Onset of alveolar recirculation in the developing lungs and its consequence on nanoparticle deposition in the pulmonary acinus

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Henry FS, Tsuda A. Onset of alveolar recirculation in the developing lungs and its consequence on nanoparticle deposition in the pulmonary acinus. J Appl Physiol 120: 38–54, 2016. First published October 22, 2015; doi:10.1152/japplphysiol.01161.2014.—The structure of the gas exchange region of the human lung (the pulmonary acinus) undergoes profound change in the first few years of life. In this paper, we investigate numerically how the change in alveolar shape with time affects the rate of nanoparticle deposition deep in the lung during postnatal development. As human infant data is unavailable, we use a rat model of lung development. The process of postnatal lung development in the rat is remarkably similar to that of the human, and the structure of the rat acinus is indistinguishable from that of the human acinus. The current numerical predictions support our group’s recent in vivo findings, which were also obtained by using growing rat lung models, that nanoparticle deposition in infants is strongly affected by the change in the structure of the pulmonary acinus. In humans, this major structural change occurs over the first 2 yr of life. Our current predictions would suggest that human infants at the age of ~2 yr might be at most at risk to the harmful effects of air pollution. Our results also suggest that dose estimates for inhalation therapies using nanoparticles, based on fully developed adult lungs with simple body weight scaling, are likely to overestimate deposition by up to 55% for newborns and underestimate deposition by up to 17% for 2-yr-old infants.

Structural Changes in the Infant Acinus

FROM BIRTH TO ADULTHOOD, the human pulmonary acinus undergoes dramatic structural change, not only in size but also in internal architecture. At birth, the human lungs are in the early stage of the alveolation phase, consisting of only about 30 million alveoli at birth (more than 10 times fewer alveoli compared with the fully developed adult lungs; 19). Rapid structural alveolation continues during the first few years after birth, followed by an increase in alveolar size until later childhood. The lung volume also increases, by about 13 times from birth to age 6 in children, and by 3 times from age 6 to adulthood by lengthening the acinar and conducting airways. However, there is no doubt that the formation and deepening of alveoli are the major events during the first 2 yr of lung development.

While the development of the human lung is known in broad terms, the details of the time history of the growth of individual alveoli are much less well known. Such detailed data are difficult to acquire in humans because of obvious ethical concerns which preclude in vivo experiments on human infants. The morphometry of the postnatal rat lung, on the other hand, has been well characterized (for review, see Refs. 3, 5). Burri has shown that the postnatal development of the rat lung is remarkably similar to that of the human (5, 80, 81) and noted that the structure of human and rat lung are indistinguishable at the level of the acinus (4), although the morphology of conducting airways of these two species are very different (dichotomous branching vs. monopodial branching, respectively). Because rats are born before the start of the alveolation phase (explained below) and because postnatal rat lung maturation occurs over a much shorter time scale than that of human beings (5), the rat lungs are a convenient postnatal model system for our investigation with which to simulate particle deposition phenomena accompanying alveolar formation. Finally, we have recently addressed the question of deposition in the developing lung experimentally by using rat infants (58), and hence we have a comprehensive set of data with which to compare our numerical calculations.

Postnatal Rat Lung Maturation

Detailed analyses on the postnatal changes of parenchymal ultrastructure have been performed in rats (e.g., see Refs. 2, 3, 5). Rats are born in the late stage of the saccular phase and undergo an expansionary phase from birth to postnatal day 4 (2) (Fig. 1, left) before progressing to the alveolar stage (82). Bulk alveolation occurs during postnatal days 4-13 (75); the rapid structural alveolation in early postnatal development is achieved by the formation of new septa (denoted secondary septa) from shallow indentations (denoted primary septa) lining the saccules and transitory ducts present at birth (Fig. 2A). Interalveolar septa are formed in concert with the folding up of one of the two capillary layers. When bulk alveolation wanes, lung development enters the stage of microvasculature maturation and septal interstitium thinning (5) which produces a miniature version of adult lung morphology after ~3 wk in rats (compare Fig. 1, middle vs. right). The key feature of the septal thinning stage is the restructuring of the double-layer septal capillaries (Fig. 2B) to more mature single capillaries (Fig. 2C). The four stages of lung development are summarized in Table 1. Note that the phenomenon of late alveolation has been recently reported (e.g., see Refs. 56, 66).

Particle Transport and Deposition in Infants

In general, particle mixing/deposition processes in conduits are strongly conditioned by the nature of the fluid flow in the conduit, and the structure of the flow is strongly influenced by the shape of the conduit (31, 59). Thus we know that particle mixing/deposition processes are strongly conditioned by the shape of the conduits. Hence, the rapid change in lung architecture is likely to affect the rate of particle transport and deposition in the conduits, i.e., respiratory airways, of the infant lung.
The question arises as to what degree the changing structure of the infant lung affects the transport, mixing, and deposition of nanoparticles. For the past two decades, we have studied acinar fluid mechanics and particle mixing/deposition processes in the pulmonary acinus (e.g., see Refs. 27–30, 67, 69, 71). From these studies, we know that 1) the rate of particle mixing/deposition is intricately linked to acinar duct structure, and 2) the structure of the human infant lung (acinus) changes dramatically postnatally. Combining 1) and 2), we suggest that particle mixing/deposition in the infant lung must be very different from that of the adult case. Except for our in vivo study (58), no one has studied the effect of postnatal changes in alveolar geometry and breathing patterns during lung development on the rate of deposition of nanometer-size particles. The goal of this paper is to address this question by analyzing the results of a set of detailed numerical calculations of the transport and deposition of particles in models of infant rat alveoli at various stages of development compared with our recent in vivo experimental data (58).

**Acinar Fluid Mechanics: Dimensionless Parameters**

While many flows are characterized by the Reynolds number, Re (a measure of the relative importance of inertial and diffusive transport), the flow in the acinus has little inertia (as we demonstrate later), and hence Re cannot be used to describe the difference in flow structure in the alveoli of the acinar entrance compared with that in the terminal sacs. Tsuda et al. (67) showed that the ratio of the flow entering an alveolus, \( Q_A \), to that passing over the alveolar entrance in the duct, \( Q_D \), is the factor that defines the type of flow occurring in mature alveoli. That is, when \( Q_A/Q_D \) is relatively small, as in the alveoli of the entrance region of the acinus, the flow in an alveolus rotates; when \( Q_A/Q_D \) is relatively large, as that in the terminal generation, the flow is directed radially. Henry and Tsuda (29) and Henry et al. (30) predicted that recirculation occurs, to some degree, in all but

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1 We note that other groups (e.g., see Refs. 9, 15, 38, 43, 61, 62) have more recently investigated the effects of acinar structure on particle deposition, and have broadly agreed with our earlier findings. However, none have considered the effects on deposition of the change of the alveolus shape during maturation.
the terminal alveoli. Flow rotation, or recirculation, is important because it is believed to be a requirement for the existence of chaotic mixing, which results in flow irreversibility and, in turn, enhanced particle mixing/deposition in the pulmonary acinus (28, 67, 71).

