in the last interval of the increasing ΔE sequence.

Acoustic evidence of airway opening during recruitment (Study 1)

Diaphragmatic lung lobes were isolated from 6 mongrel dogs. The animals were anaesthetized, heparinized and exsanguinated. After thoracotomy, the lungs were removed, and the lobes were suspended in a glass box. The translobar pressure (P_{tl}) and the bronchial $\dot{V}$ were measured, while P was slowly increased from 0 to 30 hPa by decreasing the box pressure (P_{box}).

The LF energy content of the crackles was obtained by digital low-pass filtering at 60 Hz and squaring the sound pressure amplitude. The crackles were often accompanied by transients in the corresponding raw $\dot{V}$ signal, which returned either to the same or to a somewhat higher level than before the transient. The signals of $\dot{V}$ were integrated to obtain V, and were also processed to identify the discrete ΔVs corresponding to the transient spikes in $\dot{V}$. Whenever the successive $\dot{V}$ transients were separable and the background noise allowed accurate identification of the beginning and end points of a $\dot{V}$ transient, ΔV was determined. For the calculation of ΔV, the post-

transient level of $\dot{V}$ was taken as the baseline. To examine the relationship between recruitment and crackle sound, an energy package (ΔE_{LF}) for the duration of each identified $\dot{V}$ transient was also calculated from the LF sound data and correlated with the corresponding ΔV.

Crackle recording to monitor recruitment in bronchoconstriction (Study 2)

Six adult mini-pigs were anaesthetized, tracheotomized and mechanically ventilated. Following chest opening, a PEEP of 4 hPa was applied. $\dot{V}$ and P_{tr} with reference to atmosphere (P_L) were measured. The tracheal cannula was detached from the respirator at end expiration (at a PEEP of 1 or 4 hPa) and connected to a buffered compressed air source. A water column was employed for limitation of P_L to 30 hPa at the end of the reinflation.

The mechanical ventilation was suspended at a PEEP level of 4 hPa, and Z_L was recorded during the resulting short apnoeic period. The lungs were then slowly inflated to a peak P of 30 hPa, during which period the intratracheal crackles were recorded. Following an ~2-5-min period of mechanical ventilation, the baseline Z_L and crackle measurements were repeated. Resumed mechanical ventilation was followed by the *iv* administration of a Mch bolus; 30 s after the injection, Z_L was measured, and immediately afterwards the lungs were inflated and the crackles were recorded. Subsequent Mch challenges were made by administering increasing doses of Mch. After completion of the Mch challenges the PEEP was decreased to 1 hPa to facilitate lung derecruitment. The experimental procedure was then repeated while this P level was maintained during the Z_L recordings, and the subsequent inflation was also started from 1 hPa.

Crackles and airway function in emphysematous mice (Study 3)

Female CBA/Ca mice were anaesthetized and intubated. The treated animals received PPE via intratracheal instillation, the control animals received saline only. Three weeks thereafter, the mice were anaesthetized, tracheotomized and ventilated transmurally in a body plethysmograph with a PEEP of 2 hPa.

The TGV at end expiration at zero transrespiratory pressure (FRC) was measured with the recently modified plethysmographic technique[5]. Following the measurement of the FRC, 14 control (group C1) and 14 PPE-treated mice (group E1) were used to obtain $V(t)$ and the TGV as a function of time. Transrespiratory pressure (P_{rs}) was calculated as P_{tr} - P_{box}. In 5 control (group C2) and 5 treated mice (group E2), the measurement of the FRC was followed by deflation to the RV, which was accomplished by elevating P_{box} to 20 hPa; subsequently, P_{box} was lowered to -35 hPa to allow an estimate of the TLC. The box was then opened to atmosphere via a resistor to permit passive deflation. During this deflation-inflation-deflation manoeuvre, $\dot{V}$ was measured.

[5] *Janosi et al. J. Appl. Physiol. 101: 454 – 459 (2006)*

The expiratory reserve volume (ERV) was determined by integration, and the RV was obtained as FRC - ERV.

For the measurements in groups C2 and E2, the setup was modified so that a microphone was connected to the tracheal tube and sound was recorded during reinflation from the RV to TLC. The tracheal $\dot{V}$ was measured while the microphone recorded the sound, which was high-pass filtered at 2 kHz and individual crackle events were identified.

