A rat lung model of instilled liquid transport in the pulmonary airways

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A rat lung model of instilled liquid transport in the pulmonary airways. J Appl Physiol 90: 1955–1967, 2001.—When a liquid is instilled in the pulmonary airways during medical therapy, the method of instillation affects the liquid distribution throughout the lung. To investigate the fluid transport dynamics, exogenous surfactant (Survanta) mixed with a radiopaque tracer is instilled into tracheae of vertical, excised rat lungs (ventilation 40 breaths/min, 4 ml tidal volume). Two methods are compared: For case A, the liquid drains by gravity into the upper airways followed by inspiration; for case B, the liquid initially forms a plug in the trachea, followed by inspiration. Experiments are continuously recorded using a microfocal X-ray source and an image-intensifier, charge-coupled device image train. Video images recorded at 30 images/s are digitized and analyzed. Transport dynamics during the first few breaths are quantified statistically and follow trends for liquid plug propagation theory. A plug of liquid driven by forced air can reach alveolar regions within the first few breaths. Homogeneity of distribution measured at end inspiration for several breaths demonstrates that case B is twice as homogeneous as case A. The formation of a liquid plug in the trachea, before inspiration, is important in creating a more uniform liquid distribution throughout the lungs.

surfactant replacement therapy; respiratory distress syndrome; liquid ventilation; drug delivery; exogenous lung surfactant; liquid bolus

LIQUID MAY BE INSTILLED into the pulmonary airways during medical treatments such as surfactant replacement therapy (SRT), partial liquid ventilation (PLV), and drug delivery. SRT is a common treatment for respiratory distress syndrome (RDS), particularly in premature infants. Typically, liquid is instilled into the trachea, and ventilation assists in propagating the liquid toward the alveoli, coating airways along the way. Studies show that SRT can decrease infant mortality by up to 50% of infant RDS patients (15). Surfactant can be instilled as a preventative measure soon after birth or as a rescue measure within the first 24 h (11) and may treat meconium aspiration syndrome (27). In adults, SRT is a therapy for RDS and for lung damage due to smoke inhalation (20) and sepsis (19, 26). During PLV, a perfluorochemical liquid is instilled into the airways, which has been shown to improve respiratory function in RDS cases (1, 12, 13). Some forms of drug delivery involve piggybacking drugs or genetic material with instilled surfactant (9, 10, 16, 22) or perfluorochemical liquid (14).

Several studies have used an animal lung model to evaluate the distribution of liquid instilled into the pulmonary airways. In some experiments modeling SRT, the instilled surfactant-lipid may be radiolabeled and mixed with a suspension of dye-labeled microspheres (17, 25). After the surfactant treatment, the lung is flash frozen and then cut into many (50–120) horizontal slices and analyzed for surfactant content. Several animal studies have been conducted to measure the effects of varying surfactant type (5), size and number of doses (25), lung lobes targeted (17, 25), instillation techniques (17, 23, 25), and ventilation methods (18, 21). Although measurement using this type of imaging quantifies the final liquid distribution in the lung regions, it does not provide information about the local fluid transport in the airways, which affects the final distribution through the lungs. Does instilled liquid distribute via gravitational drainage, propagate as a liquid plug, or become aerosolized? The fluid dynamic process is key in determining how and when the material reaches its destination. Knowledge of the transport dynamics will enable the treatment method to be chosen to suit the needs of the patient.

When a liquid or surfactant is instilled into the pulmonary airways, a sequence of transport phenomena occurs that determines its path and ultimately its
distribution. For the clinician, the strategy of liquid instillation may be governed by the mode of therapy or the type of lung injury. Optimal delivery may require homogeneous distribution through the airways, or it may be advantageous to target specific lung lobes, airways, or alveoli. Some physical parameters that may affect the resulting distribution include gravity, liquid viscosity, liquid density, surface tension, surface activity, airflow speed, airway geometry, lung compliance, liquid bolus size, respiratory rate, tidal volume, and previous treatments.

Recently, theoretical studies have investigated the transport of instilled liquid through the airways as a model of SRT (3, 7). Halpern et al. (7) predict that surfactant delivery can be divided into four distinct transport regimes. The first regime is the propagation and distribution of an instilled liquid plug, driven mainly by gravity and forced air due to mechanical ventilation. As the plug propagates, it leaves a liquid coating on the walls of the airway. The second regime is gravitational drainage of the liquid film, which is significant mainly in the larger airways. A third regime is surfactant flow in the small airways, and a fourth regime is surfactant uptake in the alveoli. For the first regime, the ratio of deposited film thickness to liquid film thickness, $h/a$, is predicted as a function of the capillary number, $C_a = \mu U a / \sigma$, where $\mu$ and $\sigma$ are the exogenous surfactant viscosity and surface tension, respectively, and $U$ is the trailing meniscus velocity of the instilled liquid plug. Given the plug volume and $C_a$ in the trachea, they predict the plug volume at each airway generation $n$ through rupture. Espinosa and Kamm (3) also predict a similar first regime; their results predict the local $C_a$ and $h/a$ for a range of surfactant viscosities and accumulated film volume as a function of airway generation $n$. These models (3, 7) assume a symmetric, dichotomous system and therefore do not include the local fluid dynamics through a bifurcation that may be sensitive to asymmetric geometry, downstream conditions, or asymmetric tilt with respect to gravity.