In the developing lung, there is a second parameter that controls the ability of the alveolar flow to rotate; that is, the aspect ratio, \( L_D/L_W \) (=depth/width), of the alveolus (35). As stated above, at birth the acinar airways are relatively smooth with little or no discernable alveoli, and hence there is no opportunity for the flow to rotate. As the secondary septa grow, alveoli are formed and at some point they are deep enough to allow rotation, providing \( Q_A/Q_D \) is sufficiently small. The results of Karl et al. (35) suggest that a single large rotation of the flow cannot occur in alveoli with \( L_D/L_W < \frac{1}{3} \). We note that without rotation, convective mixing cannot occur in the alveolus (28, 67, 71). Hence, the point in the development of the lung at which this mechanism becomes operative is of ultimate interest.

In fluid mechanics, dimensionless parameters are used to identify similarities in seemingly different flows and are used to construct models that when studied produce results that can be used to predict the behavior of a full-scale prototype (77). For meaningful comparison, model and prototype have to be geometrically, kinematically, and dynamically similar. Geometric similarity requires that the model and prototype be identical in shape but differ only in size. Kinematic similarity requires that the ratio of velocities at all corresponding points in the model and the prototype flows be the same. Dynamic similarity requires that the ratio of forces (or pressures) at all corresponding points in the model and prototype flows be the same.

We have mentioned above that the nature of the flow in the developing acinus is governed by the magnitude of the ratio \( Q_D/Q_D \) and by the parameter \( L_D/L_W \). As, in our model, \( Q_D/Q_D \) is proportional to \( V_D/V_D \), \( Q_D/Q_D \) is both a measure of kinematic similarity and geometric similarity. Hence, for the flow in the rat and the human acinus to be considered similar, a necessary condition is that the value of \( Q_D/Q_D \) in the acinus of the two species should be of the same order. Figure 3A shows that \( Q_D/Q_D \) for the adult rat and the human acinus are similar throughout the acinus. In the case of the parameter \( L_D/L_W \), while quantitative comparison on the similarity of this parameter between human and rat alveoli is not possible because data for the human infant do not exist, Burri (4) stated that except for the size difference of the air spaces there is a striking anatomical similarity between the developing human acini and rat acini. Therefore, it is not unreasonable to assume that the change in \( L_D/L_W \) is similar between these species during the process of lung growth.

The basic measure of dynamic similarity for steady flow is the Reynolds number. As mentioned above, \( Re \) is a measure of the relative importance of convective and diffusive transport. However, \( Re \) is also a measure of the relative importance of inertial forces to viscous forces. In most of the acinus, \( Re \ll 1 \), which means that viscous forces dominate inertial forces. Also, we note that acinar flows are basically quasi steady (70), and hence forces due to temporal acceleration are negligible. Even though the Reynolds number in the acinus is small, for dynamic similarity it should be of the same order for the rat and the human acinus. Figure 3B shows that the Reynolds number for the adult rat and the human acinus are similar throughout the acinus. If instead of the transport of momentum we consider the transport of a scalar, such as concentration, then rather than the Reynolds number, the parameter that defines dynamic similarity is the Pécelt number, i.e., \( Pe = VL/D \), where \( D \) is the molecular diffusivity of the scalar in the carrier fluid. Hence, for two concentration fields to be dynamically similar, the Pécelt numbers should be of the same order. As \( Pe = Re v/D \), and the Reynolds numbers for the adult rat and the human acinus have been shown to be similar, we can expect that the concentration fields will be dynamically similar. As the concentration fields are similar, it is to be expected that the rate of deposition of particles, which depends on the gradient of concentration near the wall, will be similar in the rat and the human acinus.

**Objective**

The objective of this study was to investigate how alveolar secondary septa growth affects particle deposition on a local level, that is, how the pattern of deposition within an individual alveolus is affected by changes in the alveolar flow structure. We were particularly interested in how the start of recirculation in those alveoli in which rotation can occur affects the pattern of deposition. Specifically, we have considered how \( Q_A/Q_D \) and \( L_D/L_W \) affect deposition patterns within model alveoli at various stages of lung development. We calculate total acinar deposition at each stage of development from estimates of the deposition efficiencies of typical alveoli in each generation. Alveolar deposition is...
computed from the concentration fields at each time step over a full breathing cycle. The ultimate goal of this study is to provide numerical support to our recent experimental finding (58) that nanoparticle deposition peaks at day 21 in the rat.

In the following, we first outline the geometrical and mathematical models used to compute the deposition of particles onto the surface of a typical alveolus. We then present the basic deposition results obtained from applying our models to typical alveoli in various generations of the rat acinus at various stages of growth. The basic results are distributions of particle deposition along the alveolar surface, and we use these to compute the deposition of particles on a generational basis and in the acinus as a whole. Finally, we discuss our findings in terms of how the changes in deposition with age might affect an infant’s response to airborne particulates, and the possible implications our findings might have on nanoparticle dose calculations for therapeutic drug delivery.

MODELS AND METHODS

Acinar Geometry

The model topology is identical to that described in Henry and Tsuda (29). The axisymmetric model of the rat acinar airway is shown in Fig. 4A. By comparing basic flow structures of 1) a rhythmically expanding alveolar duct with realistic wall shape imaged by synchrotron radiation-based X-ray tomographic microscopy [see Fig. 3 of Henry et al. (30)] and 2) a fully three-dimensional model of a rhythmically expanding single alveolus [see Figs. 6–10 of Henry et al. (30)], we confirmed that our axisymmetric model captures the essential features of alveolar flow, namely that the alveolar flow is rotational in the entrance region of the acinus, and the flow is mainly radial in the peripheral alveoli. Values of alveolar width, \( W \), inner diameter, \( D \), and outer diameter, \( C \), given in Table 2, were estimated for the adult rat (90 day old) acinar airways from the data of Rodriguez et al. (54). Below we outline the development of the model geometries for each phase of development (Table 1).

The outer diameter of the infant rat acinar airway was taken to scale as the cube root of the lung volume at total lung capacity (TLC).

Fig. 4. Schematic of three-cell model alveolated duct (A) and detail of center alveolus (B); local grid refinement in the near-wall region of the three-cell model (C). Grey double-headed arrows in A used to indicate that the geometry expands and contracts.
Table 2. Duct dimensions of the rat lung model at functional residual capacitya

<table>
<thead>
<tr>
<th>Age, days</th>
<th>VT TLC, ml</th>
<th>C, μm</th>
<th>D, μm</th>
<th>W, μm</th>
<th>t, μm</th>
<th>θ, deg</th>
</tr>
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<tbody>
<tr>
<td>7</td>
<td>1.54</td>
<td>121</td>
<td>97.8</td>
<td>35.1</td>
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<td>7.8</td>
<td>208</td>
<td>87.7</td>
<td>60.2</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>90</td>
<td>16.7</td>
<td>268</td>
<td>113.0</td>
<td>77.6</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

V_{TLC}, lung volume at total lung capacity; C, alveolar outer diameter; D, alveolar inner diameter; W, alveolar width; t, secondary septum thickness; θ, primary septum angle. *See Fig. 3 for definitions; †values of VT TLC were taken from Bolle et al. (2008).