Crackles and airway structure/function in emphysematous rats (Study 4)

Fourteen male adult Sprague-Dawley rats were anaesthetized, intubated and treated with an intratracheal instillation of 50 IU PPE or saline (controls). Six weeks after the treatment, the rats were re-anaesthetized, tracheotomized and mechanically ventilated in a custom-built body plethysmograph. The FRC was measured plethysmographically. The inspiratory capacity (IC) and the ERV, respectively, were defined as the V changes resulting from decreasing P_{box} to -35 hPa and increasing it to 20 hPa, respectively. Z_{rs} was measured at the FRCand R_{aw}, H, G and I were estimated from 5-6 successive recordings.

Intratracheal sounds recorded during slow inflations from the degassed state of the lungs to the TLC were high-pass filtered at 2 kHz and individual crackle events were identified.. Degassing was accomplished by 10-min ventilation with 100% O_2 and the subsequent ERV manoeuvre, followed by tracheal occlusion.

After the measurements, the rats were euthanized, the heart and lungs were removed *en bloc*, and stored in formaldehyde before embedding in paraffin. Transversal sections were made and stained for standard morphometry, and with elastin and collagen.

The whole sections were scanned for the identification of bronchi. From the readings of bronchial perimeter (P_b) the diameter of an equivalent circular cross-section (D_b) was calculated. The mean wall thickness (T_w) and the number of septal attachments (N_s) was determined for each airway. The septal attachment density was calculated as N_s/P_b. Quantitative analysis of the elastin and collagen densities was performed on randomly selected lung sections.

RESULTS

Study 1. In the early phase of inflation, the crackles possessed large amplitudes and were well separated from each other. As inflation continued, the density of acoustic events increased, to the accompaniment of a decrease in crackle amplitudes. In these dense intervals, the crackles became inseparable from each other in the raw recordings. P_{tl} steadily increased throughout the course of the entire inflation. The $\dot{V}$ traces consisted of a series of spikes superimposed on a slowly varying mean $\dot{V}$ level. During the first inflation, the mean $\dot{V}$ exhibited a single maximum, whereas the

second inflation was characteristically biphasic, with two distinct maxima: the first rise in the mean $\dot{V}$ included massive $\dot{V}$ transients accompanied by a significant crackle activity. The mean $\dot{V}$ then decreased temporarily before a second rise, which was similar in character to that during the first inflation at the same V_L.

During the first inflation, the *P*–*V* curves always exhibited a single characteristic lower knee. The corresponding acoustic activity, as characterized by the values of ΔE, increased quickly and irregularly and remained high until a P_{tl} ~10 hPa was attained. The acoustic activity then decreased very regularly by 4 to 5 orders of magnitude, which coincided with the steep rise in *V* until the *P* reached the upper knee in the *P*–*V* curve. This was followed by another irregular pattern, during which epochs of large values of ΔE emerged from the low acoustic activity, indicating the occurrence of sparse, but still relatively large crackles. Although crackles were detected throughout the entire inflation process, the cumulated energy as a function of P_{tl} had reached 94-98% of its final value by the lower knee. During the second inflations, the *P*–*V* curves always exhibited two distinct lower knees, corresponding to the two maxima observed in the mean $\dot{V}$. The large values of ΔE during its fast rise occurred around the first knee in the *P*–*V* curve, and the increase in energy was even steeper, reaching its plateau value at much lower values of *P* than during the first inflation.

When a short segment of a recording from an early phase of inflation was taken, where crackles were relatively rare and the transients were well separated, the relation between crackles and transients could be examined. Every $\dot{V}$ transient was clearly marked by a crackle or a burst of crackles, whereas not every acoustic event was accompanied by a detectable transient in $\dot{V}$. It was also clear, however, that crackles of similar amplitude may correspond to either a relatively large or a much smaller $\dot{V}$ transient. Through determination of the LF (<60 Hz) and HF (>1 kHz) components of each crackle, it could be demonstrated that crackles with significant LF energy were always associated with detectable transients in $\dot{V}$, whereas those that had low LF energy were not. Another interesting feature of these data was that, after a $\dot{V}$ transient marked by crackles with significant LF energy, the mean level of $\dot{V}$ was usually elevated.

