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Effect of alveolar volume and sequential filling on the diffusing capacity of the lungs: I. Theory

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Abstract

The diffusing capacity, DL, is a critical physiological parameter of the lung used to assess gas exchange clinically. Most models developed to analyze experimental data from a single breath maneuver have assumed a well-mixed or uniform alveolar region, including the clinically accepted Jones–Meade method. In addition, all previous models have assumed a constant DL, which is independent of alveolar volume, VA. In contrast, experimental data provide evidence for a non-uniform alveolar region coupled with sequential filling of the lung. In addition, although the DL for carbon monoxide is a weak function of VA, the DL of nitric oxide depends strongly on VA. We have developed a new mathematical model of the single breath maneuver that considers both a variable degree of sequential filling and a variable DL. Our model predicts that the Jones–Meade method overestimates DL when the exhaled gas sample is collected late in the exhalation, but underestimates DL if the exhaled gas sample is collected early in the exhalation phase due to the effect of sequential filling. Utilizing a prolonged constant exhalation method, or a three-equation method, will also produce erroneous predictions of DL. We conclude that current methods may introduce significant error in the estimation of DL by ignoring the sequential filling of the lung, and the dependence of DL on VA.

Keywords: Diffusing capacity, heterogeneous lung; Gas exchange, diffusing capacity, heterogeneous lung; Heterogeneity, lung, diffusing capacity; Mediators, NO; Model, single breath DL

1. Introduction

Krogh first introduced the single breath technique for the measurement of the diffusing capacity, DL, of the lung in 1915 (Krogh, 1915). He described the lung as a single well-mixed alveolar compartment during the period of breathholding. The increasing clinical significance of estimating the rate of diffusion across the alveolar membrane prompted researchers to provide more robust methods of measuring DL. A significant source of error in Krogh's method is due to the fact that

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carbon monoxide (CO) and nitric oxide (NO) are absorbed continuously during inspiration and expiration as well as during breathholding. Ogilvie et al. (1957) modified the above method by introducing the inert gas dilution for the estimation of the initial alveolar concentration of CO. In addition, they tried to standardize the method by introducing a significant breathhold time of 10 sec, with rapid inhalation and exhalation to reduce the error of neglecting the inspiratory and expiratory phases.

Jones and Meade were the first to solve a three-equation model (i.e. one equation for each of the three phases of the single breath maneuver) (Jones and Meade, 1961). In the same fashion with the previous attempts, they assumed a uniform (well-mixed) alveolar region throughout the single breath maneuver. They demonstrated that the Ogilvie–Forster method still had a significant error from assuming instantaneous inspiration and expiration. However, they concluded that the one-equation (Krogh) method could be corrected to account for the exchange during inspiration and expiration. They proposed two modifications: (1) the breathhold time included 70% of the inspiratory time, and (2) the exhaled concentration was estimated from a small collection sample (~ 85 ml) immediately after the dead space washout.

Cotton et al. (1979) suggested two corrections to the single breathhold equation which would also account for the effects of inspiratory and expiratory flow and position of exhaled breath sampling: (1) a time-averaged alveolar volume, and (2) an effective residence time of gas in the alveolar region. They also assumed that the lung filled sequentially during inspiration and thus the first bolus of gas inspired was the last bolus of gas expired. This 'first in, last out' approach (sequentially filled model) was in marked contrast to the well-mixed uniform alveolar compartment used by previous investigators since it creates an effective axial concentration gradient in the alveolar region.

As rapid response CO analyzers and computers became available more sophisticated models and methods were developed. These new approaches included variations of the 3-Eq. method (Graham et al., 1980, 1981; Martonen and Wilson, 1982; Graham et al., 1984; Saumon et al., 1984; Brenner et al., 1994), as well as the continuous-exhalation technique (Newth et al., 1977; Stokes et al., 1981; Graham et al., 1983). In the 3-Eq. method, the equations. proposed by Jones and Meade (uniform alveolar compartment) are used sequentially. In the continuous-exhalation technique, D_L can be estimated by the rate that the alveolar concentration decreases during exhalation. This approach provides an estimation of D_L that is independent of inspired concentration, inspiratory flow rate, and breathhold time.

All of the aforementioned reports assume that D_L remains *constant* throughout the single exhalation. Several researchers have provided evidence for a constant $D_{L_{CO}}$ (Newth et al., 1977; Cotton et al., 1979), while others have reported a slightly increasing $D_{L_{CO}}$ with increasing lung volume (Gurtner and Fowler, 1971; Weibel et al., 1973; Rose et al., 1979; Borland and Higenbottam, 1989). In contrast, the D_L of NO, $D_{L_{NO}}$, is a strong function of alveolar volume (Borland and Higenbottam, 1989; Tsoukias et al., 2000). Hence, the previous models developed for determining $D_{L_{CO}}$ are inappropriate for describing $D_{L_{NO}}$, and new theoretical methods must be developed.

The goal of this manuscript is to formulate a new theoretical model that is able to simulate the single breath maneuver used to estimate $D_{L_{CO}}$ and $D_{L_{NO}}$. Hence, the model development will: (1) incorporate a D_L that is a positive function of V_A, and (2) will consider the effect of sequential filling of the lung due to parallel and stratified inhomogeneities. The model will be tested using experimental data in the companion manuscript (Tsoukias et al., 2000).

2. Model development

The following model development will consider the exchange of gas (i.e. CO or NO) during a single breath maneuver, and, in terms of estimating the diffusing capacity of the lungs, will incorporate the new features of the effect of alveolar volume and sequential filling. There is substantial evidence which suggests that the lungs tend to fill

and empty in a sequential fashion; that is, the first air inspired tends to be the last air expired (first in, last out phenomenon) (Dollfuss et al., 1967; Fukuchi et al., 1980; Engel and Paiva, 1981; Meyer et al., 1983). In order to capture the effect of sequential filling one must consider the mechanisms responsible for this observed phenomenon.

The lung is inhomogeneous in many aspects, including the filling and mixing of air by convection and diffusion. Parallel inhomogeneities result from parallel convective pathways in the lungs whose convective conductance is heterogeneous (Paiva and Engel, 1981). The result is that certain regions of the lungs tend to fill first (usually the apical regions) and empty last. Stratified inhomogeneities result from a diffusion limitation in the gas phase along a single axial pathway (Scheid et al., 1981; Six et al., 1991). When convective flow is very slow (i.e. distal to the $\sim$15th generation), gas transport is dominated by diffusion. As a gas diffuses distally, the result is an axial concentration gradient or stratified inhomogeneity. Both parallel and stratified inhomogeneities or convective–diffusive interactions contribute to a sloping alveolar plateau during a single breath washout experiment of an inert gas such as nitrogen or helium (Paiva and Engel, 1981). In terms of estimating the diffusing capacity, the important physiological sequelae of sequential filling due to parallel and stratified inhomogeneities is a distribution of residence times of gas boli in the lungs.

The model development will first consider two extreme cases (model 1 and model 2), then combine these two cases to formulate the final model (model 3). In the first approach (Fig. 1A), and in agreement with previously developed models (Jones and Meade, 1961; Graham et al., 1980; Martonen and Wilson, 1982; Graham et al., 1983, 1984; Saumon et al., 1984), the alveolar region of the lung is represented by a single well-mixed compartment (model 1). Alveolar concentration, $C_A(t)$, like alveolar volume, $V_A(t)$, is only a function of time while the diffusing capacity (D_L) can be constant or a function of V_A. In the second approach (Fig. 1B), the alveolar compartment is modeled as a series of parallel/axial compartments that fill and empty in a completely sequential fashion; each parallel/axial compartment is completely filled before the next compartment starts filling (and vice versa on expiration). This system can be alternatively represented by a series of boluses of gas that do not interact or mix with neighboring boluses and enter and leave the lung sequentially (model 2). This model structure will introduce a concentration gradient in the alveolar region that models sequential filling due to parallel and/or stratified inhomogeneities; thus, we will refer to this as an *effective* alveolar concentration gradient. Finally, model 1 and model 2 will be combined to form the complete robust model (model 3) that includes a variable degree of alveolar compartment or acinar mixing.

To simplify the problem and preserve analytical solutions, we assume that inspiratory flow ($\dot{V}_I$), expiratory flow ($\dot{V}_E$), and inspired concentration (C_I) remain constant for any individual maneuver, while the alveolar gas concentration prior to the inspiration is zero. The derivation of the model equations is presented for the simpler case of constant D_L, while only the solutions for a volume dependent D_L are presented.