There remains a need to connect the theoretical predictions of liquid transport and distribution in the lungs to experiments in actual airways. To facilitate investigation of the dynamics of liquid plug flow in the airways, it is essential to employ an improved imaging technique over those previously utilized. The current experimental studies involve imaging methods that allow for real-time detection of liquid transport dynamics in the pulmonary airways. The goal of this study is to determine how the distribution of instilled liquid is affected by the delivery method. In particular, the objective is to identify differences in flow dynamics and the resultant fluid distribution when a plug either does or does not exist in the upper airways.

**METHODS**

**Experimental Methods**

Two experimental methods are investigated for liquid transport and distribution in rat lungs. For each, a surfactant mixture is instilled by constant infusion into the trachea during continuous ventilation. *Case A* involves liquid that is inserted into the trachea and rapidly falls into the airways. The instilled liquid drains briefly from gravity and then is driven through the lungs by an inspired breath. *Case B* involves instilling liquid that forms a plug in the trachea tube before the inspired air propagates the liquid through the airways. Natural exogenous surfactant is mixed with a radiopaque material and instilled into the tracheae of excised rat lungs during continuous ventilation. The imaging technique involves recording X-ray images of the experiment in real time, enabling the viewer to observe and evaluate large-scale liquid dynamics in the airways. The images are then transferred to digital image format for data analysis.

**Surfactant material.** The surfactant used in this study is a natural surfactant preparation made from bovine lung surfactant, Survanta (Ross Laboratories, Columbus, OH). The liquid surfactant is mixed with a radiopaque tracer, meglumine diatrizoate (Sigma Chemical, St. Louis, MO). The tracer is available as a powder and is mixed with the surfactant in the quantity $0.6 \text{ g}/\text{ml}$. Hereafter the surfactant + meglumine diatrizoate mixture is referred to as SMD. We measure the viscosity of the surfactant as $45 \text{ cStokes}$, and the viscosity of SMD is $10 \text{ cStokes}$. Both are measured by use of an Oswald bulb viscometer at room temperature. Using a platinum-iridium ring tensiometer, we measure the surface tension of the surfactant at room temperature to be $48 \text{ dyn/cm}$, and SMD is $54 \text{ dyn/cm}$. The density of SMD is measured to be $1.22 \text{ g/cm}^3$. The SMD is removed from refrigeration ~30 min before usage, as the experiments are conducted at room temperature.

**Matching dimensionless parameters between experimental and clinical values.** In order for experiments on liquid plug transport in animal lungs to model accurately the treatments in infant and adult lungs, the experimental systems are designed such that the relevant dimensionless parameters will match typical clinical values. First examine the dimensional parameters for SRT and PLV in infants and adults. Halpern et al. (7) estimate typical clinical values for the velocity of an inspired surfactant bolus in the trachea of an infant and adult; values are listed in Table 1. During PLV in infants, the ventilatory frequency can be up to $50 \text{ breaths/min}$ (4). The perfluorocarbon Peflubron (Alliance Pharmaceu-

<table>
<thead>
<tr>
<th>System Type</th>
<th>$\alpha$, cm</th>
<th>$V_t$, ml</th>
<th>$f$, breaths/min</th>
<th>Inspiration-to-Expiration Ratio</th>
<th>$T_i$, s</th>
<th>Tracheal Velocity, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT infant</td>
<td>0.15</td>
<td>6</td>
<td>30</td>
<td>1.2</td>
<td>0.7</td>
<td>125</td>
</tr>
<tr>
<td>SRT adult</td>
<td>0.89</td>
<td>500</td>
<td>10</td>
<td>1.2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>PLV infant</td>
<td>0.15</td>
<td>6</td>
<td>50</td>
<td>1.1</td>
<td>0.6</td>
<td>140</td>
</tr>
<tr>
<td>Current rat experiments</td>
<td>0.162 ± 0.008</td>
<td>4</td>
<td>40</td>
<td>1.2</td>
<td>0.5</td>
<td>96</td>
</tr>
</tbody>
</table>

The surfactant replacement therapy (SRT) values estimated from Ref. 7; partial liquid ventilation (PLV) frequency from Ref. 5. $\alpha$, Tracheal radius; $V_t$, tidal volume; $f$, ventilation frequency; $T_i$, inspiration time. Tracheal velocity ($U$) equals $\pi a^2 \cdot V_t \cdot T_i$. 


tential, San Diego, CA) has a density of 1.93 g/cm³, viscosity of 1.10 cStokes, and surface tension of 18 dyn/cm (8). Assuming an inspiratory-to-expiratory ratio near 1:1 at this higher frequency, we estimate the typical clinical values for PLV in infants, also shown in Table 1.

Dimensionless parameters are calculated for typical clinical values and for the current rat experiments and are shown in Table 2. The capillary number, Ca = μU/σ, quantifies the ratio of viscous forces to surface tension forces. Again, μ and σ are the exogenous surfactant viscosity and surface tension, respectively, and U is the velocity of the trailing meniscus of the air-blown liquid plug. The Bond number, Bo = gρa²/σ, quantifies the ratio of gravitational forces to surface tension forces; g is acceleration due to gravity. Here, ρ is the exogenous surfactant density and a is the tracheal radius. The Reynolds number, Re = Ua/ν, where ν is the kinematic viscosity, quantifies inertial forces to viscous forces. A dimensionless tidal volume can be calculated as V/T a³, where V/T is the dimensional tidal volume and a is the tracheal radius. The Strouhal number is Str = ωa/U, where ω is the frequency of ventilation.