Values of lung volume at TLC with age (Table 2) were based on the experimental measurements on age-matching developing rats (1). As there is evidence (5) that rat alveoli attain their mature shape by day 21, we scaled the inner diameter for the 21-day-old and the 35-day-old rat as the cube root of the lung volume at TLC. That is, the 21- and 35-day-old acinus was a scaled version of the adult case.

Burri (5) demonstrated that secondary septa grow to form alveolar ducts from the saccular stage over the first 21 days of the life of a rat. However, the exact time history of this growth remains unclear, thus we have set the growth of the secondary septa linear with time as a first approximation. As mentioned above, Burri (5) showed that by day 21, the alveolar shape had attained a fixed aspect ratio. We set the aspect ratio equal to one. Hence, at day 21 and beyond, the width of the alveolus was equal to the height of the secondary septa for that age. In the case of 7- and 14-day-old rats, the width of the alveolus was calculated based on the outer and inner diameters. The important point here is that the secondary septa for those immature animals were set shorter than their mature height. In this study, therefore, we set the alveolar aspect ratios at 1/3 and 1/2 for the 7- and 14-day-old rats, respectively.

Burri (5) has shown that the septa are initially thick and become thinner over a period between 14 to 21 days in rats (5). However, Karl et al. (35) have shown that the flow within model alveoli is not similar sinusoidal fashion, the length scale changes as, (23, 24, 68). In a model that expands and contracts in a geometrically similar fashion, the Reynolds number of the duct, $Re$, is the lung volume at functional residual capacity (FRC). As the model is at all times geometrical similar, the volume flow rate in the duct, $Q_{D}$, is proportional to acinar volume distal of the generation of interest, $V_{A}$ (the time-mean value of the acinar air volume distal of the model; i.e., the part of the lung that is fed by the airflow leaving the model duct), that is, $Q_{D} = dV_{A}/dt$, $V_{A}(t) = (V_{D})(\text{mean})^{t}(t)$, hence, $Q_{D} = 3(V_{D})(\text{mean})F/dt$. As lung flow has zero mean, we define the Reynolds number of the flow by using a root-mean-square bulk velocity, i.e.,

$$Re = \frac{U_{rms}(d/2)}{v}, \quad (1a)$$

where $U_{rms} = (Q_{D})_{\text{max}}\pi(d/2)^{2}/\sqrt{2}$, and $v$ is the kinematic viscosity of air.

Although some investigators (e.g., see Refs. 5, 16, 32, 64) suggested the creation of new generations by septation after birth, Burri (5) states that this issue remains an open question (e.g., see Ref. 7) since evidence to the contrary is unavailable. Thus we considered that all generations were present at birth. Rodriguez et al. (54) determined that on the average the adult rat acinus had seven generations (designated here as 0–6). Values of $V_{D}$ at each acinar generation were estimated for the adult rat by using the duct data of Rodriguez et al. (54). Values of $V_{D}$ for the infant rats were estimated by assuming that $V_{D}$ scaled as the TLC volume. Values of TLC volume for the infant rats (Table 2) were estimated from the data of Bolle et al. (1).

From the above, it can be seen that $Re$ is a function of the duct diameter, the volume distal of the alveolus of interest, the tidal volume, $V_{FRC}$, the breathing frequency, $f$, and the kinematic viscosity of air. Values of $V_{D}$, $V_{FRC}$, and $f$ with age were estimated from the data of Bolle et al. (1) and Semmler-Behnke et al. (58). The kinematic viscosity of air at 37°C was taken to be $1.66 \times 10^{-5} \text{ m}^2/\text{s}$. By using these data, the breathing period, $T = 60f$, and $C$ were computed for each age (Table 3). While Re can change over two or three orders of magnitude in the acinus, mainly because of the change in $V_{D}$ with generation number (Fig. 3B), it is always below unity. Hence, as stated above, it can be expected that the effects of flow inertia are small.

Table 3. Period and volume excursion for the rat lung model

<table>
<thead>
<tr>
<th>Age, days</th>
<th>$f$, bpm</th>
<th>$V_{R}$, ml</th>
<th>$V_{FRC}$, ml*</th>
<th>$T$, s</th>
<th>$C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>154</td>
<td>0.15</td>
<td>0.56</td>
<td>0.390</td>
<td>0.268</td>
</tr>
<tr>
<td>14</td>
<td>147</td>
<td>0.27</td>
<td>0.70</td>
<td>0.409</td>
<td>0.386</td>
</tr>
<tr>
<td>21</td>
<td>126</td>
<td>0.36</td>
<td>1.10</td>
<td>0.477</td>
<td>0.327</td>
</tr>
<tr>
<td>35</td>
<td>127</td>
<td>1.28</td>
<td>2.20</td>
<td>0.474</td>
<td>0.582</td>
</tr>
<tr>
<td>90</td>
<td>99</td>
<td>2.38</td>
<td>4.10</td>
<td>0.608</td>
<td>0.580</td>
</tr>
</tbody>
</table>

$f$, breathing frequency (breaths per minute); $V_{R}$, tidal volume; $V_{FRC}$, lung volume at functional residual capacity; $T$, period; $C$, volume excursion. *Values of $V_{FRC}$ were taken from Bolle et al. (2008), and values of $V_{R}$ and $f$ were taken from Semmler-Behnke et al. (58); these data were then scaled to standard values for the adult rat given at http://www.ratbehavior.org/Stats.htm. From the scaled data, the breathing period, $T = 60f$, and $C = V_{R}/V_{FRC}$ were computed for each age.
tion and the vorticity. Because of the low Re of the flow, we can expect the flow to be fully developed over one duct diameter, or one alveolus. Hence, we have concentrated our calculations on the middle alveolus with the view that any artificiality introduced by the model boundary conditions will not impact greatly on the flow (and concentration) in the middle alveolus. Essentially, we define the outlet velocity profile to be the same as an inlet profile plus a velocity due to the increase in volume per unit time of the expanding solution domain. For each flow configuration, it was found that a repeating flow pattern was achieved after one quarter of a breathing cycle. Hence, the flow program was run for one and a quarter cycles and a full set of velocity values was dumped, starting after the first quarter cycle. Subsequently, this velocity data set was used in the concentration calculations. More details of the numerical methods employed can be found in Henry and Tsuda (29).