2.1. Case I: D_L = constant (no dependence on V_A)

2.1.1. Model 1 (single well-mixed alveolar compartment)

A simple mass balance on the well-mixed compartment of Fig. 1A results in the following differential equation:

$$\frac{d}{dt}(V_A C_A) = \dot{V}_E C_A + \dot{V}_I C_I - D_L C_A \quad (1)$$

Here D_L has the units of ml/sec as we are using units of concentration in the mass balance. In the figures that follow, we convert to the more commonly encountered units for D_L of ml/min/mmHg in which units of partial pressure are employed as the driving force for mass transfer. Eq. (1) can be solved for each of the three phases of the single breath maneuver: (1) inspiration; (2) breathhold; and (3) expiration. These three solutions have already been introduced by others (Jones and Meade, 1961; Graham et al., 1980; Martonen and Wilson, 1982), and are presented here with minor modifications:

Inspiration $\quad 0 < t < t_{insp}, \quad \dot{V}_E = 0$:

$$\hat{C}_A(t_{insp}) = \frac{C_A(t_{insp})}{C_{Ao}} = \frac{V_{Ao}/t_{insp}}{D_L + \dot{V}_I}\left(1 - \left(\frac{V_{RV}}{V_{Ao}}\right)^{(D_L + \dot{V}_I)/\dot{V}_I}\right) \quad (2a)$$

$$C_{Ao} = \frac{\dot{V}_I C_I t_{insp}}{V_{Ao}} \approx \frac{C_I C E_{CH_4}(V_A)}{C I_{CH_4}} \quad (2b)$$

$\hat{C}_A(t_{insp})$is the alveolar concentration normalized by C_{Ao}, and V_{Ao} is the alveolar volume at the end of inspiration. C_{Ao} represents the hypothetical alveolar concentration after instantaneous dilution of the inspired gas with the pre-inspiratory or residual volume ($V_A(0) = V_{RV}$). C_{Ao} is estimated with the dilution of an inert gas (we will use CH_4 in our simulations). V_{RV} is estimated by dilution in the inspired and exhaled concentrations of the inert gas (CI_{CH_4}, CE_{CH_4}). The decay in the exhaled *inert* gas concentration ($CE_{CH_4}(V_A)$) is used in Eqs. 2(a) and (b) to correct for the components of the effective alveolar concentration gradient (and

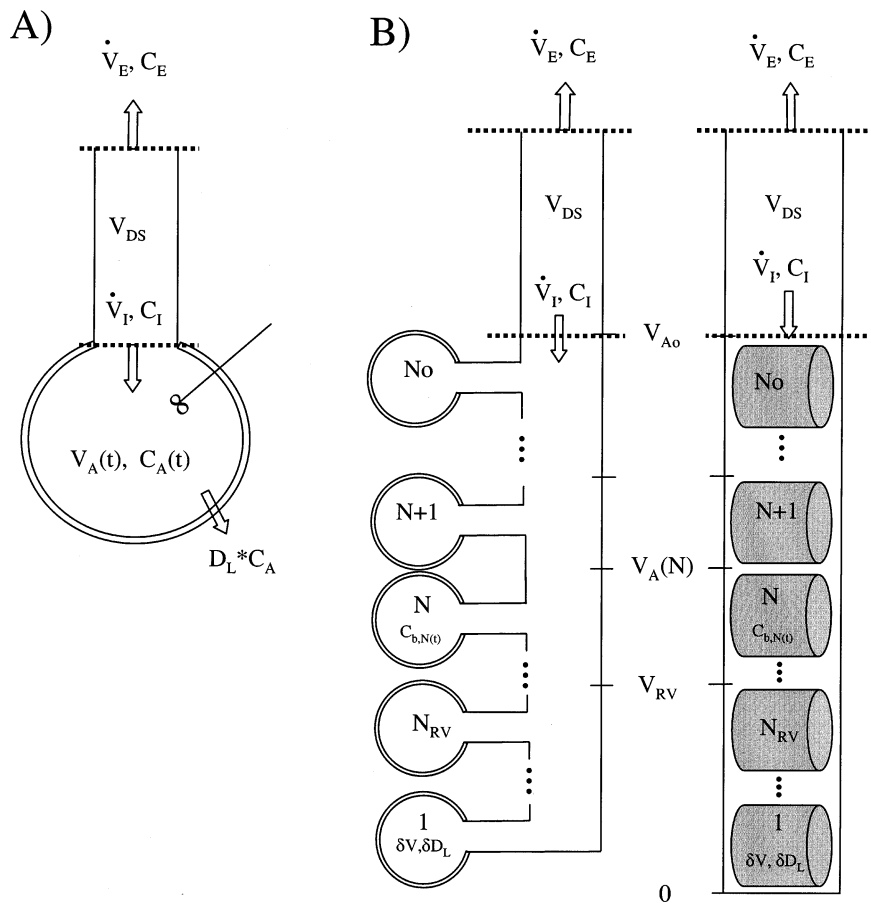

Fig. 1. (A) Uniform alveolar model (model 1). A single well-mixed compartment describes the alveolar region. Alveolar concentration $C_A(t)$, like the alveolar volume $V_A(t)$, is only a function of time while the diffusing capacity D_L can be constant or a function of V_A. The inspiratory flow rate ($\dot{V}_I$), the expiratory flow rate ($\dot{V}_E$) and the inspired concentration (C_I) are assumed constant while the exhaled concentration C_E is equal to C_A delayed by the time needed for the gas to transverse the dead space of volume (V_{DS}). (B) Sequentially filled alveolar model (model 2). The alveolar compartment is modeled as a series of well-mixed compartments, or boluses of gas, which do not interact or mix with neighboring compartments and enter and leave the lung sequentially. Every individual bolus has a unit volume δV, a unit diffusing capacity δD_L, and a uniform, time dependent concentration $C_{b,N}(t)$ where N represents the bolus number. The total number of such boluses in the lung $N(t)$ changes with time to account for the change of V_A.

thus exhaled concentration) that are not due to diffusion through the alveolar membrane (Newth et al., 1977; Cotton and Graham, 1980).

Breathhold

$$t_{insp} < t < t_{insp} + t_{bh}, \quad \dot{V}E = 0, \quad \dot{V}I = 0,$$

$$VA = V_{Ao}:$$

$$CA(t_{insp} + t_{bh}) = CA(t_{insp})\ e^{-(DL/V_{Ao})t_{bh}} \tag{3}$$

Expiration $t_{insp} + t_{bh} < t, \quad \dot{V}I = 0:$

$$CA(t) = CA(t_{insp} + t_{bh})\left(\frac{VA(t)}{V_{Ao}}\right)^{DL/\dot{V}E} \tag{4}$$

Eqs. 2–4 can be combined to yield the normalized exhaled concentration ($\hat{C}E$) as a function of time:

$$\ell n\hat{C}E\left(t + \frac{VDS}{\dot{V}E}\right) = \ell n\frac{CE(t + VDS/\dot{V}E)}{C_{Ao}(VA(t))}$$

$$= DL\left(\frac{1}{\dot{V}E}\right)\ell n\frac{VA(t)}{V_{Ao}} - \frac{DL}{V_{Ao}}t_{bh}$$

$$+ \ell n\left(\frac{V_{Ao}/t_{insp}}{DL + \dot{V}I}\left(1 - \left(\frac{VRV}{V_{Ao}}\right)^{(DL + \dot{V}I)/\dot{V}I}\right)\right)$$

$$= DL\left(\frac{1}{\dot{V}E}\right)\ell n\frac{VA(t)}{V_{Ao}} - \frac{DL}{V_{Ao}}t_{bh}$$

$$+ \ell n\left(\frac{\dot{V}I}{DL + \dot{V}I}c\right) \tag{5a}$$

$$c = \frac{1 - (VRV/V_{Ao})^{(DL + \dot{V}I)/\dot{V}I}}{1 - VRV/V_{Ao}} \tag{5b}$$

The exhaled concentration CE is equal to alveolar concentration CA delayed by the time needed by the alveolar gas to transverse the dead space volume VDS.

2.1.2. Model 2 (completely unmixed alveolar region)

In model 2 the alveolar compartment consists of a series of compartments (boluses), which do not mix or interact with neighboring compartments (Fig. 1B), and effectively represents sequentially filled parallel compartments in the lungs and/or stratified inhomogeneities. The volume of the unit compartment, δV, is constant throughout the single breath maneuver, while the number of these units, $N(t)$, within the alveolar volume changes with time to account for changes in VA. Thus,

$$\delta V = \frac{VA(t)}{N(t)} = \frac{VRV}{NRV} = \frac{V_{Ao}}{N_o} \tag{6}$$

where N_{RV} and N_o are the number of filled compartments prior and at the end of inspiration, respectively. The concentration of the Nth bolus, $C_{b,N}(t)$, depends completely on the initial concentration $C_{b,N}(0)$ of the specific unit, and the residence time of the bolus inside the alveolar compartment. The residence time is simply the difference between the time when the bolus enters, $t_{in,N}$, and when it exits, $t_{out,N}$, the alveolar region. This model assumes a 'first in, last out' approach in the filling and emptying of the alveolar compartment and represents the opposite extreme of model 1. Thus, at the time when the Nth compartment fills or empties (Nth bolus enters or exits), the alveolar volume VA_N is the same (i.e. $VA_N = VA(t_{in,N}) = VA(t_{out,N})$). It can be shown that for an alveolar region consisting of N uniform compartments, the total diffusing capacity DL is just the sum of the compartment diffusing capacities (Tsoukias, 1999). Then the following relationships hold:

$$\delta DL(t) = \frac{DL}{N(t)} \tag{7a}$$

$$\frac{\delta DL(t)}{\delta V} = \frac{DL}{VA(t)} \tag{7b}$$

Note in order to preserve a constant total DL, δDL has to be dependent on VA.

The differential equation that describes the gas exchange inside the Nth compartment in all three phases of the single breath is derived from a mass balance on the compartment and is given by:

$$\frac{dC_{b,N}}{dt} = -\frac{\delta D_L}{\delta V}C_{b,N} = -\frac{D_L}{V_A(t)}\ C_{b,N} \tag{8}$$

The choice for the initial condition $C_{b,N}(0)$ (i.e. the entering concentration of the Nth bolus) is very important. A general description can be acquired by assuming an entering concentration equal to the following:

$$C_{b,N}(0) = c\ C_{Ao}(VA_N) = c\frac{C_I}{C_{ICH_4}}C_{ECH_4}(V_{AN}) \tag{9}$$

where c is defined in Eq. (5b). This choice lies in between the extreme cases of absolutely no mixing between the inspired gas and the residual volume ($C_{b,N}(0) = C_I$), and complete mixing between the inspired gas and the residual volume ($C_{b,N}(0) = C_{Ao}$). It should be noted that for the important limiting case of $D_L \to \infty$, Eq. (9) reduces to the case of no mixing with the residual volume, and for the limiting case were $D_L \to 0$, Eq. (9) reduces to the case of mixing with the residual volume. For the general case ($D_L > 0$), Eq. (9) provides an estimation for the effective alveolar concentration profile with a uniform decay in both the inspired and the residual volumes and an average concentration over the alveolar volume for model 2 identical to model 1 (Appendix A). Eq. (8) can be solved for $C_{b,N}(t)$ in a fashion analogous to that presented for model 1.