To quantify the observed relationship between the LF energy of crackles and the recruited volumes associated with the $\dot{V}$ transients, the corresponding pairs of ΔE_{LF} and ΔV pooled for all lobes were plotted. Linear regressions were carried out in the log-log data, and according to the unpaired *t*-test on these data, the slopes of the regression lines were significantly larger than unity for both the first and the second inflation. Additionally, the slope corresponding to the second

inflation was significantly larger than that corresponding to the first inflation. The relationship was stronger for the second than for the first inflation.

To characterize the irregularities in ΔV and to calculate their probability density distribution, all data were combined. The distribution probability of ΔV peaked between 0.02 and 0.05 ml and then decreased linear by in the log-log graph, indicating that the tail of the distribution follows a power law.

Study 2. At a P_L of 4 hPa, the bottom knee in the P_L–V diagram was not apparent until the highest doses of Mch, whereas it was typically observed even under the control conditions at 1 hPa. It is worth noting that, in general, the crackle intensity increased significantly before the P_L–V curve displayed any alteration indicative of the development of the bottom knee. The histograms of the crackles exhibited their maxima at or slightly above the bottom knee and decayed rapidly around the highest slope of the P_L–V curve. The crackles disappeared before the upper knee was reached in the P_L–V diagram.

Increasing doses of Mch induced progressive increases in the number of crackles (N_C), with statistically significant elevations from the control values first being observed at the dose of 20 $\mu g \cdot kg^{-1}$ at the PEEP of 4 hPa; overall, an average 38-fold rise occurred in N_C between the control level and the highest Mch dose. Statistical significance was reached already at the lowest dose at 1 hPa, where the crackle activity was already considerable under the control conditions, whereas at the highest 3 doses the PEEP dependence of the crackle number disappeared.

Study 3. PPE treatment resulted in statistically highly significant changes in V_Ls. The FRC and TGV at a P_{rs} of 20 hPa) increased by 52 and 45% in group C1 and group E1, respectively, relative to the control. The average TGV *vs* P_{rs} curves also reflected significantly different values between the groups at all P_{rs} levels. The ratios of the TGV between the groups at the same P_{rs} were fairly constant, suggesting a nearly proportional increase in TGV at all P_{rs} values, *i.e.*, an unchanged shape of the P–V loops. The inspiratory volume increased by 37% in the treated animals.

The changes in the mechanical parameters were most marked in H, which decreased by 57 and 27% at P_{rs} values of 0 and 20 hPa, respectively. Small, but statistically insignificant decreases were observed in R_{aw} at both 0 hPa (-2%) and 20 hPa (-10%).

The vital capacity (VC) manoeuvres performed in the group C2 and group E2 animals also revealed marked differences in all V_Ls but the RV. Interestingly, the P at the bottom knee in the TGV–P_{rs} curve decreased on average by 5.5 hPa in the treated animals.

N_C detected during inflation from the RV to the TLC was ~150 and did not differ between the group C2 and group E2 mice. In group C2, the cumulative crackle energy rose steeply from 0 to above 95% within a narrow range of P_{rs} values between 2.5 and ~5 hPa. In contrast, the

cumulative energy in the group E2 rose almost immediately at the start of inflation, but reached 95% only by a P_{rs} of ~9 hPa. Despite the grossly different rates of crackle energy release during inflation, the probability distributions (Π) of the crackle amplitudes in the two groups were very similar. Since both distributions decreased linearly in a log-log graph, Π can be described by a power law as $\Pi \sim s^{-\alpha}$, where s is the crackle amplitude and α is the exponent of the distribution.

Study 4. The FRC and RV were statistically significantly higher in the PPE group as compared with the controls; however, the increase in TLC in the treated rats was not significant. Whereas the tissue mechanical parameters G and H were statistically significantly smaller in the PPE-treated rats than in the controls, there was no difference in R_{aw} between the groups. Because of the larger differences in H, tissue hysteresivity was elevated in the treated rats. The lower knee in the inflation P–V curves from the degassed state was shifted to higher Ps (19.3±1.0 *vs* 17.8±1.2 hPa; p=0.021) and the asymptotic volume level (TLC) was slightly larger in the PPE group than in the control rats.

The number of bronchial cross-sections analysed in each rat ranged between 40 and 50. According to the bronchial morphometry, there was no difference between the D_b of the airways of the control and the PPE-treated animals. The attachment density N_s/P_b was mildly, but statistically significantly lower in the PPE group. However, the mean value of T_w was higher in the PPE group than for the controls. The average, the spatial variability of the elastin density and the total elastin content within the bronchial wall did not differ between the groups. The collagen density and its intra-bronchial SD were increased by 12% and 17% in the treated group. Furthermore, the inter-airway variance of the collagen density was substantially higher in the treated animals.