SRT typically involves instilling 100–200 mg surfactant kg body wt (20–80 mg surfactant/ml solution) at a dose of 2–5 ml/kg body wt. The dose is often divided into several smaller aliquots inserted between breaths over a period of a few minutes. If we assume that each aliquot forms a plug, a dimensionless aliquot volume may be calculated as V/a³. Table 2 shows the estimated V/a³ value for each aliquot, assuming that an infant with body mass of 1,000 g has a tracheal diameter of 0.3 cm receives 2–5 ml of surfactant instilled over 60 breaths and that an adult with 70 kg body mass receives 140–350 ml of surfactant over 50 breaths. The Stokes number and the Froude number are two dimensionless parameters that are not independent but are worth a closer look. The Stokes number St = Ca/Bo quantifies the ratio of viscous forces to gravitational forces. The Froude number Fr = ReCa/Bo = U²/ɡa represents the ratio of inertial forces to gravitational forces. This is an important parameter to investigate as a way of relating plug propagation to gravitational drainage. Experimental estimates of St and Fr values are listed in Table 2.

The current experimental parameters are listed in Table 1 (dimensional) and Table 2 (dimensionless). Estimates for the current rat experiments are based on SMD properties mentioned above. The average tracheal diameter measures 3.25 ± 0.19 mm (mean ± SD). The SMD liquid is instilled at approximately V = 0.053 ml/breath. Because the dimensionless parameters for these experiments fall within the clinical ranges, these experiments are suitable to model SRT and plug flow dynamics in the lungs.

Animal preparation. All of the animals used in this study are handled in accordance with the Animal Welfare Act. Ten healthy adult male Wistar rats, with an average mass ~500 g (494 ± 39) and lungs ~3 cm in length, are used. The animals are anesthetized with 40 mg/kg of Demutol, then the heart and lungs are removed via a thoracotomy, and the heart is completely dissected out to eliminate any cardiogenic motion during imaging. A flared polyethylene tube (PE 240) with 0.2-cm OD is inserted into the proximal end of the trachea and tightly secured. The lungs are suspended vertically from this tracheal cannula. Ventilation is established at 40 breaths/min before liquid instillation is begun. The experiments are performed on the freshly isolated rat lungs immediately after the thoracotomy so that the endogenous surfactant layer is essentially intact.

Experimental setup and procedure. The imaging system used in this experimental setup consists of a microfocal X-ray source capable of producing focal spots as small as three microns, coupled with a precision X-Y-Z-theta stage and an image-intensified digital detector (see Fig. 1). Projected images of radiopaque liquid motion in excised lungs are captured at video rates of 30 frames/s. This system has been used to image the pulmonary circulation down to the arteriolar and capillary level (30-µm diameter) and track the time course of a vascular bolus (K. Cassidy and J. B. Grothberg, unpublished results). The X-ray images are acquired by a high-resolution camera (Sony AI-01-CCD) and recorded to SVHS video format and later digitized on a Mac computer using Fusion Recorder and Adobe Premiere software.

The lungs are mounted in the X-ray chamber, inflated to total lung capacity, and then deflated to approximately FRC (4 mmHg transpulmonary pressure). A small (PE 50) tube is introduced through the tracheal cannula for instillation of the SMD. The lungs are continuously ventilated with forced inspiration and passive expiration by using a Harvard Model 683 rodent ventilator. The SMD is injected through the small PE tubing from a 10-ml syringe driven by a Harvard model 903 infusion/withdrawal pump. After ventilation is established, SMD is pumped into the trachea from the syringe pump at a rate of 2.10 ml/min (calibrated 0.053 ml/breath). For case A experiments, the tip of the SMD tubing is lowered past the end of the tracheal tube. The liquid drips directly into the upper airways; this method encourages gravitational drainage. For case B, the tip of the SMD tubing is pulled up into the tracheal tubing. As the liquid exits the small tubing it has a tendency to wet the wall and form a plug within the tracheal tubing; this method encourages plug formation in the trachea. The dose is instilled during constant ventilation, and experimental measurements are taken for the first 10 breaths after the onset of instillation. The average end-expiratory pressure for these experiments is 2.9 Torr.

Figure 2 shows schematic diagrams simplifying the difference between experimental methods for the two sets of experiments. For case A (n = 5), the instilled liquid initially drains down the side of the trachea into the lungs. The liquid tends to pool in medium-sized airways and form liquid plugs there (Fig. 2A). The timed inspiration follows (Fig. 2B), and the liquid acts as air-blown liquid plugs starting from the

<table>
<thead>
<tr>
<th>System Type</th>
<th>Capillary Number (Ca = μU/σ)</th>
<th>Bond Number (Bo = gρa²/σ)</th>
<th>Reynolds Number (Re = Ua/ν)</th>
<th>Tidal Volume (V/T a³)</th>
<th>Strouhal Number (Str = ωa/U)</th>
<th>Aliquot Volume (V/a³)</th>
<th>Stokes Number (St = Ca/Bo)</th>
<th>Froude Number (Fr = ReCa/Bo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT Infant</td>
<td>1.2</td>
<td>0.46</td>
<td>42</td>
<td>1800</td>
<td>3.5 × 10⁻²</td>
<td>10 - 25</td>
<td>2.6</td>
<td>55</td>
</tr>
<tr>
<td>SRT Adult</td>
<td>0.94</td>
<td>16</td>
<td>200</td>
<td>700</td>
<td>8.9 × 10⁻²</td>
<td>4 - 10</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>PLV Infant</td>
<td>0.17</td>
<td>2.4</td>
<td>1900</td>
<td>1800</td>
<td>5.3 × 10⁻²</td>
<td>10 - 25</td>
<td>7.1</td>
<td>68</td>
</tr>
<tr>
<td>Current rat</td>
<td>experiments</td>
<td>0.22</td>
<td>0.58</td>
<td>160</td>
<td>6.7 × 10⁻²</td>
<td>12.4</td>
<td>0.38</td>
<td>29</td>
</tr>
</tbody>
</table>