Inspiration $\quad 0 < t < t_{insp}, \quad \dot{V}_E = 0$:

$$\ell n(\hat{C}_{b,N}(t_{insp})) = \ell n \frac{C_{b,N}(t_{insp})}{C_{Ao}(VA_N)} = \ell n \frac{C_{b,N}(t_{insp})}{c^{-1}C_{b,N}(0)}$$

$$= \frac{D_L}{\dot{V}_I} \ell n \frac{VA_N}{V_{Ao}} + \ell n\ c \qquad (10)$$

Breathhold

$$t_{insp} < t < t_{insp} + t_{bh}, \quad \dot{V}_E = 0, \quad \dot{V}_I = 0:$$

$$C_{b,N}(t_{insp} + t_{bh}) = C_{b,N}(t_{insp})\ e^{-(D_L/V_{Ao})\ t_{bh}} \qquad (11)$$

Expiration $\quad t_{insp} + t_{bh} < t, \quad \dot{V}_I = 0$:

$$C_{b,N}(t_{out,\ N}) = C_{b,N}(t_{insp} + t_{bh}) \left(\frac{VA(t_{out,\ N})}{V_{Ao}}\right)^{D_L/\dot{V}_E} \qquad (12)$$

Eqs. (11) and (12) represent the concentration of the Nth compartment at the end of breathhold, and at the time that the gas exits the alveolar compartment during expiration ($t_{out,N}$), respectively, and are identical with Eqs. (3) and (4).

By combining Eqs. (10)–(12), and the assumption of an infinite number of unit compartments ($\delta V \to 0$), the following expression for the normalized exhaled concentration as a function of time is derived:

$$\ell n \hat{C}_E(t + V_{DS}/\dot{V}_E) = \ell n \frac{C_{b,N}(t_{out,N})}{c^{-1}\ C_{b,N}(0)}$$

$$= D_L\left(\frac{1}{\dot{V}_I} + \frac{1}{\dot{V}_E}\right) \ell n \frac{VA(t)}{V_{Ao}} - \frac{D_L}{V_{Ao}} t_{bh} + \ell n\ c \qquad (13)$$

The exhaled concentration $C_E(t + V_{DS}/\dot{V}_E)$ is equal to the concentration of the gas which exits the alveolar compartment at time $t = t_{out,N}$.

2.1.3. Comparison of model 1 and model 2

Inspecting the output of the two models (i.e. Eqs. 5a,b and 13) we can conclude that for the two limiting cases of $D_L \to 0$, or $\dot{V}_I \to \infty$, the two models are equivalent. For the general case were $D_L > 0$ and $\dot{V}_I$ is finite, the two approaches differ only during inspiration. For model 1, the alveolar concentration $C_A(t_{insp})$ has a profile similar to the decay of the inert gas (i.e. CH_4), resulting from stratified and parallel inhomogeneities ($\hat{C}_A(t_{insp}) = \text{constant}$). The corresponding profile in model 2 has a steeper axial decay resulting from the sequential filling of the lung (i.e. gas in the regions that fill first have a lower concentration due to increased residence time in the alveolar region). Nevertheless, despite the different effective alveolar concentration profiles in the two models, the average alveolar concentration at the end of inspiration is the same due to the homogeneous D_L (Tsoukias, 1999).

The above concepts are presented in Fig. 2. In this figure, the natural logarithm of the normalized alveolar concentration, (corrected for the inert gas decay), is presented as a function of the natural logarithm of V_A. As suggested by Eqs. 5a,b and 13, the dependence is linear. Three pairs of lines are shown representing the solution for model 1 and model 2 at three different times during the single breath maneuver: (1) at the end of inspiration, $t = t_{insp}$; (2) at the end of breath holding, $t = t_{insp} + t_{bh}$; and (3) during exhalation $t > t_{insp} + t_{bh}$. For the first two pairs of lines (inspiration and breathhold), V_A represents different effective axial positions in the alveolar region (Fig. 1B), while for the last pair (expiration), V_A represents different times during the exhalation. For model 1 (thin lines), the effective alveolar axial concentration gradient or slope, $S_{alv,1}$, is zero at t_{insp} and $t_{insp} + t_{bh}$. During exhalation there is a decay in concentration that reflects uptake by the pulmonary blood and creates a slope, $S_{exh,1}$, equal to $D_L/\dot{V}_E$. For model 2 (thick lines) there is a

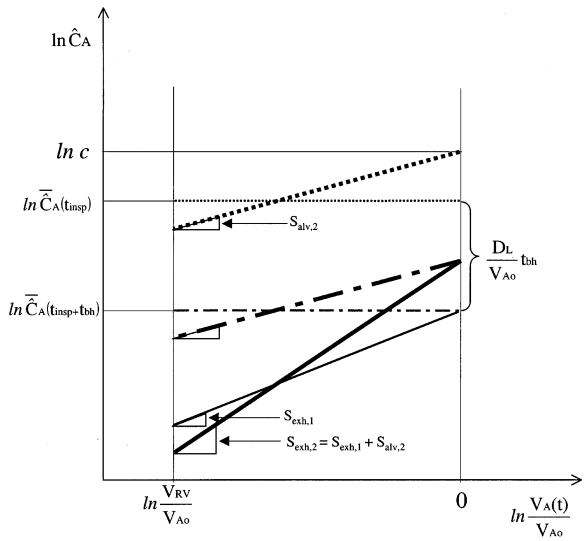

Fig. 2. The natural logarithm of the effective normalized alveolar concentration, as predicted by models 1 and 2 is presented as a function of the natural logarithm of V_A. Three pairs of lines are plotted, one for each model's output at three different points of the single breath maneuver (i.e. at the end of inspiration t_{insp}, at the end of breath holding $t_{insp} + t_{bh}$, and during exhalation $t > t_{insp} + t_{bh}$). The thin lines represent model 1, while the bold or dark lines represent model 2. For the first two pairs of lines (inspiration and breathhold represented by dashed lines), V_A represents different *position* in the alveolar region, while for the last pair (expiration represented by solid lines), V_A represents different *time* during the exhalation. The generated slope during exhalation according to model 2 ($S_{exh,2}$) is steeper than the corresponding slope of model 1 ($S_{exh,1}$) by $S_{alv,2}$ (the effective alveolar concentration gradient prior to exhalation).

slope, $S_{alv,2}$, in the effective alveolar concentration profile at t_{insp} and $t_{insp} + t_{bh}$ due to uptake during inspiration equal to $D_L/\dot{V}_I$. This additional effective gradient creates a steeper exhalation slope, $S_{exh,2}$, equivalent to the sum $S_{exh,1} + S_{alv,2}$ (or $S_{exh,2} = D_L\left(\frac{1}{\dot{V}_E} + \frac{1}{\dot{V}_I}\right)$).

2.1.4. Model 3 (combination of model 1 and model 2)

The actual lungs do not behave as a perfectly mixed compartment, nor as a series of completely sequentially filling independent compartments. Models 1 and 2 represent extreme cases; thus, the actual behavior of the lungs lies somewhere in between. To generate a model which behaves somewhere in between these two extremes one can combine Eqs. 5a,b and 13 using two restrictions or assumptions: (1) the effective alveolar concentration gradient should lie between the minimum value of model 1 and the maximum value of model 2; and (2) $\bar{\hat{C}}_A(t < t_{insp} + t_{bh})$ should be in agreement with both models (Tsoukias, 1999). The result is the following equation (model 3), which introduces a free parameter k (Fig. 3):

$$\ell n\hat{C}_E(t + V_{DS}/\dot{V}_E) = D_L\left(\frac{1}{\dot{V}_E} + \frac{k}{\dot{V}_I}\right)\ell n\,\frac{V_A(t)}{V_{Ao}} - \frac{D_L}{V_{Ao}}t_{bh} + \ell n\left(\frac{kD_L + \dot{V}_I}{D_L + \dot{V}_I}\,c\right) \quad (14)$$

For $k = 0$, Eq. (14) reduces to model 1 (5a,b), while for $k = 1$, Eq. (14) reduces to model 2 (Eq. (13)). For $0 < k < 1$, the slope of the effective alveolar concentration profile, $S_{alv,3}$, generated during inspiration is equal to $kD_L/\dot{V}_I$ and lies between the minimum slope of Model 1 ($S_{alv,1} = 0$) and the maximum slope of Model 2 ($S_{alv,2} = D_L/\dot{V}_I$).

k represents an index of the rate of decrease in C_E due to the sequential filling of the lung. The term $(1/\dot{V}_E + k/\dot{V}_I)$ represents the change in the effective residence time of each gas bolus in the alveolar region (τ_{res}) per unit change in volume. Thus, k could be independently estimated if the relationship between τ_{res} and V_A during exhalation is known. Regardless of the value of k, $\bar{\hat{C}}_A(t_{insp},k)$ will remain the same (equal to $\hat{C}_A(t_{insp})$), while the decay in alveolar concentration during exhalation (i.e., $S_{exh,3}$) and the average value of the exhaled concentration may change. Theoretically, $-1 < k < 1$, where negative values of k represent a lung which fills 'first in, *first* out'. For example, if a lung filled in a completely 'first in–first out' fashion and $\dot{V}_I = \dot{V}_E$, then all gas boli would have the exact same residence time in the alveolar region, and thus the same concentration on exhalation. In other words, the exhaled concentration would be constant in time. This is precisely the case for $k = -1$, which causes the first term in the left hand side of Eq. (14) to be zero.