The N_C per inflation were not different between the two groups; however, their distributions expressed as relative frequencies (N_C/N_{total}) were statistically significantly different. In the control rats, N_C peaked at lower Ps than it did in the PPE group, and exhibited another maximum. This latter fact suggests a bimodal distribution of the sounds of reopening, which is also expressed by the $\log(N_C/N_{total})$ *vs* V graphs exhibiting a second peak in the control group.

DISCUSSION

Study 1. We investigated the possibility that the process of airway opening, as indicated by the crackles, contributes to alveolar recruitment and the lower knee in the P–V curve of isolated dog lung lobes. The main findings were that *1*) recruitments occurred in discrete ΔVs; *2*) the discrete ΔVs were accompanied by crackles carrying LF energy; *3*) in the presence of trapped air, the

inflation exhibited a biphasic behaviour resulting in two distinguishable lower knees in the *P*–*V* curve; and *4*) the distribution of ΔV followed a power law.

A characteristic feature of the results was that, for both the first and second inflations, the vast majority of the crackles occurred before $\dot{V}$ reached its maximum value. Since crackles are successively attenuated at every bifurcation as they propagate from the site of generation towards the trachea, this pattern suggests that the opening phenomena progressed from the central to the peripheral airways. The sound energy decreased by several orders of magnitude when the inflation reached the lower knee in the *P*–*V* curve, and this is consistent with the decreasing envelope of the crackle time series.

During the second inflation, the cumulative sound energy reached its 95% level at a lower inflation *P* as compared with the first inflation, which was accompanied by a biphasic shape of the $\dot{V}$ *vs* time curve and two separate lower knees in the *P*–*V* curve.

Crackles can be regarded as direct signatures of individual airway openings, which can be considered "microscopic" events contributing to the "macroscopic" *P*–*V* curve of the lung. The mechanism by which a crackle sound is generated is not well understood. We believe that the mechanism that is most likely in the excised lungs is that crackles are a consequence of the rapid break-up of the fluid meniscus inside a closed airway, with a small amount of air behind the closure. In terms of temporal properties, we argue that crackles of short duration, consisting of the initial sharp sound of the break-up of the fluid meniscus alone, may mark the opening of a single airway segment without a noticeable increase in lung *V*, whereas crackles that also include an elongated ringing with a significant LF energy mark the entry of $\dot{V}$ into a larger peripheral region. This latter crackle type indicates the sudden recruitment of lung *V*, which we have termed a discrete ΔV, to distinguish it from the continuous *V* change corresponding to the elastic expansion of the recruited airspaces. Theoretically, if all $\dot{V}$ transients could be detected and the corresponding discrete *V* increments ΔV cumulated, we would be able to reconstruct that part of the *P*–*V* curve that is formed by the discrete *V* recruitments. The distribution of the recruited ΔVs has been predicted to be a power law with an exponent of 2. Our data reflect a direct assessment of the distribution of alveolar recruited ΔVs. During both the first and second inflations, the distribution displays a plateau-like region for small ΔV, followed by a region of about two decades over which the distribution decreases linearly in the log-log graph, *i.e.* it follows a power law form. The plateau region is obviously due to the limitation of the measurement. Nevertheless, for the first inflation, the data were in excellent agreement with the model prediction since the theoretical value of the exponent is 2, whereas the estimated average one was 2.02. The distribution obtained

from the second inflation had an exponent of 1.88, which is also close to the theoretical value of 2. The reason for this small discrepancy is unclear, but it may be due to the fact that the presence of significant trapped air terminates the avalanches in larger alveolar regions than in the fully collapsed lung.

Study 2. In order to characterize the recruitment processes during an acute pulmonary constriction in mechanically ventilated animals, intratracheal recordings of crackles during slow inflations were made in open-chest pigs. The results of the experiments revealed that: 1) the development of the lower knee in the P_L–V curve was always associated with a significant increase in crackle activity; 2) the N_C increased with the dose of Mch administered before the reinflation from the PEEP level and 3) while the PEEP level (1 *vs* 4 hPa) was an important determinant of the crackle activity in the control group and at low doses of Mch, at high Mch doses the bronchoactive factor predominated.