Values are calculated for the trachea. See text for definitions of abbreviations.
regions where the liquid has blocked an airway. For case B (n = 5), a liquid plug forms in the trachea tube just before inspiration begins (Fig. 2C) so that the liquid becomes an air-blown liquid plug starting from the trachea on inspiration (Fig. 2D). End inspiration is chosen as an appropriate point for investigating liquid distribution because the lung shape is repeatable, the motion is quasi-static, the liquid would be the furthest reaching for that breath, the lung volume would be the greatest, and the distribution would be most directly affected by the delivery method (as opposed to reflux).

**Analytical Methods**

Two analytical studies are used to quantify the liquid transport and distribution through the airways. The first study explores the liquid dose transport dynamics during the first few inspired breaths after the onset of liquid instillation. The experimental results are quantified through statistical measures and then compared with a simple theoretical model of liquid plug propagation. The second study assesses an index of homogeneity to quantify liquid distribution throughout the lungs at end inspiration for the first 10 breaths. The homogeneity index results are compared for case A and case B.

The transport of the SMD is visible in the two-dimensional image as the liquid spreads through the lung. The digitized images are raw 640- by 480-pixel bitmap files in which a numerical intensity value I(x,y) is assigned to each of the pixels, ranging from 0 (black) to 255 (white) with a gray scale level between. Areas of the lung containing the radiopaque SMD exhibit a greater intensity than lung regions without it. A separate image of the lung is captured before any liquid has been instilled, where I₀(x,y) represents the intensity at
Each pixel. For both of the studies, the image of a lung with liquid is compared with the image of the lung without liquid to track the liquid location.

Liquid dose dynamics. The liquid transport during a single dynamic breath is quantified by statistical methods. As liquid travels from the trachea toward smaller airways, quantities for the liquid location change with time, including the leading edge, the center of liquid mass, and the standard deviation, skewness, and kurtosis of spatial distribution. Each image of the lung containing liquid (sampled at 6/s) is loaded into Matlab (MathWorks) and converted to a double-precision matrix, as is a similar matrix corresponding to the lung before liquid instillation. Let $I_L(x,y)$ be the intensity at each pixel (matrix element) of the image of the lung containing liquid, and $I_T(x,y)$ is the intensity at each pixel for the image of lung tissue without liquid.

Beer’s law (24) quantifies the illumination of radiopaque material in an X-ray

$$I(x, y) = I_0(x, y)e^{-mz}$$  \hspace{1cm} (1)

where $I$ is the intensity of an image containing material, $I_0$ is the intensity of an image without material, $m$ is the absorption coefficient of the material, and $z$ is the path length through the material. If the material under investigation is tissue, then the equation becomes

$$I_T(x, y) = I_0(x, y)e^{-m_Tz}$$. \hspace{1cm} (2)

where $I_T$ is the image with the lung tissue, $I_0$ is the image of the lung tissue without liquid, $m_T$ is the absorption coefficient of the tissue, and $z_T$ is the path length through the tissue. For an image which contains tissue and radiopaque liquid, the material under investigation is the liquid, such that

$$I_L(x,y) = \frac{I_0(x,y)}{I_T(x,y)}$$ \hspace{1cm} (3)

where $I_L$ is the image of the lung containing liquid, $I_T$ is the image of the lung tissue without liquid, $m_L$ is the absorption coefficient of the liquid, and $z_L$ is the path length through the liquid; the term $m_Lz_L$ is a measure of the mass of liquid. To quantify the illumination of radiopaque liquid, the matrix elements are determined by substituting Eq. 2 into Eq. 3 and using scalar math (element-by-element) to solve for $m_Lz_L$. The scaled intensity matrix ($\hat{I}$) is

$$\hat{I}(x,y) = m_Lz_L(x,y) = -\log\left[\frac{I_0(x,y)}{I_T(x,y)}\right]$$ \hspace{1cm} (4)

The matrix $\hat{I}(x,y)$ is then run through a threshold-limiting filtering process to minimize digital noise and then cropped to ignore regions outside of the lung. The origin of the ($x,y$) coordinates is positioned on the image where the trachea meets the edge of the lung. Positive $x$ points vertically along the axis of the trachea toward the first bifurcation (in the direction of gravity) and positive $y$ points horizontally toward the left (appears right) lung.

The statistical analysis determines the location and distribution of liquid by using the spatial center of mass of liquid in the lungs, $\bar{x}$. The distribution of the liquid mass can be characterized by its probability density function, $P(x_0,y_0)$, where $x_0$ and $y_0$ are dummy variables in the $x$ and $y$ directions, respectively

$$P(x_0, y_0) = \frac{\hat{I}(x_0, y_0)}{\int_{-5}^{5} \int_{-5}^{5} \hat{I}(x,y) dx dy}$$ \hspace{1cm} (5)

To determine the vertical statistics, first find the moments about the $x$-mean ($\bar{x}$)

$$M_n(y) = \int_{-\infty}^{\infty} (x - \bar{x})^n P(x,y) dx$$ \hspace{1cm} (6)

Then the average value for the $k$th moment is

$$M_k = \int_{-\infty}^{\infty} M_k(y) dy = \int_{-\infty}^{\infty} (x - \bar{x})^k g(x) dx = \sum_{x=1}^{m} (x - \bar{x})^k g(x)$$ \hspace{1cm} (7)

where the marginal probability density function is

$$g(x) = \frac{\int_{-\infty}^{\infty} \hat{I}(x_0, y) dy}{\int_{-\infty}^{\infty} \hat{I}(x_0, y) dx dy}$$ \hspace{1cm} (8)

From the first four moments, the four statistical quantities are defined

$$\bar{x} = M_1$$, mean value

$$\text{skew}_x = \frac{M_3}{\sigma_x^3}$$, skewness

$$\sigma_x = (M_2 - M_1^2)^{1/2}$$, standard deviation

$$\text{kurt}_x = \frac{M_4}{\sigma_x^4} - 3$$, kurtosis

For the horizontal statistics, Eqs. 5–9 may then be computed in the opposite direction.