The Concentration Field

The particles under consideration (20 nm, 50 nm, and 80 nm) have negligible inertia and are unaffected by gravity. Hence, their concentration can be modeled by the equation governing the transport of a scalar in a constant density flow, which can be written, in polar coordinates (r, z), in the following form:

$$\frac{\partial C}{\partial t} + \frac{\partial}{\partial z}(u_r C) + \frac{1}{r} \frac{\partial}{\partial r}(u_r r C) = D \left( \frac{\partial^2 C}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( \frac{\partial C}{\partial r} \right) \right)$$

(2)

where D is the coefficient of diffusion of the particles in the carrier fluid. The velocity field (u_r, u_z) of the carrier gas is given by the Navier-Stokes equations (in this case, in the form of equations for stream function and vorticity).

Equation 2 was solved by using the same finite volume method as employed in the solution of the stream function and vorticity equations. As indicated above, the velocity field of the carrier gas was solved separately and written to file, once a fully repeating cycle had been achieved. A full cycle of velocity data was then read into the concentration program and used repeatedly for as many breathing cycles as required to reach a repeating concentration profile. In all cases, a repeating cycle was achieved within three breathing cycles. The axial boundary conditions on the concentration were as follows. Over inspiration, the inlet concentration was set to unity and the outlet concentration computed by extrapolation from the inner solution. Over inspiration, the concentration profile at the distal boundary at each time step was recorded and an inspiration average concentration was computed. This average concentration was then adjusted to account for deposition in the generations distal of the duct of interest (see Appendix A). This modified concentration was then used as the boundary condition at the distal boundary over expiration. The proximal boundary concentration over expiration was computed by extrapolation from the inner solution. At the wall, the concentration was set to zero, which represents mathematically the fact that particles leave the flow once they deposit.

Grid Dependence Checks

While it was shown by Henry and Tsuda (29) that the grid used for the flow calculations produced grid-independent results for the flow field, the concentration calculations required a finer grid. This is because the Péclet numbers are much higher for the concentration fields compared with the equivalent Reynolds numbers in the flow calculations. Hence, a grid refinement procedure was conducted for the concentration calculations (Fig. 4C). The flow in generation 0 of the adult rat model was recomputed with a refined grid in the near-wall region (where the gradients would be largest). Then, by using the recomputed flow field, the concentration in generation 0 of the adult rat was computed for the 80-nm particle on the same refined grid. This combination of flow location and particle size was chosen because it produced the largest Péclet number, and hence would require the finest grid. The grid refinement procedure was continued until a grid-independent concentration field was achieved for all cases.

Deposition

There is no doubt that particles in nano size eventually deviate from the airflow streamlines by diffusion and deposit onto the alveolar surface, but it is important to recognize that particles are largely transported from the airway opening to the deposition sites in a piggyback fashion with the airflow (convection), not by diffusion. In other words, the strength of convection and the complexity of the airflow patterns along the airways are the crucial factors one needs to know to study particle deposition in the acinus.

At each time step, the rate at which particles deposit on the surface of the center alveolus, S, defined in Fig. 4B, was computed by using dm/dt = ∫ S (dC/dt) dS, where the gradient of concentration normal to the surface, dC/dt, is calculated from the numerical solution of Eq. 2 at that time step. Hence, the number of particles depositing on the alveolar surface over one breathing period, T, is given by m_p = ∫ S (dm/dt) dt. The deposition efficiency of a typical group of alveoli in generation z is defined as η_z = m_p/m_p, where m_p is the number of particles depositing on the middle alveolar surface, S, over one breath, and m_p is the number of particles entering the center alveolus through the plane below the proximal secondary septum over the same time period (Fig. 4B).

The deposition efficiency of the typical duct in generation z is given as, η_z = 1 - (1 - η_z) where p is the number of alveolar deposition areas in the streamwise direction on each duct. Based on Weibel (76), p is considered proportional to the number of alveoli in the lung. The number of alveoli at each age was estimated from the data of Burri et al. (4), and an equation of alveoli growth derived from one proposed by Durnill (19) for the human infant (see Appendix B for more details of the derivations of η_z and p).

The number of particles depositing in generation z is given by N_{D0z} = 2^n_{D0z}, where n_{D0z} = γ_{ND0z} - 2^n_{ND0z} (assuming a dichotomously bifurcating geometry and an equal division of particles as they leave generation z-1 and enter generation z). The total deposition in the acinus is the sum of the deposits in each generation, i.e., N_{ND0z} = ∑ n_{ND0z} (see Appendix B, for more details of the derivations of N_{D0z} and N_{ND0z}).

RESULTS

Flow

As mentioned above, viscous effects generally far outweigh inertial effects in acinar flow (typically Re ≪ 1 in the acinus, see Fig. 3B); the flow pattern within an individual alveolus is governed by \( Q_i/Q_D \) (ranging \( 10^{-3} < Q_i/Q_D < 10^2 \) along the acinar tree, see Fig. 3A) and its geometry (specifically, the aspect ratio, \( L_y/L_w \), of the alveolus). The instantaneous streamline patterns indicate that the aspect ratio for the 7-day-old alveoli is insufficient to allow flow rotation for any value of \( Q_i/Q_D \) in any generation (Fig. 5); the flow direction in the alveoli is largely radial in all generations (i.e., from the entrance generation to the terminal generation).

By day 21, however, the secondary septa have grown to a sufficient extent that rotation occurs in most of the acinar generations except the last generation, as predicted by Henry and Tsuda (29) (Fig. 5). The extent and strength of the recirculating flow is especially high, and a large area of rotating flow occurs in the alveoli of the acinar entrance region. As the shape of the alveolus is considered to be fully developed at this age (2), the flow in the alveoli of the 21-day acinus resembles that of the adult. The flow patterns in the 35- and
90-day alveoli mirrored those in the 21-day acinus (results not shown).

The shape of the acinus in animals at day 14 is between the one at day 7 and the one at day 21. The secondary septa have grown to a sufficient extent that rotation occurs in the alveolus for the small $Q_A/Q_D$ (e.g., flow conditions in the proximal region of the acinar tree), but still not sufficient for alveolar flow to rotate for the large $Q_A/Q_D$ (e.g., flow conditions in the peripheral region of the acinar tree; Fig. 5).

### Concentration

The distribution of particle concentration in the alveoli is the result of a balance between diffusive and convective transport.
This balance is characterized by the Péclet number, $Pe = \frac{U_{rms} \tau_p D}{\nu}$, hence $Pe = \frac{vR_e D}{\nu}$ (72). Tsuda et al. have shown that even though $Re < 1$ in the acinus, $Pe \gg 1$ in most of the acinus because the kinematic viscosity, $\nu$, of air is many times greater than the diffusivity, $D$, of nanoparticles. Thus even in the lung periphery, convection dominates the transport of nanoparticles tested in this study.