The fact that N_C plateaued with increasing doses of Mch may reflect the limit set by the noise to the sensitivity of crackle detection, and explain why the progression of reopenings towards the periphery could be followed until N_C reached a value of ~4,000 (corresponding to the 11^{th} airway generation). Although the acoustic tracking of the recruitment process may have been incomplete, it is important to note that this is the first *in vivo* study in which the development of the full inspiratory P–V curve was accompanied by the recording of reopening sounds. Whilst the crackles were hardly recognizable (either acoustically or visually) in the original sound recordings because of the strong cardiogenic sounds, simple but appropriate high-pass filtering revealed the abundant crackles accompanying the reopening process.

The use of an open-chest preparation made it possible to lower the lung V below the closing V, thereby facilitating closure of the airways even without a bronchoconstrictor challenge. Indeed, re-inflation from a P_L of 1 hPa under control conditions resulted in a reopening activity comparable in N_C to that reached at the third dose of Mch at the higher P_L level.

While the experimental model was chosen to mimic situations characterized by airway obstruction, such as in anaphylactic reactions, the exacerbation of asthma or COPD, it was not intended to include capillary filtration/resorption abnormalities typical of an acute lung injury, where excessive intrabronchial fluid and foam production in pulmonary oedema may increase the number of crackle-like sounds not uniquely connected to airway opening.

Study 3. The purpose of this study was to characterize the behaviour of the airways during slow inspiratory VC manoeuvres in a mouse model of emphysema. We used a standard PPE treatment protocol and confirmed the presence of emphysema in the treated mice 3 weeks following the treatment on the basis of lung Vs and respiratory mechanics.

The main findings of the study are as follows. (1) We observed increases in TGV similar to those seen in FRC and TLC in patients with emphysema; (2) the dynamic elastance was significantly lower in the treated mice, whereas the R_{aw} was not different. Measurements made in two additional groups of mice revealed that (3) the PPE treatment did not lead to any increase in RV, whereas the bottom knee in the *P–V* curve was shifted to lower P_{rs}, and (4) although the cumulated crackle energy increased more slowly during inflation in the treated mice, the probability distributions of crackle amplitudes were very similar.

Since the RV is most sensitive to small airway collapse, the little or no change in RV implies that the small airways were not influenced by PPE treatment. Interestingly, the lower knee in the *P–V* curve shifted to a lower P_{rs}, and the cumulative crackle energy started to increase at a much lower P_{rs} in the treated mice. Both of these findings suggest that a significant fraction of the airways had a lower critical opening P_{rs} in the treated animals. How is it possible then that, whereas the RV and R_{aw} at the same P_{rs} are the same in the control and treated mice, the lower knee and the crackle energies are different in the two groups?

We are unsure about the exact mechanism responsible for the reduction in the opening P_{rs} following treatment. We can argue that whereas the lower knee in the inflation curve is associated with massive airway opening, the crackles that were detectable in our experiments may have come from larger airways than those that determine the RV. Thus, although the site of airway closure and the trapped air behind the small airways that determine the RV are similar in the two groups, the relative locations of the knee imply that some of these airways are easier to open in the emphysematous group.

Study 4. In this rat model of emphysema, we investigated the alveolar and bronchial structural changes underlying the alterations in lung *V* and mechanics, and the acoustic manifestations of the airway function. The combination of structural and functional measurements revealed that (1) the PPE treatment caused significant increases in the FRC and the RV, but only a small change in the TLC; (2) the tissue mechanical parameters were significantly lower in the treated group, whereas there was no detectable alteration in the total R_{aw} as measured at the FRC; (3) the alveolar attachment density was lower and T_w was higher in the emphysematous animals; (4) while no difference was found in the elastin content per unit T_w of the bronchial wall, the collagen content was higher and more heterogeneous in the treated animals; (5) while the N_C per inflation was similar, the distributions of crackles as a function of P_{rs} or lung *V* were statistically significantly different in the PPE-treated and control groups, and (6) the lower knee in the inflation *P–V* curve was shifted to higher P_{rs} in the treated rats.

We attribute the increase in opening P_{rs} in the emphysema group to the decrease in parenchymal tethering forces transmitted to the walls of the sequentially opening segments of the airway tree. This shift in the inflation $P–V$ curve was consistent with a delayed recruitment process, also indicated by the crackle intensity.