2.2. Case II: $D_L = f(V_A)$

Eq. (14) was derived assuming that D_L is constant. The same approach can be used when D_L is a simple function of V_A. We present two different functions for $D_L(V_A)$. The first is a simple linear dependence of D_L on V_A (i.e. $D_L(V_A) = a + bV_A$). The second has a physical and anatomical basis, and is derived from a simple model for the membrane diffusing capacity (Appendix B) that suggests a dependence of the form $D_L(V_A) = \alpha V_A^{\beta}$. These two functional forms for $D_L(V_A)$ can be inserted into the governing equations described previously and be solved to derive a solution for the exhaled concentration for model 3:

$$D_L(V_A) = a + bV_A:$$

$$\ell n\, \hat{C}_E \left(t + \frac{V_{DS}}{\dot{V}_E}\right) = a \left(\frac{1}{\dot{V}_E} + \frac{k}{\dot{V}_I}\right) \ell n \frac{V_A(t)}{V_{Ao}} + b \left(\frac{1}{\dot{V}_E} + \frac{k}{\dot{V}_I}\right) (V_A(t) - V_{Ao}) - \frac{a + bV_{Ao}}{V_{Ao}} t_{bh} + \ell n \left[\frac{V_{Ao}}{V_{Ao} - V_{RV}} \frac{\int_{V_{RV}}^{V_{Ao}} \exp[b/\dot{V}_I(V_A(t) - V_{Ao})]\, [V_A(t)/V_{Ao}]^{a/\dot{V}_I}\, dV_A}{\int_0^{V_{Ao}} \exp[kb/\dot{V}_I(V_A(t) - V_{Ao})]\, [V_A(t)/V_{Ao}]^{ka/\dot{V}_I}\, dV_A} \right] \quad (15)$$

$$D_L(V_A) = \alpha\, V_A^{\beta}$$

$$\ln \hat{C}_E \left(t + \frac{V_{DS}}{\dot{V}_E}\right) = \frac{\alpha}{\beta} \left(\frac{1}{\dot{V}_E} + \frac{k}{\dot{V}_I}\right) [V_A(t)^{\beta} - V_{Ao}^{\beta}] - \alpha V_{Ao}^{\beta-1} t_{bh} + \ell n \left[\frac{V_{Ao}}{V_{Ao} - V_{RV}} \frac{\int_{V_{RV}}^{V_{Ao}} \exp[\alpha/\beta\dot{V}_I(V_A^{\beta} - V_{Ao}^{\beta})] dV_A}{\int_0^{V_{Ao}} \exp[k\alpha/(\beta\dot{V}_I)(V_A^{\beta} - V_{Ao}^{\beta})] dV_A} \right] \quad (16)$$

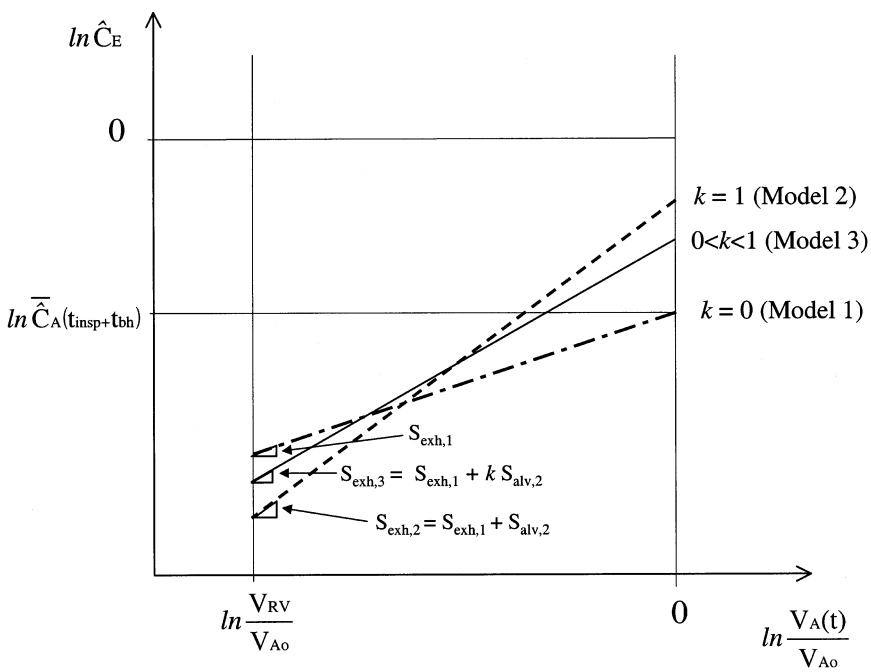

Fig. 3. Graphical representation of models 1–3 with constant D_L. The natural logarithm of the normalized exhaled concentration, as predicted by models 1, 2 and 3 is presented as a function of the natural logarithm of the normalized alveolar volume. See text for definitions of the slopes of the effective alveolar concentration gradient.

Table 1
Parameter values for single breath simulation

Parameter	Value	Units
Single breath		
V_{RV}	1150	ml
V_{A_o}	5150	ml
$\dot{V}i$	2000	ml/sec
$\dot{V}e$	350	ml/sec
D_L	380 (30)	ml/sec (ml/min/mmHg)
C_I	0.3	%vol
t_{bh}	0.5	sec
Inert gas		
$C_{I,CH4}$	0.3	%vol
$C_{E,CH4}$	$(225.28+0.003\ V_A)\times 10^{-3}$	%vol

The estimation of the integrals in Eqs. (15) and (16) can be done numerically, or using infinite series sum formulas.

2.3. Calculating D_L

2.3.1. Single breath – prolonged breathhold

The Jones–Meade method (Jones and Meade, 1961) is the standard method used clinically to determine $D_{L_{CO}}$ in a prolonged breathhold maneuver. D_L determined using the Jones–Meade method, $D_{L_{JM}}$, is calculated using the following equation:

$$D_{L_{JM}} = \frac{V_{A_o}}{t - 0.3t_{insp}} \ell n \frac{C_E(t + V_{DS}/\dot{V}_E)}{C_{A_o}(V_A(t))} \quad (17)$$

Jones and Meade (1961) suggested the collection of a small exhaled sample (~ 85 ml) for the measurement of V_{A_o}, C_{A_o} and C_E, while others have used larger samples (500 ml, Graham et al., 1980). In every case the time interval is estimated until the midpoint of the collection of the sample. For our simulation the size of the collected exhaled sample did not affect the results (examined sizes 1–500 ml) and thus a minimum exhaled sample was used.

2.3.2. Single breath – constant exhalation

D_L can be estimated using two analysis techniques following a prolonged constant exhalation maneuver. The first utilizes only data from the constant exhalation phase and will be referred to as $D_{L_{CE}}$ (Newth et al., 1977; Stokes et al., 1981; Graham et al., 1983). The second considers all three-phases of the breathing maneuver and will be referred to as $D_{L_{3\text{-}Eq}}$ (Graham et al., 1980, 1981; Saumon et al., 1984). $D_{L_{CE}}$ is estimated from the slope of $\ln(\hat{C}_E)$ versus $\ln(V_A)$, which according to Eq. 5, should be equal to the ratio $D_L/\dot{V}_E$. Thus, $D_{L_{CE}}$ is calculated from the following expression:

$$D_{L_{CE}} = S_{exh,1} \cdot \dot{V}_E \quad (18)$$

$D_{L_{3\text{-}Eq}}$ is estimated using Eqs. (5a,b) (i.e. combination of the equations. for the three phases of the single breath). $D_{L_{3\text{-}Eq}}$ is the optimum value of D_L for which the model-predicted (Eqs. (5a,b)) C_E best fits the experimental data. For our case, the estimation of $D_{L_{3\text{-}Eq}}$ was made using an optimization algorithm to minimize the sum of squares of error between predicted and model generated ('experimental') exhaled profiles over the entire exhaled interval from V_{A_o} to V_{RV}.

3. Results

3.1. Effect of sequential filling

The simulations are performed using model 3 as a hypothetical lung with a constant diffusing capacity $D_{L_{CO}} = 30$ ml/min/mmHg (380 ml/sec). Unless otherwise stated, a single breath is simulated using the control parameters summarized in Table 1, and includes inspiration from V_{RV} to V_{A_o}, breathhold, and expiration to V_{RV}. The single breath-prolonged breathhold was simulated at two different breath hold times ($t_{bh} = 5$ and 10 sec), the effect of inspiratory flow rate was examined in the range from 350 to 3000 ml/sec, and three different values of k were used (0, 0.5 and 1.0). Using these 'experimental' data from model 3, one can then estimate D_L of our hypothetical lung using the standard Jones–Meade method ($D_{L_{JM}}$), utilizing exhaled gas samples collected at different points throughout the exhalation, the 3-Eq. analysis ($D_{L_{3\text{-}Eq}}$), or the constant exhalation analysis ($D_{L_{CE}}$).

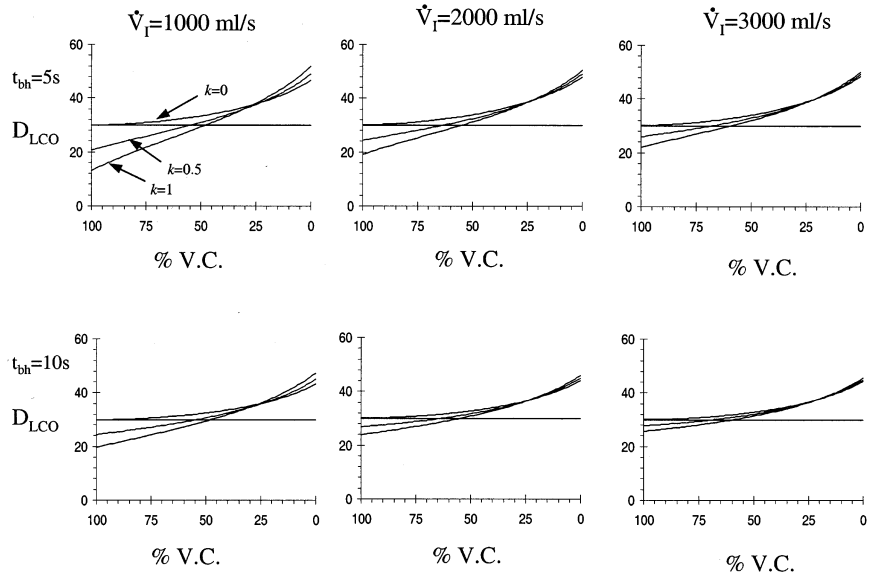

Fig. 4. $D_{L_{CO}}$ measurement with the Jones–Meade method. $D_{L_{JM}}$ estimation as a function of the alveolar volume (% of vital capacity) at the time of collection of the exhaled sample. Two different breath hold times and three different inspired flow rates are examined. The effect of a possible effective alveolar concentration gradient due to sequential filling is examined through three different values of k (0, 0.5, and 1). Experimental data were generated using Eq. (14) and a constant $D_{L_{CO}}$ of 30 ml/min/mmHg.