Skewness indicates the degree of asymmetry of a distribution around its mean. A positive (negative) skewness indicates a distribution with an asymmetrical tail extending out toward more positive (negative) $x$. The kurtosis indicates the relative peakedness or flatness of a distribution relative to a normal distribution. A positive (negative) kurtosis indicates a distribution that is more peaked (flat) than a normal distribution. These statistics indicate the shape and uniformity of the distribution of liquid in the lung in the $x$ direction.

Homogeneity index of distribution. The time-varying distribution of liquid is evaluated for the first 10 breaths after the onset of liquid instillation for case A and case B. Data are measured at end inspiration (after breath 1, after breath 2, etc.) when the lung volume is a maximum and the liquid motion is quasi-static. For each successive breath, the liquid mass in the lung increases and the distribution of the liquid changes. The lungs are divided into four main quadrants, and liquid deposition between these quadrants is compared to assess the homogeneity of distribution.

The preinstillation image is digitally subtracted, pixel by pixel, from each of the subsequent images in SigmaScan (Jandel Scientific) so that only the radiopaque shadow remains visible, removing background lung structures. This method also compensates for differences in beam intensity and attenuation and lung position between experiments. This processed image then is a map of the distribution path of the instilled liquid. A digital window is placed over the outer regions of the image, covering the border of the image, the time/date stamp from the video recorder, and any regions with no lung, so that these pixels are ignored. A thresholding method is used, in which pixels having a numeric intensity above a threshold value are marked, so that portions of a lung region containing the liquid can be identified and mea-
sured. Figure 3A shows a sample digital image in raw pixel form, lightened here for clarity to the reader. Figure 3B is the same image processed via image subtraction and thresholding. Dark lines define the four lung quadrants, and the window covers nonlung regions that are ignored during analysis.

To the viewer the quadrants are labeled, clockwise from the trachea, as the upper left (UL), lower left (LL), lower right (LR) and upper right (UR) regions of the lungs. The thresholding process marks the pixels where the liquid has reached, which can then be counted. Within each quadrant, the two-dimensional area reached by the liquid is divided by the area of the quadrant. This value is presented as a percentage of the area reached (AR) within the quadrant and accounts for any variation in area between the lung quadrants. We define a homogeneity index (HI) as the smallest value of AR in a lung quadrant divided by the largest AR in another quadrant of the same image (this actually represents the minimum value of HI for distribution of liquid between any two lung quadrants). For example, if the lung quadrants are reached at a level of AR = 40% in the LL region, 50% in LR, 20% in UL, and 25% in UR, then HI = 40%. If all four quadrants have the same AR value, then the distribution is considered to be 100% homogeneous, regardless of which breath is being measured or how much of the entire lung is filled. Thus the HI is a measure of the amount of liquid that reaches a lung quadrant compared with the values in the other quadrants.

RESULTS

Liquid Dose Dynamics

Images of liquid transport during inspiration are shown in Fig. 4. This sample corresponds to case A but demonstrates both gravitational drainage and liquid plug propagation. The images are cropped and lightened for clarity to the viewer. During the first breath, instilled liquid begins to drain into the trachea, and we designate $t = 0$ as the point at which liquid passes the $(x, y)$ origin, which occurs about midway through inspiration. Figure 4A is taken at 0.43 s after the liquid instillation began, at the end of inspiration (EI) for the first breath (abbreviated as EI-1). The liquid (dark) begins to drain from the trachea into the first bifurcation. Gravitational drainage continues through expiration. Figure 4B is taken at 1.33 s, at the end of expiration (EE) for the first breath (EE-1). A liquid plug is visible in the large airway branching in the positive $x$ direction, and liquid travels rapidly toward the periphery of the lung. Figure 4C is taken at 1.5 s, during the next inspiration. The liquid plug moves distally and branches into the entrance of several more generations of airways. Figure 4D corresponds to 1.7 s, at EI-2, when liquid reaches smaller airways and alveoli, mainly in the UR and LL lung regions.

A statistical evaluation is performed on the first few breaths after the onset of liquid instillation for the animal shown in Fig. 4. The statistical results for liquid distribution include center of mass, standard deviation, skewness, and kurtosis. The leading edge of liquid is also traced as a function of time in the $x$ direction and in the positive and negative $y$ directions. The leading edges are shown in Fig. 5A scaled on the lung length. For the first 1.3 s, the vertical leading edge moves under gravitational drainage. The horizontal leading edges demonstrate little branching, which can be expected because the liquid appears to drain down the right side of the trachea (see Fig. 4, A and B). The average vertical plug velocity $U_{vert} = 0.28$ cm/s for $0.4 < t < 1.2$ s. During the second inspiration, $1.3 < t < 1.7$ s, the leading edges move more quickly in both the vertical and horizontal directions, as rapid liquid transport disperses liquid to multiple branches of smaller airways (see Fig. 4, C and D). Here, the average vertical plug velocity is $U_{vert} = 4.0$ cm/s corresponding to $Ca = 9 \times 10^{-3}$. At the end of inspiration, $t \approx 1.7$ s, the leading edges have reached a local maximum. The time course of the leading edge positions increase at a slower rate for the remainder of the experiment.