Although the transport of nanoparticles is largely convective in the acinus, the effect of the difference in diffusivity, $D$, of the three particles sizes ($D$ of a 20-nm particle is roughly six times that of a 50-nm particle and 12 times that of an 80-nm particle) can be still seen in the distribution of particle concentration in the alveoli. Since our interest is, in particular, the interplay between alveolar recirculation and particle diffusion, we examined the concentration profile of particles with three different sizes in the fully developed alveoli of the 21-day rats (see Fig. 6, for example). While the effect of flow recirculation on the concentration pattern in the alveoli is clearly visible for all particle sizes in the generations 0, 2, and 4, the strength of the rotation in the alveoli decreases as the terminal generation is approached. This can be seen in that the higher diffusivity of the 20-nm particle generally produces concentration patterns that have more gradual gradients (shown by the color bands being further apart in Fig. 6, left) than is the case in the concentration patterns for the less diffusive 50- and 80-nm particle (Figs. 6, middle and right). While inspection of the

![Fig. 6. Predicted concentration fields at end inspiration in the middle alveoli of the three-cell model for 20-nm particles (left), 50-nm particles (middle), and 80-nm particles (right) for selected generations in the 21-day rat case. Red indicates relatively high concentration and blue relatively low. Note that the septa are at zero concentration.](image-url)
contours for generation 6 might appear to give evidence to the contrary, it is noted that the convection strength is the same for each particle size at a particular generation. The reason that the 80-nm case appears to have more convection (because there is more red) than the 20-nm particle is because the latter deposits at a much greater rate in the first alveolus (not shown) than does the 80-nm particle. Hence, in the case of the 20-nm particle, there are few particles entering the middle alveolus.

To emphasize the importance of a combination of convection and diffusion over pure diffusion in the deposition of nanoparticles in the alveolus, we compare the deposition based on our full solutions (with recirculating convective flow in the alveolus) to that of the case in which the alveolar flow is set to zero (Fig. 7). As we can see in Fig. 7, deposition rates for all three particle sizes are much higher in the presence of alveolar recirculation compared with the case in which alveolar flow is set to zero (20, 50, 80 nm). Further, this difference is more significant for the less diffusive particles. In the case of no alveolar flow, deposition in the alveolus is solely due to diffusion, and thus the differences in the deposition rates in three different size particles roughly follow the differences in their diffusivity. Conversely, in the case with alveolar recirculation (i.e., our full solutions), deposition in the alveolus must be greatly enhanced by convection since the differences in the deposition rates between the three particle sizes do not correspond to the differences in their diffusivity.

Local Particle Deposition

The distributions of particle deposition patterns along the alveolar wall were compared between the case of our full solutions with recirculating convective flow in the alveolus and the case that alveolar flows set to zero (data not shown). First, deposition in the presence of alveolar recirculation is substantially higher than that in the case of no alveolar flow. Second, while in both cases the highest deposition occurs at the tip of the secondary septa, the patterns of deposition on the walls of the secondary septa are different. While the pattern is smooth (i.e., exponential decay) in the case of no flow in the alveolus, suggesting the driving mechanism is solely diffusion, the pattern in the presence of alveolar recirculation shows two steps: a sharp drop near the tip of the septa and a gradual decrease toward the corner of the alveolus. This deposition pattern on the secondary septal wall likely reflects the flow pattern in the alveolus; the intersection of these two distinct slopes occurs at the change in flow patterns [e.g., near the saddle point (67)]. Third, the deposition patterns on the walls of the primary septa appear to be very sensitive to the wall shape in the presence of alveolar recirculation (appearing to be less sensitive in the case of no flow), indicating that convective flow patterns play an important role in the deposition process. It is worth noting that detailed flow patterns of a viscous flow, like alveolar flow, are known to be highly sensitive to the wall shape (25, 64, 67, 77).

Deposition Along the Acinar Tree

Based on the deposition efficiency of a typical single alveolus ($\eta$), acinar airway deposition—a fraction of particle deposited in generation ($z$) denoted as $N_{D@z}/N_{in@z}$ in APPENDIX B—was plotted against generation number, $z$, with a family of animal ages (Fig. 8). Key parameters determining deposition are 1) convection, 2) diffusion, 3) alveolar size, and 4) alveolar shape. First of all, since the Péclet number is...
extremely high in the first few generations in the acinus (say, 0 < z < 3), the role of diffusion relative to convection would be negligible in the proximal regions of the acinus. We have noted previously, however, that very near the wall, where the convection velocity approaches zero and the concentration gradients are high, the particles diffuse down the concentration gradient, crossing the flow streamlines, and deposit on to the wall.

It is obvious that animal age (secondary septa development) has an effect on deposition efficiency in the more peripheral generations (4 ≤ z ≤ 6) for all particle sizes considered (Fig. 8). However, because the deposition is high in the terminal generations, the deposition efficiencies in the proximal generations (0 ≤ z ≤ 3) appear to collapse onto a single curve. In fact, looking at the actual numerical values, it is apparent that animal age (i.e., alveolar size/shape) also affects deposition in the proximal generations. For example, comparing the deposition efficiencies for day 7 and day 21 in a typical alveolus in generation 0, we found the following: for 20-nm particles, the deposition at day 7 was 68% of that at day 21; for 50-nm particles, the deposition at day 7 was 52% of that at day 21; and for 80-nm particles, the deposition at day 7 was 42% of that at day 21. These values clearly illustrate the effect of both secondary septa length and particle size on deposition efficiency in the proximal region of the acinus.

The low deposition efficiencies in the proximal generations is an indication that convection dominates. That is, particles are convected through the alveolated ducts at such a rate as to allow little time for the particles to diffuse to the septa. Note that while the deposition efficiency is low (less than 10% for 20-nm particles and less than 5% for 50- and 80-nm particle, for instance) at the entrance of the acinus, the amount of particles deposited could be nonnegligible if the amount of particles entering this region is appreciable. Also, there are far fewer acinar ducts in the entrance region than in the deeper lung area, and hence deposition density (i.e., an amount of deposited particle per unit surface area) is likely high in the entrance region.

A transition from the dominant role of convection to an increased role of other factors in terms of deposition can be seen. The predominance of diffusion, which is the only mechanism by which particles of the size considered deviate from the streamlines for deposition, is evidenced by a decrease in deposition efficiency with an increase in particle size. As the terminal generation is approached, the extent of convection diminishes owing to the dichotomous structure of the acinar tree (the flow velocity roughly halves at each bifurcation); the deposition increases according to particle diffusivity (note that convection, however, still much outweighs diffusion, for the tested particles, in the entire acinus). The interplay between age, particle size, and deposition is illustrated in the different spreads of the deposition curves for each particle size. Put another way, roughly the same spread of deposition curves is seen at z = 2 for 20 nm, at z = 3 for 50 nm, and at z = 4 for 80 nm (note the difference in scales between the 20-nm and the 50- and 80-nm graphs).