Fig. 4 plots $D_{L_{JM}}$ as a function of the V_A at the time of collection of the exhaled gas (presented as a percent of the vital capacity). The data presented here are derived using a point (minimal size) collection sample. Three different inspiratory flow rates are shown ($\dot{V}_I = 1000$, 2000 and 3000 ml/sec). For $k = 0$ (well-mixed alveolar region or model 1), the Jones–Meade method can accurately estimate $D_{L_{CO}}$, when the exhaled gas sample is collected immediately following the dead space wash-out (i.e. in the early part of the exhalation). Using a sample collected later in the exhalation will result in an overestimation of $D_{L_{CO}}$. This overestimation will be less pronounced if the breathhold time or the inspiratory flow rate is increased. For $k = 0.5$ or 1 (i.e. part of the decay in the exhaled concentration is generated from the sequential filling of the lung), the Jones–Meade method again overestimates $D_{L_{CO}}$ when samples are collected later in the exhalation. In contrast with before, however, collection of the exhaled sample immediately following the dead space wash-out will *underestimate* $D_{L_{CO}}$. As is the case for $k = 0$, the estimation of $D_{L_{CO}}$ with the Jones–Meade method is improved by increasing the breathhold time or the inspiratory flow rate.

Fig. 5 plots $D_{L_{CE}}$ as a function of $\dot{V}_I$. When there is no significant contribution on the decay of the exhaled concentration from the sequential

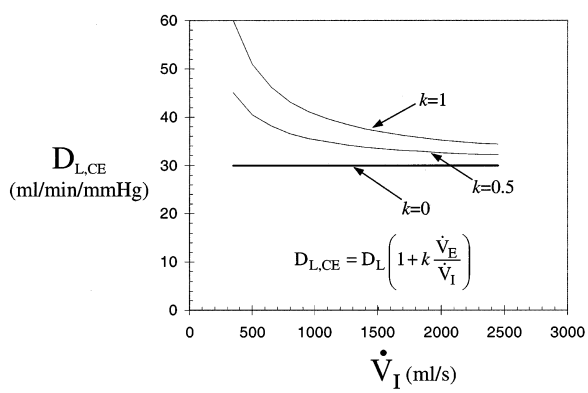

Fig. 5. $D_{L_{CO}}$ measurement with the continuous exhalation technique. $D_{L_{CE}}$ estimation of $D_{L_{CO}}$ as a function of the inspiratory flow rate. The effect of a possible effective alveolar concentration gradient due to sequential filling is examined through three different values of k (0, 0.5, and 1). A constant value for $D_{L_{CO}}$ of 30 ml/min/mmHg was used.

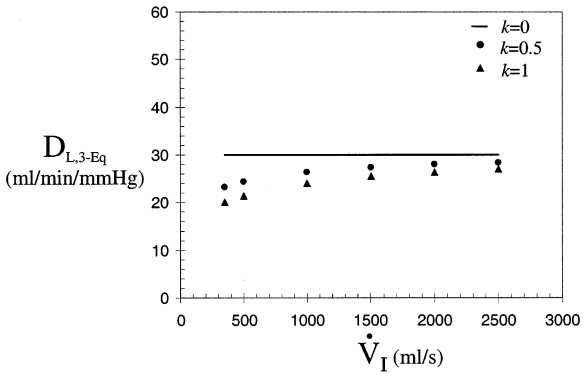

Fig. 6. $D_{L_{CO}}$ measurement with the 3-Eq. approach. $D_{L_{3\text{-}Eq}}$ estimation of $D_{L_{CO}}$ as a function of the inspiratory flow rate. The effect of a possible effective alveolar concentration gradient due to sequential filling is examined through three different values of k (0, 0.5, and 1). Experimental data were generated using Eq. (14) and a constant $D_{L_{CO}}$ of 30 ml/min/mmHg.

filling of the lung ($k = 0$), there is, again, no difference between $D_{L_{CE}}$ and $D_{L_{CO}}$. On the other hand, if the lung behaves as a completely ($k = 1.0$), or partially ($k = 0.5$) sequentially filled compartments, then $D_{L_{CE}}$ *over*estimates the actual $D_{L_{CO}}$ and this phenomenon is exaggerated at low inspiratory flows. Inspection of Eqs. 5a,b and 14 indicates that this overestimation is proportional to the value of k and the ratio$\dot{V}_E/\dot{V}_I$ (i.e. $D_{L_{CE}} = D_L(1 + k\dot{V}_E/\dot{V}_I)$.

Fig. 6 presents $D_{L_{3\text{-}Eq}}$ as a function of $\dot{V}_I$. For $k = 0$, Eqs. 5a,b and 14 become equivalent and there is no difference between $D_{L_{3\text{-}Eq}}$ and the hypothetical lung. For $k > 0$, $D_{L_{3\text{-}Eq}}$ *under*estimates $D_{L_{CO}}$ and, as before, the error becomes more significant at slow inspiratory flow rate maneuvers.

3.2. D_L as function of V_A

The small dependence of $D_{L_{CO}}$ on V_A can be approximated as linear ($D_{L_{CO}} = a + bV_A$), while the dependence of $D_{L_{NO}}$ can be described by an exponential function ($D_{L_{NO}} = \alpha V_A^{\beta}$) (Appendix B). Eqs. (15) and (16) can then be used to simulate the hypothetical lung for CO and NO, respectively, to determine the impact of a variable D_L. Preliminary data from normal subjects indicate approximate values for a and b to be 220 ml/sec and 20 ml/sec/L, respectively, and for α and β to be 500 ml/sec/$L^{-\beta}$ and 0.8, respectively, which provide the following functional dependence of D_L on V_A: $D_{L_{CO}}(V_A) = 17.37 + 1.58\ V_A$ (ml/min/mmHg), and $D_{L_{NO}}(V_A) = 39.5V_A^{0.8}$ (ml/min/mmHg) with V_A in liters. We used Eqs. (15) and (16) as our hypothetical lung for CO and NO respectively, and the relationships above for $D_{L_{CO}}$ and $D_{L_{NO}}$, to generate 'experimental' data using the same range of values for k, t_{bh}, and $\dot{V}_I$ as before for the case of a constant D_L. We then analyzed this data to determine $D_{L_{CO}}$ and $D_{L_{NO}}$ using Jones–Meade method, the 3-Eq. method, or the constant exhalation technique.

Fig. 7 plots the estimated $D_{L_{CO}}$ and $D_{L_{NO}}$ using the Jones–Meade method as a function of V_A at the end of inspiration. Simulations were performed using different inspired volumes and a prolonged breathhold time of 10 sec. Since most of the gas exchange occurs during breathholding, $D_{L_{JM}}$ should approximate the value of D_L at V_{A_O} (i.e. $D_{L_{JM}} \approx D_L(V_{A_O})$). Thus, one can utilize different inspired volumes to investigate the dependence of D_L on V_A. For the calculations an exhaled sample collected at 100% of V.C. (i.e. immediately following the dead space washout) is used.

In Fig. 7A,B simulations are performed for $k = 0$ and three different values of $\dot{V}_I$ (500, 1000 and 2000 ml/sec). In Fig. 7C,D, $\dot{V}_I$ is set to the control value (2000 ml/sec) while we investigate the effect of different k (0, 0.5, 1). In the absence of a significant effective alveolar concentration gradient from the sequential filling ($k = 0$), $D_{L_{JM}}$ can sufficiently approximate the actual D_L at different lung volumes, for both gases, provided that sufficient inspiratory flows have been utilized (i.e. $\dot{V}_I > 2000$ ml/sec). Thus, the Jones–Meade method can provide a satisfactory description of the dependence of D_L on V_A. As $\dot{V}_I$ decreases, the method progressively underestimates D_L. This underestimation is much more significant for NO than for CO. In addition, when $k > 0$ the Jones–Meade method significantly underestimates D_L for both gases even at high $\dot{V}_I$ (2000 ml/sec). Since the absolute error is more significant at high volumes the method in this case will underestimate the dependence of $D_{L_{CO}}$ and $D_{L_{NO}}$ on V_A.

Fig. 8A,B plots the estimated DL_{CO} and DL_{NO} using the single breath-constant exhalation maneuver with either the 3-Eq. approach ($DL_{3\text{-}Eq}$) or the continuous exhalation technique (DL_{CE}) as a function of VA. Both methods provide a constant value for DL independent of VA (i.e. horizontal lines) even though DL in the 'experimental' lung is changing with VA (thick solid line). For $k = 0$, both methods provide an estimation of DL, which is significantly smaller than the estimation acquired with the Jones–Meade method for the same inspired volume ($DL_{JM} \approx DL(VAO)$). DL_{CE} is less than the mean value of DL over the examined range of VA ($VRV - VAO$) while $DL_{3\text{-}Eq}$ is higher. In agreement with the case of constant DL, DL_{CE} is a positive function of k while $DL_{3\text{-}Eq}$ is a negative function of k for both gases.