R_{aw} was not different in the two groups, which suggests that the overall resistance of the bronchial tree was not affected by the PPE treatment. The knee in the $P–V$ curve was shifted statistically significantly to higher P_{rs} in the treated rats, which was in contrast with the observations in mice (Study 3). Since the lower knee in the $P–V$ curve signifies airway openings, this shift implies that the critical opening P_{rs} at which massive airway opening allows the alveoli to start filling up was higher in the treated lungs. The reasons for this discrepancy between the mice and rats are not clear; perhaps, the difference in the elastic properties of the chest wall transmitting the P_{rs} to the lung surface is one possible explanation.

We quantified the strength of parenchymal tethering by measuring the number of attachments per unit airway wall perimeter and found that the treatment reduced the average attachment density, probably due to loss of septa around airways.

The elastin density of the walls was similar in the two groups. Since T_w was larger in the treated animals, it is likely that the total elastin content was also larger in the bronchial walls of the treated rats, implying cellular remodelling of the airway walls. The increased collagen density of the wall with the elevated intra-wall heterogeneity also suggests a disordered cellular remodelling following PPE treatment.

The results from the crackle sounds also revealed major differences in the distribution of N_C as a function of airway P or lung V. The amplitude distribution of the crackle sounds followed a power law, in agreement with previous studies in normal dog lungs and in PPE-treated mice (Study 3).

It can be predicted that the airway diameters decrease faster along the airway tree in the treated animals than in the control animals, which seems to contradict the fact that R_{aw} did not differ between the two groups. However, whereas R_{aw} characterizes flow resistance around FRC, the distribution of crackle amplitudes mostly reflects diameters at much higher lung Vs than FRC. Furthermore, the reduced diameter ratio obtained from crackles is also in accord with both a stiffer wall due to increased collagen content and T_w, and a reduced parenchymal tethering as a result of the lower attachment density and septal stiffness. We therefore conclude that the reduced diameter ratio inferred from the crackles and the shift in the knee in the $P–V$ curve imply an important deterioration in airway patency under conditions such as airway reopening and high lung V that the FOT-based R_{aw} measured around the FRC does not detect.

SUMMARY AND CONCLUSIONS

1. We have observed that in the reinflation of isolated collapsed lungs the elastic expansion of the alveoli is intermittently interrupted by discrete volume increments, which are accompanied by acoustic events. The majority of crackles were detected near the lower knee in the inspiratory pressure–volume curve. Hence, in contrast with the prevailing view that recruitment occurs at the alveolar level, our data provide strong evidence that the recruitment of alveolar regions is a highly irregular process triggered by avalanche-like airway openings. We also found that the lower knee in the pressure–volume curve shifts to lower pressures, in the presence of trapped air, but the knee is still dominated by airway openings. Thus, airway opening is an important determinant of the recruitment process of the partially collapsed and also fluid-filled lung in ARDS.

2. We have demonstrated that the quantification of airway closure on the basis of subsequent recruitment is feasible by recording and processing intratracheal crackle sounds in vivo. The measurements revealed the importance of airway closure and reopening in the development of the pressure–volume characteristics following the administration of methacholine at a normal lung volume, and especially at a lung volume below the closing volume. Lung sound recording appears to be a sensitive tool with which to indicate early impairments in the mechanics of the lung periphery. Thus, crackle recordings may have the potential to serve as a bedside monitoring tool for detection of the cyclic recruitment-derecruitment of the airways during mechanical ventilation, and hence contribute to the guidance of the optimum ventilation strategy.

3. We have investigated the effects of elastase treatment on the airway function in established rodent models of human emphysema exhibiting enlarged airspaces, in order to ascertain whether or not the treatment also leads to enhanced collapsibility and hindered reopening of the airways, as a result of weakened parenchymal tethering. While the treatment resulted in marked changes in lung volumes, the airway resistance measured at the level of the FRC did not change either in mice or in rats. There was no alteration in the size distribution of crackles between the control and treated animals; however, subtle changes in recruitment dynamics and airway morphometry were observed. We conclude that the reduced parenchymal tethering and an increased stiffness of the remodelled bronchial wall acted oppositely, and these rodent models therefore need to be refined and followed on a longer time scale in order to mimic the human disease to a better extent.

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