Figure 5B shows the time-dependence of $x$, $y$, $\sigma_x$, and $\sigma_y$ for these first three breaths. For this sample, there is slightly more lung tissue in the right lung (negative $y$) than the left. Although the width of the lung is

Fig. 3. Method of analysis performed on a case B sample after the sixth aliquot. A: original image. B: after image processing.
typically smaller than the lung length, the mean and standard deviation in both directions are scaled on the lung length. Here the span of $y$ is $\sim 90\%$ of the lung length, so mean value and standard deviation are expected to be smaller in the $y$ direction than the $x$ direction.

During the first breath, the vertical center of mass increases from $\bar{x} = 10\%$ to $22\%$ during inspiration and then decreases to $16\%$ during expiration. During the second breath, $\bar{x}$ rapidly increases to $42\%$ during inspiration and then decreases to $25\%$ during expiration. The cycle continues for the third breath, in which $\bar{x}$ returns to $42\%$ during inspiration and then decreases to $34\%$ during expiration. The cyclic pattern is due to the dynamics of inspiration and expiration, although lung shape and liquid reflux may also be a factor. The statistical values are scaled on a single value of the maximum lung length, but the actual lung increases in size on inspiration and decreases during expiration that may amplify the cyclic behavior. The horizontal center of mass stays near the negative side of $\bar{y} = 0$ or revolves about the centerline of the lung. The standard deviation in the $x$ and $y$ directions has a cyclic pattern for the first breath, which is damped for successive breaths. As discussed in the previous paragraph, rapid liquid transport occurs from $1.3 < t < 1.7$ s. Here, the mean values and standard deviations change at a rapid rate.

The skewness and kurtosis in the $x$ and $y$ directions are shown in Fig. 5C. The skew$_x$ is positive at $t = 0$ (asymmetric tail pointing away from origin) as the liquid is mainly in the trachea. The skew$_y$ is also positive at $t = 0$, demonstrating the offset of the trachea from $y = 0$. During gravitational drainage ($t < 1.3$ s), skew$_x$ and skew$_y$ decrease slightly. Rapid liquid transport ($1.3 < t < 1.7$ s) causes the skewness in both
directions to drop rapidly toward zero, where they remain. The \( \kurt_x \) is strongly positive, and the \( \kurt_y \) is also positive, at \( t = 0 \), representing a peaked distribution that accurately represents the liquid mass localized in the first few generations. For the remainder of the first breath, the \( x \) and \( y \) kurtosis decrease in magnitude but remain positive in value, and increases somewhat during the end of expiration. During the first part of the rapid liquid transport, \( \kurt_x \) and \( \kurt_y \) decrease rapidly toward zero for \( 1.3 < t < 1.5 \) s. Because a negative kurtosis represent a more flat distribution than normal, negative values for kurtosis are desirable for a homogeneous distribution in the lung. For \( t \geq 1.4 \) s, \( \kurt_x \) remains negative. There is little kurtosis in the \( y \) direction except at the end of expiration (\( t \approx 2.8 \) s) where it becomes positive and then drops again to zero.

To evaluate the relevance of the statistical measures, consider a simple theoretical one-dimensional model for liquid plug flow through a straight tube. Let a plug with initial length \( L_0 \) propagate at constant velocity \( U \) through a tube with radius \( a \) (see Fig. 6A). At time \( t \), the trailing meniscus of a liquid plug is located at position \( x_T \) (where \( T \) is time), the plug length is \( L \), and the leading meniscus is located at position \( x_T + L \) (see Fig. 6B). The trailing film has thickness \( h \) and \( h/a \) is a function of \( Ca \), or \( h/a = f(Ca) \). As the plug propagates, the length decreases as film is transferred onto the tube wall until finally the plug will rupture at \( T_F = L_0/2U \). For liquid plug propagation, Halpern et al. (7) estimate

\[
f(Ca) = 0.36[1 - \exp(-2Ca^{0.523})]
\]  

(10)

Fig. 5. Experimental statistical values for the instilled liquid mass during the first 2 breaths. A: leading edge of liquid in the \( x \) direction, \( y \) direction toward the left lung, and \( y \) direction toward the right lung. B: mean \( \bar{x} \), \( \bar{y} \), and standard deviation (std) \( \sigma_x \), \( \sigma_y \). C: skewness, \( \text{skew}_x \), \( \text{skew}_y \), and kurtosis \( \kurt_x \), \( \kurt_y \). Small top figures show lung inflation between end expiration (EE) and end inspiration (EI).
Using simple conservation of mass arguments, the volume density of the liquid $\rho(x)$, or volume per unit length, is approximately

$$\rho(x) = \frac{2\pi \alpha^2 f}{\pi \alpha^2} \frac{0 \leq x \leq x_T}{x_T \leq x \leq x_T + L} \quad (11)$$