Fig. 9 plots the exhaled NO concentration CE as a function of VA. The thick solid line represents the 'experimental' lung as predicted by Eq. (16) with $DL_{NO}(VA) = 39.5\ VA^{0.8}$. No additional effective alveolar concentration gradient from the sequential filling of the lung (i.e. $k = 0$) is assumed. As described above, the continuous exhalation technique and the 3-Eq. approach provide a constant value for DL_{NO} (i.e. $DL_{CE} = 89$, $DL_{3\text{-}Eq} = 127$ ml/min/mmHg). Simulations using these constant values for DL_{NO} (Eq. (14)) result in exhalation profiles (dotted lines) which differ significantly from the 'experimental' data. DL_{CE} pre-

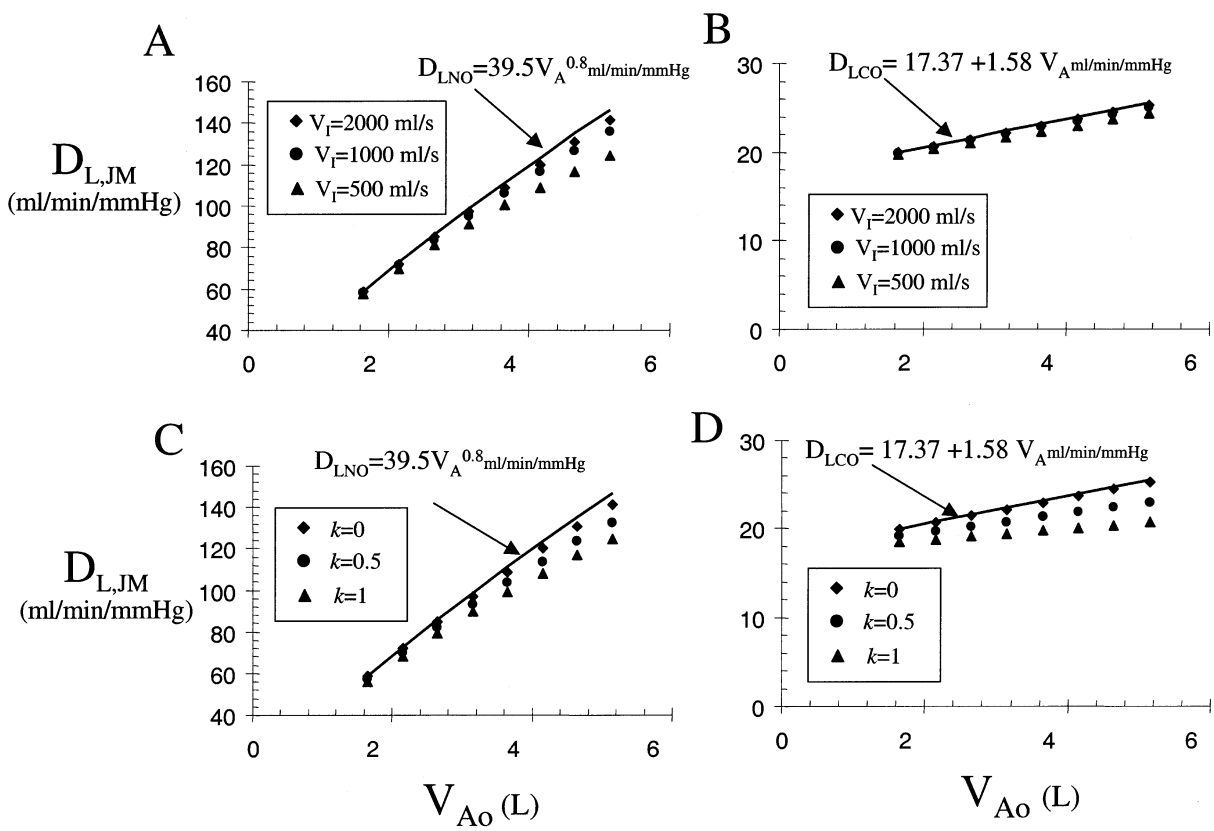

Fig. 7. Jones–Meade method for the measurement of volume dependent DL_{NO} and DL_{CO}. Predictions, using the Jones–Meade method as a function of the alveolar volume at the end of inspiration. 'Experimental' data were generated using Eq. (15) or Eq. (16) and the relationships: $DL_{NO} = 39.5VA^{0.8}$ and $DL_{CO} = 17.37 + 1.58VA$. Simulations performed using different inspiratory flow rates and different values for k. (A) DL_{JM} for NO at three different inspiratory flow rates. (B) DL_{JM} for CO at three different inspiratory flows. (C) DL_{JM} for NO at three different values of k. (D) DL_{JM} for CO at three different values of k. Solid lines represent the 'actual' dependence of DL_{NO} and DL_{CO} on VA.

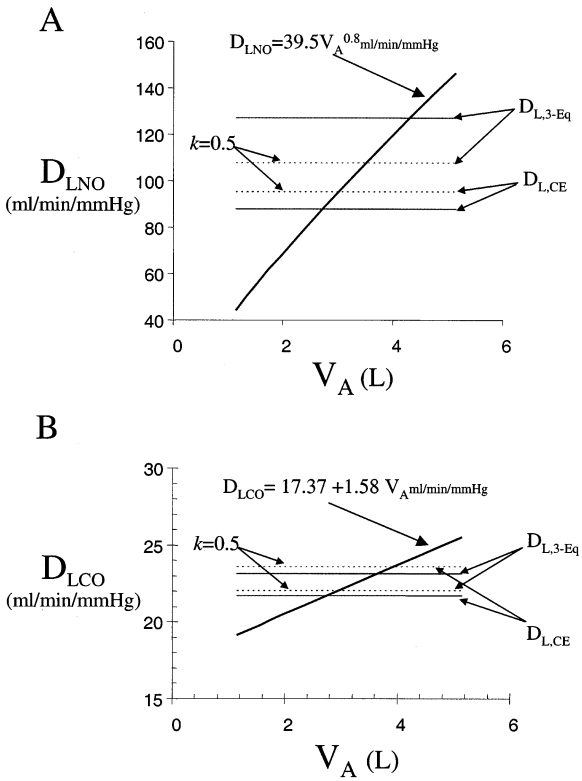

Fig. 8. DL_{CE} and $DL_{3\text{-}Eq}$ estimations of volume dependent DL_{NO} and DL_{CO}. Estimation of DL_{NO} (A) and DL_{CO} (B), using the continuous exhalation technique or the three equations approach, as a function of the alveolar volume. 'Experimental' data were generated using Eq. (15) or Eq. (16) and the relationships: $DL_{NO} = 39.5VA^{0.8}$ and $DL_{CO} = 17.37 + 1.58VA$. Simulations performed using two different values for k (k = 0, 0.5). The 'actual' dependence of DL_{NO} and DL_{CO} on VA is also plotted.

dicts exhaled concentrations much higher than 'experimental'. $DL_{3\text{-}EQ}$ predicts an exhalation profile that fits the 'experimental' data in the early part of exhalation, but fails to predict the rate of decay in the latter part. Similar behavior, but to a lesser extent, can be observed for CO (data not shown).

4. Discussion

4.1. Effect of sequential filling

Previously developed models for the estimation of the diffusing capacity have assumed a well-mixed alveolar compartment. However, there is strong experimental and theoretical evidence that this represents a rough approximation, and that the lung inflates and deflates, at least in part, in a sequential fashion (Dollfuss et al., 1967; Cotton et al., 1979; Fukuchi et al., 1980; Engel and Paiva, 1981; Meyer et al., 1983). Researchers have attempted to account for the incomplete mixing in the alveolar compartment by correcting the decay in the exhaled gas concentration with the simultaneous decay of an inert gas such as methane or helium (Newth et al., 1977). The mechanism that accounts for a heterogeneous distribution of inert gas alveolar concentrations is primarily parallel inhomogeneities in the convective–diffusive interactions during filling and emptying of the lung (Paiva and Engel, 1981). However, a consequence of parallel inhomogeneities is sequential filling and emptying of the lungs; thus, one can anticipate a discrepancy in the residence times of gas boli in the alveolar region of lungs. Hence, regions of the lungs that fill first will have a lower concentration (relative to those which fill last) of a gas such as CO or NO which are not inert, but are diffusing into the pulmonary circulation. Thus, CO and NO would have a steeper effective alveolar concentration gradient than an inert gas such as CH_4.

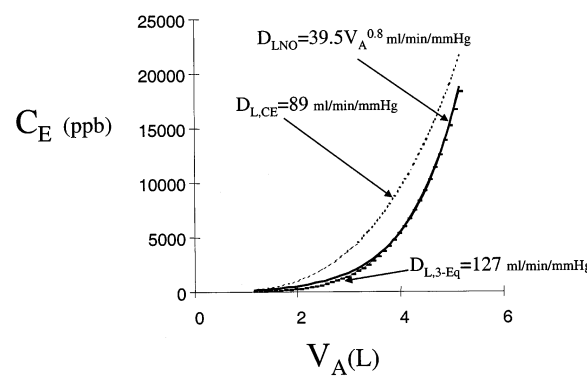

Fig. 9. DL_{CE} and $DL_{3\text{-}Eq}$ prediction of the exhalation profile. Prediction of the exhaled NO concentration using Eq. 5a,b and the DL_{CE} or $DL_{3\text{-}Eq}$ estimations for DL_{NO}. 'Actual' data (solid line) derived using Eq. (16) and the relationship: $DL_{NO} = 39.5VA^{0.8}$. Simulations performed using the control parameter values (Table 1) and for k = 0.