A closer look at Fig. 8 reveals that the deposition curves for the 7-day-old rat follow a different trend to those followed by the rest of the ages. That is, the curves for all the ages but the 7-day-old rat are fairly closely bunched together, but the 7-day-old rat deposition is noticeably lower than the rest. We believe that the reason for this difference is twofold: 1) the 7-day-old rat secondary septa are short, and thus there is less septal area on which particle can deposit, and 2) the 7-day-old rat is the only case considered that has no recirculation in any generation (Fig. 5). Finally, it is interesting to see that generational deposition is not ordered base on animal age (e.g., ND@z=5@day7 < ND@z=5@day90 < ND@z=5@day14 < ND@z=5@day35 < ND@z=5@day21 for 80 nm), suggesting that age-dependent alveolar shape and associated alveolar flow patterns play an important role in deposition.

Total Acinar Deposition

Summing the number of particles deposited in each generation (Fig. 8), the total number of particles depositing in the acinus as a fraction of those entering the acinus was estimated (Fig. 9). We see that the amount of deposition is strongly particle-size dependent regardless of animal age. Specifically, the 20-nm particles appear to deposit in the acinus over 80% (discussed below), whereas the larger particles (50, 80 nm) appear to deposit about or less than 50%. Nonetheless, the pattern of particle deposition with age is consistent across the particle-size range. Namely, there is a rapid increase in deposition during structural alveolation (from day 7 to day 21) followed by a decrease in deposition during the enlargement phase of lung growth, over which period (from day 21 to adults) the acinar structure (aspect ratio) remains the same.

DISCUSSION

The principal finding of this report is that the pattern of alveolar flow is critical for the deposition of the particles in the acinus. Even for nanoparticles, whose diffusivity is indeed higher than that of larger particles (e.g., micron-size particles), the role of convection cannot be ignored. In this regard, the repeatedly stated notion to the effect of “Nanoparticles deposit with high efficiency . . . due to diffusion” (e.g., 22, 33, 46) is somewhat misleading because the distance that a nanoparticle travels from the airway opening to the deposition site in the acinus is nearly entirely accomplished by convection and only the last small portion (within a diffusion distance during the breathing period) is due to diffusion. It is important that one
should realize that convection in the acinus generally far outweighs diffusion for particles larger than a few nanometers.

Among many factors, the presence or absence of alveolar recirculation is the most important feature of alveolar flow in the postnatal developing alveoli. The onset of alveolar recirculation is largely determined by two parameters: 1) the aspect ratio \(L_D/L_W\) (= depth/width) of alveolar geometry and 2) the \(Q_A/Q_D\) ratio (i.e., the flow ratio between that entering an alveolus vs. that passing over the alveolar entrance in the duct). The former is solely age dependent and the latter is location (generation) dependent along the acinar tree for each age.

During the immediate postnatal period of development in both the human and the rat lung (see INTRODUCTION), the growth in the length of the secondary septa results in an increase in the aspect ratio of the alveoli. Therefore, for a particular \(Q_A/Q_D\) there is likely a critical developmental stage at which the secondary septa length is large enough to force the alveolar flow to rotate (Fig. 5). Our computational analyses revealed that if \(L_D/L_W\) is set large (say, “1” for 21-day rats and older), alveolar flow rotates for the \(Q_A/Q_D\) values less than 0.1; on the other hand, if \(L_D/L_W\) is set small (say, “\(\sqrt{3}\)” for instance; the 7-day rats case in our analysis), alveolar flow does not rotate regardless of the value of \(Q_A/Q_D\), (even for \(Q_A/Q_D < 0.01\)). By setting \(L_D/L_W\) at a middle value (say, “\(\frac{3}{2}\)” for the case of the 14-day-old rats in our analysis), we demonstrated that the presence (or absence) of alveolar recirculation depends on the \(Q_A/Q_D\) values (the cutoff of the \(Q_A/Q_D\) values when the alveolar recirculation occurs appears to be 0.01 for \(L_D/L_W = \frac{3}{2}\)). Future detailed analysis along this line of investigation would include 1) in vivo morphometric measurements of the change in \(L_D/L_W\) during lung development, and 2) fluid mechanics investigation on the onset of alveolar recirculation (i.e., theoretical investigation on the occurrence of flow separation on the secondary septa) in the case of rhythmically moving boundary.

The onset of alveolar recirculation is important in terms of particle transport. As we have demonstrated before (28, 67, 71), the occurrence of alveolar recirculation together with oscillatory flow (i.e., cyclic breathing) are conditions that promote alveolar chaotic mixing and flow irreversibility. Readers interested in this topic can find detailed numerical analyses and theoretical explanation of alveolar chaotic mixing elsewhere (28, 67, 71).

Alveolar convection has several effects on acinar deposition. As well as creating a significant increase in the rate of deposition within the alveolus, it changes the pattern of deposition along the septal wall within an alveolus (data not shown) and along the acinar tree (Fig. 8). That is, alveolar convection makes deposition significantly flow pattern dependent. We see that the patterns of deposition along the septal wall and along the acinar tree become complex as the patterns of alveolar flow (e.g., presence or absence of alveolar recirculation) become complex. That is, as the secondary septa grow.

Regarding the deposition pattern along the septal wall within the alveolus. The wall of each alveolus is mainly lined by two types of cells, squamous type I pneumocyte (covering >95% of the alveolar surface area) and cuboidal type II pneumocyte [locate preferentially at the corners of alveolus (40), covering the rest of the alveolar surface], forming the alveolar epithelium. In the developing lung, the lengthening (e.g., growth) of the secondary septa is one of the main events of structural change, and this change greatly affects the alveolar airflow patterns. This, in turn, conditions the pattern of deposition because deposition is highly sensitive to the pattern of flow (for instance, deposition is high at stagnation points on both proximal and distal septal walls).

In our previous study (18), we demonstrated that contractile elements [\(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) expressing cells] are concentrated at the tips of the septa during septation, contributing to the formation of the alveolus via micromechanics (78). The areal density of \(\alpha\)-SMA at the septal tip (indicative of myofibroblast activity and collagen/elastin fiber deposition) peaks at postnatal 21 days (in rats), and that is consistent with the phase of rapid alveolarization in the developing lung (18). Thus the developing secondary septa must be particularly vulnerable; for instance, preferential deposition of particulate pollutants at the tip of the septa could impact adversely the normal development of acinar architecture and lung function (e.g., see Refs. 6, 39, 74) as well as cell function (e.g., see Ref. 63). Conversely, preferential deposition of a therapeutic drug at the tip of the septa could be strategically used to control the shape and size of the alveolus to manipulate/intervene the disease preventing/interrupting process.

Regarding the deposition pattern along the acinar tree. As the number of alveoli increases during the lung development phase (more than 10 folds both in human and in rats), differentiation and proliferation of cells must occur. Lung stem cells have only recently been identified (17, 34, 37), and hence the adverse effects of exogenous components (chemicals, gases, particulates) on these cells’ capacity to differentiate is unknown. However, their adverse effects on nonciliated bronchiolar (Clara) cells are well documented. Clara cells are progenitor cells during bronchiolar epithelial differentiation (50) and a primary target for many environmental toxicants [e.g., naphthalene (20) and cytochrome P450 monooxygenase (51)].