The volume can be integrated from the density for the two segments, and time $t$ is scaled on $T_F$ such that $T = t/T_F$. If $M_n$ is the $n$th moment of the liquid mass, then the dimensionless $n$th moment is derived as

$$\hat{M}_n(T, Ca) = \frac{M_n}{\pi \alpha^2 L_0} = \frac{1}{n + 1} \left\{ \left( \frac{T}{2f} \right)^{n+1} (2f - 1) \right\} \quad (12)$$

valid for $0 \leq T \leq 1$. The first, second, third, and fourth moments can be used to calculate the center of mass, standard deviation, skewness, and kurtosis of the progressing plug. The leading edge, $\lambda$, of the liquid progression is the same as the leading meniscus position and can be scaled on $L_0$ and $T$, yielding an equation for the dimensionless leading edge

$$\hat{\lambda} = \frac{\lambda}{L_0} = \frac{T}{2f} (1 - 2f) + 1 \quad 0 \leq T \leq 1 \quad (13)$$

Then the experimental $x$-direction leading edge, center of mass, standard deviation, skewness, and kurtosis of the progressing plug during the first breath can be compared with the theoretical predictions of these values.

Consider the portion of the second inspired breath in Fig. 5 consisting mainly of liquid plug propagation, i.e., $1.3 < t < 1.7$ s. Figure 7A displays the experimental vertical leading edge, center of mass, and standard

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**Fig. 7.** Statistical measures for propagation of a liquid plug in 1 dimension. Vertical experimental data, dimensionless, during the first breath ($0.9 < t < 1.2$ s from Fig. 6): $\bullet \lambda$, $\circ \bar{x}$, and $\nabla \sigma_x$ (A); $\bullet$ skew, and $\circ$ kurt, (B). Theoretical predictions, dimensionless, for a plug through rupture (plug in Fig. 7, $0 < t < T$); solid line, $\lambda$, dotted line $\bar{x}$, and dashed line $\sigma_x$ (C); solid line skew, and dotted line kurt, (D). See text for details.
deviation for this time period, scaled on the maximum value of the leading edge. The corresponding skewness and kurtosis in the vertical direction are shown in Fig. 7B, each scaled on their own maximum value. Both Fig. 7A and Fig. 7B are plotted against the time T, which is the time in seconds scaled on the final time. In comparison, the dimensionless theoretical leading edge, center of mass, and standard deviation in one dimension are shown in Fig. 7C, and the corresponding skewness and kurtosis are shown in Fig. 7D, calculated for $Ca = 9 \times 10^{-3}$. The trends in statistical behavior can be compared for the experimental results and the theoretical predictions.

The experimentally determined positions of the leading edge, mean value, and standard deviation support the theoretical predictions. The leading edge has a greater magnitude than the mean value, as well as a steeper slope. The standard deviation is the smallest of the three, both in magnitude and in slope. The experimental skewness and kurtosis follow the theoretical trends for $T > 0.1$, where the slopes are negative. Note in Fig. 5C that the data just before this exploded section have a positive slope as predicted for the skewness and kurtosis. In experiments, the skewness approaches zero as $T \rightarrow 1$, and the kurtosis becomes negative for $T > 0.3$. The negative kurtosis demonstrates that the liquid is distributed to more distal regions than a normal distribution. For this application, this indicates that liquid is dispersed from a single plug of liquid to many smaller airways throughout the lung.

**Homogeneity Index of Distribution**

Images of the lungs captured at end-inspiration are shown in Fig. 8. **Case A** is shown in Fig. 8A for EI-1. Figure 8B corresponds to EI-3, Fig. 8C shows EI-6, and Fig. 8D shows EI-10. For this sample, the liquid begins to drain down the trachea and into the main bronchi. At EI-1, liquid pools in a main bronchus on the left side. At EI-3, the pooled liquid is blown into some of the smaller airways, mainly delivering liquid to the LL region. At EI-6, liquid continues to distribute well into the LL lung, and some liquid has reached into some of the UL and UR lung as well. Draining liquid in the central right region is visible in a main bronchus, and liquid begins to drain into the LR quadrant. **Case B** is shown in Fig. 8E at EI-1, in Fig. 8F at EI-3, in Fig. 8G at EI-6, and in Fig. 8H at EI-10. At EI-1, some liquid has reached into the main bronchi on both the left and right sides. At EI-3, the first few generations have liquid on the airway walls, and a light distribution of liquid has branched into a few of the smaller airways. At EI-6, the liquid has reached many of the smaller airways of the LL, LR, and UR regions. At EI-10, liquid has distributed to smaller airways in all four lung quadrants.

Using the thresholding method, the amount (area) of each lung region coated with liquid is measured at end inspiration for each breath and compared with the area of the region. A sample graph is presented for each case, corresponding to the same lung samples shown in Fig. 8. The area reached by the advancing liquid within each lung quadrant is displayed as a function of the breath number in Fig. 9. The lung regions represented are the LL ($\triangle$), LR ($\bigcirc$), UR ($\square$), and the UL ($\bigcirc$) quadrants. **Case A** results are shown in Fig. 9A. At EI-1 most of the liquid is in the UL lung. After EI-10 the least amount of liquid is in the LR region, with only 10% of the area reached, and the greatest amount is in the LL region (63%). Therefore, this **case A** sample has a homogeneity index of 15% at EI-10. Note that these quantitative results accurately reflect the visual results shown in Fig. 8, A–D. This sample is chosen with the worst distribution results to emphasize the inhomogeneity of **case A**. **Case B** results are shown in Fig. 9B. Throughout the 10 breaths, the liquid distributes fairly evenly to each quadrant, which is visible in Fig. 8, E–H. After EI-10, the least amount of liquid (lowest AR) is in the LR region (43%), and the greatest amount is in the UR region (49%). This **case B** sample, with HI = 88% at EI-10, is chosen to emphasize the improvement in homogeneity of **case B**.