Jones and Meade (1961) suggested that their method could provide an accurate estimation of the diffusing capacity if the exhaled gas sample is collected early in the exhalation (immediately following the dead space wash-out). Graham et al. (1980), using simulations for different flow and breathhold conditions, showed how the Jones–Meade method could overestimate DL if the exhaled gas was not collected early in the exhalation phase. Our data (Fig. 4) suggests an alternative explanation. If there is a contribution to the CO effective gradient from the sequential filling of the lung ($k > 0$), then the Jones–Meade method *over*estimates the true DL when the exhaled gas is sampled late in the exhalation phase, but *under*estimates DL if the sample is taken immediately after deadspace wash-out. Importantly, the average experimental value for k is 0.51 in normal subjects (Tsoukias et al., 2000) suggesting a significant component of sequential filling. As a result, even if exhaled samples are collected immediately after the dead space of the lungs is emptied, one would anticipate a weak reproducibility and accuracy for the method unless high enough inspiratory flows and sufficient breathhold times are utilized to counteract the effect of sequential filling.

The overestimation of DL is generated from the fact that the Jones–Meade method does not describe the non-exponential decay during exhalation. The concentration decays faster during exhalation than during breathholding; thus, an artificially low exhaled concentration is observed resulting in an overestimation of DL. The error can be significantly reduced if the sample is collected immediately after the dead space of the lungs is exhaled which causes the decay in the concentration during expiration to be minimal in comparison with the decay during breathhold. However, if there is an effective gradient in the alveolar concentration due to sequential filling, the early sample of exhaled air is not representative of the average alveolar concentration. This early portion of the exhaled gas has resided in the lungs for less time and thus has an artificially high concentration of gas resulting in an underestimation of DL.

A continuous single exhalation technique provides an estimation of DL independently of the inspiratory and breathhold phases of the single breath maneuver by utilizing only the rate of change of gas concentration during exhalation. This method offers several advantages over other techniques for the measurement of DL. The method does not require knowledge of breathhold or inspiration time, inspiratory flow, residual volume, or initial alveolar concentration, and, thus, eliminates error associated with the estimation of these parameters. Previous investigators have presented modifications of this method including the use of the breathhold equation to describe the exchange over small intervals during exhalation (Newth et al., 1977; Stokes et al., 1981), or the point sample and the discrete sample variations (Graham et al., 1983). Our approach to estimate the ratio of $DL_{CO}/\dot{V}E$ from the slope of ln $\hat{C}E$ versus ln VA should be in agreement with the above analyses provided that $\dot{V}E$ and DL_{CO} remain constant throughout the exhalation.

The decay in the exhaled gas concentration is generated primarily from the continuous gas uptake during exhalation according to Eq. (4). However, part of this decay is a result from the effective concentration gradient in the alveolar region due to incomplete mixing. In order to accurately estimate DL we need to distinguish the part of the decay that is attributed to diffusion into the pulmonary blood during inspiration from the part that is generated purely by gas mixing mechanisms. Newth et al. (1977) suggested using the decay of the inert gas (CH_4 or He) to correct for gas mixing inhomogeneities (Eq. (2b)), based on the assumption that gases with similar gas phase diffusivities should exhibit similar effective alveolar concentration gradients. Based on the analysis above, it is possible for a gas that is absorbed by the pulmonary blood to exhibit a much steeper effective alveolar concentration gradient due to the sequential filling of the lung and subsequent heterogeneity in alveolar residence times. Then as presented in Fig. 5 (for $k > 0$), the method overestimates the actual DL. Comparison of Eqs. 5a,b and 14 reveals that the overestima-

tion increases with the ratio $\dot{V}_E/\dot{V}_I$. Thus, to improve the accuracy of the method the minimum allowable ratio of expiratory to inspiratory flow rates should be used.

The 3-Eq. method presented here differs slightly from that introduced by Graham et al. (1980) in the way the value of DL is determined. They used an iterative scheme and comparison of the measured average exhaled concentration with that predicted from the simulation (Graham et al., 1980). Integration of Eqs. 5a,b and 14 suggests that the average exhaled concentration, over a specified interval, will be different for $k > 0$. Thus, the method will fail to predict the accurate DL if there is a significant contribution to the effective alveolar concentration gradient from the sequential filling of the lung. For consistency with the method used for the non-constant DL we used a least square method to fit the profile predicted from the simulation with the 'experimental' data, rather than comparing the average concentrations. Our results however should be in agreement with the method of Graham et al. (1980). Fig. 6 reveals a slight underestimation of DL_{CO} with the 3-Eq. method when sequential filling has an impact on the decay of exhaled CO. Although the method behaves better than those previously described, the underestimation could become significant at small $\dot{V}_I$.

4.2. DL as a function of VA

An increase in DL_{CO} with VA has been suggested from the early works of Ogilvie et al. (1957) and Miller and Johnson (1966), and more recently by Rose et al. (1979) and Borland and Higenbottam (1989). The researchers used DL_{JM} to estimate DL_{CO}. There is at least one case in the literature that exhibits the opposite behavior (i.e. a slight decrease of DL_{CO} with VA) while other reports suggest that DL_{CO} remains essentially constant at different lung volumes (Newth et al., 1977; Graham et al., 1980). Despite the controversy regarding DL_{CO}, DL_{NO} is a strong function of VA.

Appendix B, coupled with preliminary experimental data, suggests that DL_{CO} and DL_{NO} can be approximated by the functions $17.37 + 1.58$ VA and $39.5\ VA^{0.8}$, respectively, which indicates a 17 and 38% decrease, respectively, when VA is reduced from 7 to 3.9 L. Thus, our results are in close agreement with Borland and Higenbottam (1989) who reported a 34% decrease in DL_{NO}, in comparison with only 8% decrease in DL_{CO}, when VA is reduced from 7 to 3.9 L. This dependence may be attributed to: (1) an artifact arising from a possible inhomogeneous DL of the lung coupled with sequential emptying. For example, the basal compartment contributes more to flow early in the exhalation while the apical latter. Thus if the apical region had a lower DL than the basal region then the 'observed' DL should progressively decrease during exhalation; (2) an actual increase in the ability of the lung to absorb the gas at higher lung volumes due to either an increase in the available surface area for diffusion, or due to a decrease in the thickness of the barrier between the gas and the blood (Staub, 1969; Weibel et al., 1973; Davidson and Fitzgerald, 1974). Current models do not consider a variable DL with VA.

The Jones–Meade method (although developed with the assumption of constant DL) can provide a description of the dependence of DL on VA when multiple single breath maneuvers with different inspired volumes are utilized. Fig. 7A,B suggest an underestimation of $DL(V_{Ao})$ at low $\dot{V}_I$ and at high V_{Ao}. If DL is a positive function of VA then the effective DL during inspiration should be less than during breathhold where VA has the maximum value. As a result DL_{JM} will decrease when the exchange of gas during inspiration becomes significant relative to that during breathhold. This occurs when either the inspired volume is increased or $\dot{V}_I$ is decreased (in either case we have an increase in t_{insp}). The phenomenon is more profound for NO where the change of DL with VA is more significant.

DL_{JM} will also underestimate $DL(V_{Ao})$ when $k > 0$. This observation is supported by analysis of experimental data in the companion manuscript (Tsoukias et al., 2000). Thus, the Jones–Meade method appears to have two disadvantages in providing a description for DL(VA): (1) multiple single breaths are needed to estimate DL at various volumes; and (2) the method will,

in general, underestimate DL and its dependence on VA. Model 3 provides an alternative to the Jones–Meade method without the above problems, and should be utilized especially for gases such as NO where DL is a strong function of VA.

The prolonged exhalation methods (i.e. continuous exhalation and 3-Eq. method) provide a constant value as an estimation of DL(VA). DL_{CE} and $DL_{3\text{-}Eq}$ represent weighted averages of the DL over the part of the exhalation used for the analysis (i.e. in our case $VRV - VAO$). In the absence of an effective alveolar concentration gradient from sequential filling ($k = 0$), $DL_{3\text{-}Eq}$ is higher than DL_{CE} (Fig. 8). Although the two methods assume the same model to describe the exchange of gas (Eqs. 5a,b) the optimum value for DL is defined differently in the two approaches. When DL is volume dependent, Eqs. 5a,b cannot accurately describe the exchange, resulting in the discrepancy between the two values. $DL_{3\text{-}Eq}$ will have a value such as to minimize the error between the 'experimental' data and that predicted using Eqs. 5a,b. In doing so, the value for DL will be relatively high to account for the higher values of DL during breathhold or the early part of exhalation. The predicted exhaled profile will best approximate the exhaled profile in the early part (Fig. 9), but will fail to provide an accurate description in the latter part (significant relative error). DL_{CE} will have a value that describes the average rate of decay (defined from the slope of ln $\hat{C}E$ versus ln VA) during the examined part of exhalation. This value underestimates the DL during breathhold resulting in a significant overestimation of the exhaled concentration (Fig. 9). As k increases, same as for the case of constant DL, DL_{CE} will increase while $DL_{3\text{-}Eq}$ will decrease (Fig. 8).

4.3. Model limitation

In the formulation of the model we assumed a uniform distribution of the diffusing capacity in the lung ($DL/VA = \text{constant}$). It has been established, however, that the basal region of the lung exhibits a higher DL_{CO}/VA ratio (due to higher perfusion) compared to the apical region. In addition, the basal region of the lungs contributes more to the flow early in exhalation. Thus, part of the observed increase of DL with VA may be attributed to the non-uniformity of DL coupled with sequential emptying. Our new method does not provide a means of distinguishing the part of the dependence of DL on VA generated from the non-uniformity of tissue properties from that of physical changes in the tissue such as stretching of the alveolar wall. This phenomenon may be more important in diseased lungs where the heterogeneity of DL may be exaggerated. This phenomenon should not impact significantly DL_{NO} due to the very rapid reaction of NO with hemoglobin.