It is known (49, 52, 53) that Clara cells are mainly located at the bronchoalveolar junction (the entrance of the acinus, just beyond the terminal bronchioles), where \(Q_A/Q_D\) is the smallest. As we show in Fig. 5, this is the area where the influence of convection on deposition is the largest; alveolar flow cannot rotate if the secondary septa have not lengthened sufficiently, as in the case of the youngest rats we simulated. Experimentally, in the fully developed adult lung, it has been repeatedly demonstrated that the transitional zone between the conducting airways and the gas exchange region [e.g., the entrance of the acinus where the sudden increase in the cross-sectional area of the airways occurs due to the emergence of alveoli, thus the particle-laden airflow rapidly decelerates and enters a viscous flow regime (47)], is the primary site of particle deposition (Fig. 10A) and lung injury (e.g., Fig. 10B) after exposure to airborne pollutants (12–14, 26, 36, 45, 48, 55, 79). Churg and Brauer (11) reported that submicron size particles were found at the entrance of the acinus, at typically 25–100 times higher concentration than in the main stem bronchi.

Remarkably, our numerical results on the age-dependent pattern of total deposition are consistent with what we found in vivo (58). Our results of total acinar deposition (Fig. 9) strongly show age dependency; acinar deposition dramatically increases with the event of structural alveolarization from the smooth-walled saccular stage to the fully alveolated acinus, peaks when the alveolation is completed at day 21, and grad-
usually decreases as the size of the lung increases. This unique age-dependent pattern is more distinct with less diffusive particle (more than a 163% increase followed by about a 15% reduction in the case of 80-nm particles), suggesting convection (i.e., airflow pattern) conditioned by the shape of acinar airway walls plays a dominant role for acinar particle deposition.

The predictions based on our current numerical model (Fig. 9) are generally higher than the in vivo data [Fig. 4 in Semmler-Behnke et al. (58)]. We interpret this discrepancy as follows: 1) while the data in the in vivo study (58) represent a fraction of particles relative to the amount of particles entering an animal (nose-only exposure), acinar deposition estimation in the current numerical study is based on the amount of particles entering the acinus; 2) while our current axisymmetric model captures the basic flow structures of acinar flow (30), it lacks alveolar septa in an azimuthal direction. This may result in the particle-laden airflow entering deeper into the model alveolar space, causing higher deposition. Despite these shortcomings in the current model, it should be emphasized that the model faithfully captures the basic age-dependent pattern of acinar deposition found in our in vivo study (58), showing the importance of convection (i.e., alveolar airflow patterns) for acinar deposition.

Finally, our findings have several implications for inhalation therapy for infants. For instance, in current neonatal care, drug dose is simply estimated by scaling the dose (44) to the infant’s body weight, i.e., $D_{infty} = \frac{BW}{BW_{1.0}} \cdot D_{1.0}$, where $D_{1.0}$ is the dose per body weight 1.0, and this is what is implicitly assumed in the classical approach. However, Semmler-Behnke et al. (58) have shown that for infant rats, $MV \propto BW^{0.91}$. Hence, the dose per body weight is itself a function of body weight, i.e., $N_{infty}/BW = (\frac{f_D}{f_{D,0}}) \cdot (\frac{MV}{BW})^{0.91}$. The fact that the exponent of $BW$ in the equation for dose per body weight is not zero demonstrates that drug dose cannot be simply estimated as proportional to body mass (although its dependency on body mass is relatively weak).

Furthermore, and of more importance, another assumption in the classical infant drug dose estimate is that the deposition fraction ($f_D$) remains constant. Our results (Fig. 9) support the findings of Semmler-Behnke et al. (58) that $f_D$ changes significantly with age. The substantial change in $f_D$ during postnatal lung development has a direct influence on particle deposition, thus affecting the delivered drug dose.

Comprehensive knowledge of the amount of particles deposited in the acinus is fundamental for effective inhalation therapy, especially because drugs administered into the lung periphery, where there is no fast clearance mechanism, remain there for extended periods (10, 57, 58). This long biological half-life offers some advantages for therapeutic drug delivery. For instance, it prolongs the time available for a drug to be released, and it increases the chance that the particle crosses the air/blood barrier (e.g., see Ref. 10).

The fact that acinar deposition is strongly age-dependent can be explained as a consequence of the combined effects of the changes in the geometric characteristics of the developing acinus, changes in breathing characteristics, and the effects of chaotic convective mixing. Deposition peaks in 21-day-old rats, when the acinus has just completed the bulk structural alveolation stage, followed by a gradual decrease toward adulthood. At this time, the shape of each alveolus is already similar to the deep, mature alveoli, but the size is still small.

Summary

In summary, our results indicate that nanoparticle deposition in infants is strongly affected by the level of structural development.
of the secondary septa and as a consequence of this structural dependency. Based on these results, we conclude that human infants at the age of ~2 yr might be most at risk to the harmful effects of air pollution. Also, dose estimations commonly used in clinical setting for inhalation therapies using nanoparticles should be reviewed, as our results suggest that dose estimates based on simple body weight scaling are likely to be inaccurate.

**APPENDIX A: DISTAL BOUNDARY CONDITION ON CONCENTRATION**

A schematic of how the boundary conditions are applied over the full breathing cycle is given in Figure A1. More details of the definitions of the various efficiencies used below are given in **APPENDIX B**.

Over inspiration, we assume that the value of concentration over the distal boundary of the model can be estimated (extrapolated) from the internal concentration field.

Over expiration, we have to make an assumption about the number of particles that deposit distal of the generation of interest. The total number of particles that deposit distal of a typical duct in generation \( z \), \( N_{d_0z} \), may be written as

\[
N_{d_0z} = \sum_{i=z+1}^{6} N_{d_0i} \quad (A1)
\]

where

\[
N_{d_0i} = 2^{i-z} n_{d_0i} \quad (A2)
\]

For example, the distal boundary condition for a typical duct in generation 5 would be given as follows. Applying Eq. A1,

\[
N_{d_05} = \sum_{i=6}^{6} N_{d_0i} = N_{d_06}
\]

Applying Eq. A2,

\[
N_{d_06} = 2^n_{d_06} = 2n_{d_06}
\]

Also, applying Eqs. B9 and B11 (**APPENDIX B**) gives

\[
n_{d_06} = \gamma_0 n_{d_05} = \left[1 - (1 - \eta_6)^p\right] n_{d_05}
\]

Hence

\[
N_{d_06} = 2^n_{d_06} = 2\left[1 - (1 - \eta_6)^p\right] n_{d_05}
\]

Using Eq. B12 (**APPENDIX B**),

\[
A
\]