The homogeneity index values from each experiment in **case A** and also **case B** are combined and averaged. Figure 10 displays the average homogeneity index as a percent. The open circles represent **case A** and the filled circles represent **case B**. The vertical error bars represent experimental scatter. For every aliquot, HI is higher for **case B** than for **case A** and is, on average, 2.4 times higher. After EI-10, HI for **case B** is 65% whereas HI for **case A** is only 35%. Overall this indicates a significant improvement in homogeneity of the liquid distribution for **case B** over **case A**.

**DISCUSSION**

On first viewing the experiments, we notice that the motion of a liquid plug, propagated by inspiration, is very rapid and liquid can reach the periphery of the lung within a few video frames. The plug of liquid appears to explode in a spray toward distal airways. The transport occurs so quickly that the imaging sampling rate of 30 frames/s is not sufficient to resolve the issue of whether the plug in fact becomes an aerosol. However, the current study of liquid dose dynamics reveals that the propagation behavior follows trends of liquid plug flow statistics. Therefore, it is believed that the plug does not aerosolize but behaves as a rapidly progressing, air-blown liquid plug.

Figure 4, B and D, demonstrates liquid traveling from the first few generations to very small airways within the first few breaths. If we assume that all of the airways are parallel to the two-dimensional image, the distance tracked from the leading edge of the liquid in Fig. 4B to the small airways of Fig. 4D is ~1.5 cm. Airways with tilt would have a longer path than projected onto the two-dimensional image, but the velocity is estimated as upward of 4 cm/s. Closure also occurs rapidly, on the order of 1–2 video frames (~30 to ~45 s),
Fig. 8. Images of sample experiments at end-inspiration. *Case A* after the 1st (A), 3rd (B), 6th (C), and 10th (D) aliquot; *case B* after the 1st (E), 3rd (F), 6th (G), and 10th (H) aliquot.
which is visible when the flow changes direction between the end of inspiration and the onset of expiration. After reforming, the liquid plugs can then travel proximally and may even reflux out of the trachea. During the next inspiration, plugs that have moved toward the trachea may then be blown back down the same pathway and progress further than during the last breath or may branch into bifurcations previously not reached. In this sense, reflux liquid appears to promote more homogeneous distribution through the lung. However, a significant amount of reflux may be evidence of a liquid-filled airway branch, leading to compromised gas exchange. A goal to consider for clinical therapy is optimal surfactant distribution by means of film deposition with minimal remaining liquid blocking the lumen.

The motion of the gravitational drainage occurs on a much slower time scale. In Fig. 8, C and D, it is visible in a large airway in the central right lung. Between these two images, or over seven breaths (12 s), the draining liquid moves \(0.8\) cm (assuming the airway is parallel to the two-dimensional image). The velocity is therefore measured as \(0.07\) cm/s, or slower by a factor of 50 than the fast air-blown liquid plug velocity shown in Fig. 4. The opportunity of an airway to receive liquid is also governed by gravitational configuration. Therefore airflow more effectively distributes liquid through the airways when the air is propagating a liquid plug rather than flowing over a draining stream of liquid. When draining pools into liquid plugs, in an area which blocks the cross section of a branch, transport of liquid via air-blown liquid plugs occurs. A relatively uniform distribution is observed distal to pooled regions.

When liquid travels from a parent to daughter branch, it may divide unevenly between the daughters for reasons including size, branch angle, and gravitational direction. For liquid that distributes primarily by gravitational drainage, the liquid travels into bifurcations that are “lower” than the parent branch, or in the direction of gravity, at each generation. However, for liquid that is driven by a propagating liquid plug, the liquid can be seen visibly to move upward, or opposing the direction of gravity, both during experiments and via the experimental results. Therefore plug formation is key in distributing liquid to many airways in the upper lung regions, which is visible in both cases.

One of the relevant issues regarding surfactant dosing is the possibility that repeated surfactant doses tend to deliver liquid through the same path as previous doses. On the basis of our previous work (K. Cassidy and J. B. Grotberg, unpublished results) with a liquid plug passing through a symmetric bifurcation that has a liquid plug blocking one of the daughters, the test plug shows preference to delivering liquid into the unblocked daughter, because there is less resistance to flow than in the blocked daughter. The results in the current study show that, for case A experiments, repeat aliquots (via ventilated breaths) often prefer to deliver to the same pathway (see Fig. 8, A–D). The gravitational configuration strongly affects the liquid distribution for these types of experiments.

In conclusion, to achieve homogeneous distribution of liquid distribution throughout the entire lung, the
method of plug formation (case B) shows superior results to gravitational insertion (case A). Average results indicate that the homogeneity of case B is nearly twice the homogeneity of case A. Statistical results for rapid liquid transport in the lungs follow similar trends to theoretical predictions for liquid plug propagation in a tube. A plug of liquid driven by forced air can reach alveolar regions within the first few breaths. The key to even distribution of liquid in any lobe or region of the lung is plug formation in the airway that supplies liquid to that lobe or region. A liquid plug propagated by forced air tends to coat distal airways rather evenly provided that enough liquid is present. Even in a lung that receives instilled liquid by means of gravitational drainage, liquid can pool in an airway and form a plug or may reflux into an airway that previously did not contain liquid. That plug is then subject to the same means to distribute evenly to distal airways. If the objective of therapy is to target liquid delivery to a certain pulmonary lobe, it appears that this may be achieved by inserting liquid plugs in the airway branch proximal to the target lobe.

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