![Fig. A1. Schematic of the application of boundary conditions for the concentration calculation for inspiration (A) and expiration (B). See **APPENDIX A** for an explanation of symbols.]
n_{in@6} = \frac{1}{2} n_{out@5}

gives

\frac{N_{doc}}{n_{out@5}} = 1 - (1 - \eta_0)^p \quad (A2)

In general, the distal boundary condition can be written (for \(z < 6\)) as

\frac{N_{doc}}{n_{out@z}} = \left[ 1 - (1 - \eta_0)^p \right] \left[ 1 - (1 - \eta_3)^p \right] \ldots \left[ 1 - (1 - \eta_{z-1})^p \right] \quad (A3)

Hence, the concentration on the distal boundary over inspiration is given as

\left( 1 - \frac{N_{doc}}{n_{out@z}} \right) C_{out@z} \quad (A4)

where \(C_{out@z}\) is estimated as the average concentration (over time and radial distance) of the carrier gas crossing the distal boundary over inspiration. That is,

\[ C_{out@z} = \frac{4}{T_d} \int_{r=0}^{r=T_d} \left( \int_{r=0}^{r=a} C_d dr \right) dt \quad (A5) \]

As estimates of distal alveolar efficiencies are employed, this scheme requires that the concentration calculations are performed in sequence, that is, starting with the most distal generation (generation 6) and ending with the most proximal (generation 0).

**APPENDIX B: CALCULATION OF DEPOSITION IN THE MODEL INFANT RAT ACINUS**

A schematic showing the steps used to calculate the overall acinar deposition is given in Fig. B1.

The analysis given below assumes that the model alveolus is axisymmetric, that is, the model represents a ring of alveoli surrounding an airway. We assume that a typical airway has maximum of two rings of alveoli. Following Rodriguez et al. (54), we assume that a typical rat acinus has six generations.

First, we define the number of particles depositing in a typical model alveolus in a typical duct of generation \(z\) as \(m_{D@z}\), where

\[ m_{D@z} = m_{in@z} - m_{out@z} \quad (B1) \]

\(m_{in@z}\) is the number of particles entering the alveolus and \(m_{out@z}\) is the number of particles leaving the alveolus.

Second, define an alveolus deposition efficiency (assumed constant for a generation) as

\[ \eta_z = \frac{m_{D@z}}{m_{in@z}} = \frac{(m_{in@z} - m_{out@z})}{m_{in@z}} \quad (B2) \]

Hence

\[ m_{D@z} = \eta_z m_{in@z} \quad (B3) \]

and

\[ m_{out@z} = (1 - \eta_z) m_{in@z} \quad (B4) \]

If there are \(p\) clusters of alveoli on each duct in generation \(z\), then

\[ n_{out@z} = (1 - \eta_z)^p n_{in@z} \quad (B5) \]

where \(n_{out@z}\) is the number of particles leaving the duct in generation \(z\), and \(n_{in@z}\) is the number of particles entering the duct. Recall that the entrance duct of the acinus is designated \(z = 0\), and hence \(n_{in@0}\) is the total number of particles entering the acinus. Note that \(p\) represents the distribution of alveoli on the duct. For example, \(p = 1\) is taken to mean that the alveolar distribution on the duct is equivalent to the duct being encircled by a fully populated, single cluster of alveoli; \(p = 2\) is taken to mean that the duct is completely alveolated, with two fully populated clusters of alveoli. We assume that \(p\) grows in proportion to

\[ n_{in@0} = 6^n n_{in@0} \]

**Fig. B1.** Schematic illustrating the steps employed in the estimation of the total acinar deposition from the deposition efficiency of a typical alveolus in each generation. See **APPENDIX B** for an explanation of symbols.
the number of alveoli in the lung, and that for the adult case \( p = 2 \).

Hence, in general,

\[
P = 2 N / N_{\text{day}90}
\]

(B6)

where \( N \leq N_{\text{day}90} \) is the number of alveoli at a particular age and \( N_{\text{day}90} \) is the number of alveoli in the acinus at day 90.

The number of alveoli was given by Burri et al. (3) for rats of ages 7, 10, 13, and 21 days. Also, Burri (5) states that the number of alveoli increases by a factor of two after the bulk alveolation period. Hence, we assumed that the number of alveoli at day 14 was twice that at day 13 (bulk alveolation occurs over the first 2 wk of life in rats (5)). To estimate the number of alveoli at days 14 and 35, we modified an equation proposed by Dunnill (19) for the number of alveoli in the human infant. The modified equation can be written as

\[
N = N_{\text{day}90} - (N_{\text{day}90} - N_{\text{day}7})e^{-a(t-\text{age})}
\]

(B7)

where \( N \) is the number of alveoli at age \( t \) (7 ≤ \( t \) ≤ 90 days), \( N_{\text{day}90} = 2 \times N_{\text{day}13} \), the number of alveoli in the rat acinus at day 90. According to Burri et al. (4), the number of alveoli in the rat acinus at day 7, \( N_{\text{day}7} = 10.46 \times 10^6 \), and the number of alveoli in the rat acinus at day 13, \( N_{\text{day}13} = 26.11 \times 10^6 \). By curve fitting Eq. B7 to the data of Burri et al. (4), we found that \( a = 0.686 \). Equation B7 was used to estimate values of the number of alveoli and the corresponding value of \( p \) for each age considered (Table B1).

We now define a deposition efficiency of the typical duct in generation \( z \),

\[
\gamma_z = n_{D@z} / n_{in@z} = (n_{in@z} - n_{out@z}) / n_{in@z} = 1 - n_{out@z} / n_{in@z}
\]

(B8)

Hence

\[
n_{D@z} = \gamma_z n_{in@z}
\]

(B9)

and

\[
n_{out@z} = (1 - \gamma_z) n_{in@z}
\]

(B10)

Therefore, by using Eq. B5, the deposition of a typical duct in generation \( z \) is

\[
\gamma_z = 1 - (1 - \eta_z)^p
\]

(B11)

Note that we assume that the number of particles leaving the single duct of generation 0 is equally divided, i.e., both ducts of generation 1 get half the particles. Also, as the number of ducts increases by two in each subsequent generation, we can write, generally, (for \( z \geq 1 \)) that

\[
n_{in@z} = 2^n_{out@z-1}
\]

(B12)

Deposition per Generation

Each generation in an acinus has \( 2^z \) ducts, assuming the acinus starts at generation 0. Hence, the deposition in a generation, \( N_{D@z} \), is

\[
N_{D@z} = 2^n_{D@z}
\]

(B13)

Specifically, the deposition in generation 0 is given by

\[
n_{D@0} = \gamma_0 n_{in@0}
\]

(B14)

Table B1. Assumed variation of the parameter \( p \) with age

<table>
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<th>Age</th>
<th>Number of alveoli, millions</th>
<th>( p )</th>
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<td>45.50</td>
<td>1.77</td>
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