In addition the estimated value of k may be affected by the non-uniformity of DL. If a heterogeneous DL were a critical feature for the estimation of k, one would expect to see differences in the estimated k using CO versus NO (DL_{CO} will have higher heterogeneity); however, our companion manuscript (Tsoukias et al., 2000) predicts no statistical difference between k estimated with either gas. Thus, although our new method does not consider a heterogeneous DL, we do not believe this significantly affects the estimation of k. Nevertheless, for significantly inhomogeneous lungs, an independent estimation for k could be made utilizing inert gases and determining the residence time distribution of gas boli.

5. Conclusions

There is experimental evidence that the alveolar region is not well mixed, and is, in part, filled sequentially. Importantly, prevalent lung diseases such as COPD may exaggerate sequential filling. The sequential filling of the lung may increase the effective alveolar concentration gradient for soluble or diffusing gases like NO and CO in comparison with the effective gradient of an inert or insoluble gas such as CH_4 or He. Current methods ignore a possible effective alveolar concentration gradient due to the sequential filling of the lung. As a result, significant error may be introduced in the estimation of DL_{CO} and DL_{NO} especially at low inspiratory flow rates. In addition, previously developed models and methods for the measurement of DL have assumed a constant value independent of VA. Such an assumption

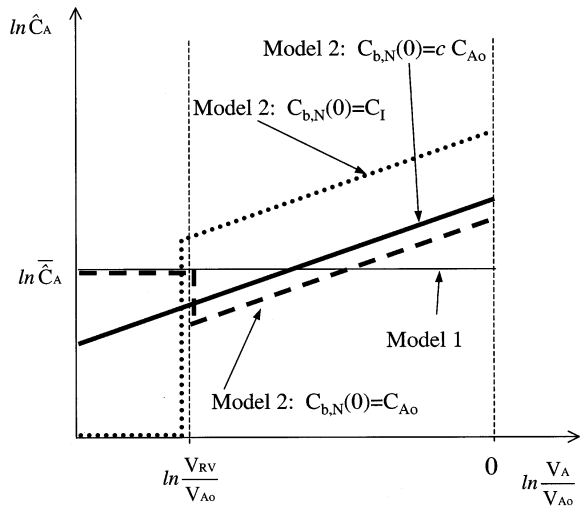

Fig. 10. Normalized alveolar concentration as a function of alveolar volume as predicted by models 1 and 2. Dashed lines represent two extreme cases for the initial condition of model 2.

may be an acceptable approximation for CO, but is not valid for NO. With the exception of the Jones–Meade method, current methods cannot describe this dependence of DL on VA. However, the Jones–Meade method needs multiple single breaths for the estimation of DL at different VA's, and will tend to underestimate DL and its dependence on VA. The model introduced in this manuscript accounts for a variable effective alveolar concentration gradient due to the sequential filling of the lung, and for a DL that changes with VA. Thus, the model represents a potentially more robust method to determine DL. The companion manuscript (Tsoukias et al., 2000) investigates the suitability of the model to estimate DL, its dependence on VA, and the degree of sequential filling in the lung through the parameter k from experimental data in normal human subjects.

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Appendix A. Initial condition for model 2

In a uniform alveolar model the uptake of gas, and thus the average alveolar concentration during inspiration or breathhold, is independent from the distribution of the gas in the region (Tsoukias, 1999). Thus, the average alveolar concentration in models 1 and 2 should be the same. Fig. 10 plots the normalized alveolar concentration as a function of the alveolar volume (axial position) as predicted by models 1 and 2. In addition, the dashed lines represent the two extreme cases for the initial condition of model 2 ($C_{b,N}(0) = C_I$ and $C_{b,N}(0) = C_{Ao}$). For model 1, $\hat{C}_A$ is uniform throughout the alveolar region (complete mixing). In model 2, we seek a value for $C_{b,N}(0)$ which provides a solution for $\hat{C}_A$ that avoids the unrealistic discontinuities which are present in the limiting cases (dashed lines), but still maintains the same average alveolar concentration as model 1, and the same effective gradient in the alveolar concentration as the two limiting cases. Under these constraints, and the relationships from Eqs. 2a,b and 10, the following relationships should hold:

$$\ell n(\hat{C}_{b,N}(t_{insp})) = \ell n \frac{C_{b,N}(t_{insp})}{C_{Ao}(VA_N)} = \frac{D_L}{\dot{V}_I} \ell n \frac{VA_N}{V_{Ao}} + \ell n \frac{C_{b,N}(0)}{C_{Ao}(VA_N)} \quad (A1)$$

$$\bar{\hat{C}}_A(t_{insp}) = \frac{\int_0^{V_{Ao}} \frac{(C_{b,N}(t_{insp})}{C_{Ao}(VA_N))} dV}{\int_0^{V_{Ao}} dV} = \frac{V_{Ao}/t_{insp}}{D_L \dot{V}_I} \left(1 - \left(\frac{V_{RV}}{V_{Ao}}\right)^{\frac{D_L + \dot{V}_I}{\dot{V}_I}}\right) \quad (A2)$$

where $C_{b,N}(0)$ is the initial concentration, and $C_{Ao}(VA_N)$ is the alveolar concentration if there was complete mixing between the inspired and residual volume. Inserting Eq. (A1) into Eq. (A2) and integrating yields:

$$\frac{C_{b,N}(0)}{C_{Ao}(VA_N)} = \frac{1 - (V_{RV}/V_{Ao})^{(D_L + \dot{V}_I)/\dot{V}_I}}{1 - V_{RV}/V_{Ao}} = c \quad (A3)$$

Appendix B. A simple model for the membrane diffusing capacity

The membrane diffusing capacity can be described by the following equation:

$$D_M = \frac{D\ \lambda\ S}{d_m} \tag{B1}$$

where D is the diffusion coefficient in the tissue layer, λ is the partition coefficient, S is the surface area available for diffusion, and d_m is the effective thickness between the gas and the blood. We assume that the alveolar region consist of N_A ($\sim 300 \times 10^6$) identical alveoli. Morphometric studies have described alveoli of different shapes including truncated spherical ones ranging from 1/4 of a sphere to 5/6 of a sphere. Our model alveolus is assumed to be a truncated sphere (Fig. 11). The following equations then hold for the volume V_α and surface area S_α of an alveolus:

$$V_\alpha = \frac{4}{3}\pi R^3\left(\frac{1}{2} + \frac{h}{2R}\right) = \frac{4}{3}\pi R^3\gamma \tag{B2}$$

$$S_\alpha = 4\pi R^2\left(\frac{1}{2} + \frac{h}{2R}\right) = 4\pi R^2\gamma \tag{B3}$$

Where γ represents the part of a sphere that describes the shape of the model alveolus ($1/4 < \gamma < 5/6$). Combining Eqs. (B2) and (B3) yields the total alveolar surface area S_A:

$$S_A = N_A S_\alpha = (36\pi\gamma N_A)^{1/3} V_A^{2/3} \tag{B4}$$

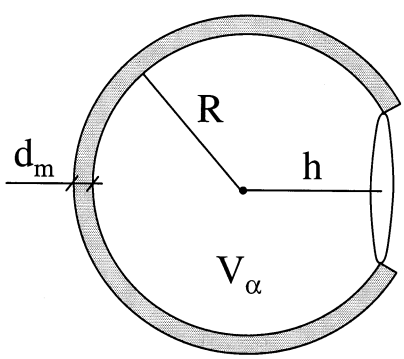

Fig. 11. Model alveolus described as part of a sphere. V_α is the volume of the alveolus and d_m is the membrane thickness between the gas and the blood.

d_m (~ 0.5 μm) is much smaller than R (~ 100 μm). Thus, the total tissue volume surrounding the alveoli can be approximated as:

$$V_t = N_A d_m S_\alpha \tag{B5}$$

In order for the tissue volume to remain constant, d_m should be inversely proportional to S_α.

Combining Eqs. (B1), (B4) and (B5) and assuming that the alveolar surface area available for diffusion S is equal to S_A one gets:

$$D_M = \frac{D\lambda(36\pi\gamma N_A)^{2/3}}{V_t} V_A^{4/3} = \alpha V_A^{4/3} \tag{B6}$$

Weibel et al. (1973) suggested that during breathing the thickness of the barrier d_m remain unchanged, and only the surface area change by folding and unfolding. In contrast, other investigators (Staub, 1969; Davidson and Fitzgerald, 1974) suggested that the surface area available for diffusion remain unchanged (surface area around the capillaries is independent of lung volume) and changes in D_L can be attributed to changes in d_m. Thus, this simplified alveolar model suggests an exponential dependence of D_M on V_A ($D_M = \alpha V_A^\beta$). We anticipate the exponent β to be somewhere between 2/3 and 4/3 depending on whether or not both the surface area and the thickness of the barrier between blood and gas change with V_A. Since NO reacts much faster with hemoglobin than CO, $D_{L_{NO}}$ should be independent of the capillary blood volume or the specific blood transfer conductance θ, and should be in close agreement with the membrane diffusing capacity (Guenard et al., 1987; Borland and Higenbottam, 1989). For CO the transfer from the blood to the gas is both diffusion and reaction limited and thus such an assumption is not valid. However, since the dependence is small, a simple linear relationship can be a satisfactory approximation for $D_{L_{CO}}$.

References

Borland, C.D.R., Higenbottam, T.W., 1989. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur. Respir. J. 2, 56–63.

Brenner, M., Babusis, A., Tran, V., Bellusa, L., Pinderski, E., Chen, C., Boyajian, J., Wilson, A.F., 1994. Measured and computer lung model predicted DLCO during alterations in the single breath maneuver. J. Appl. Physiol. 76, 2210–2215.

Cotton, D.J., Newth, C.J., Portner, P.M., Nadel, J.A., 1979. Measurement of single-breath CO diffusing capacity by continuous rapid CO analysis in man. J. Appl. Physiol. 46, 1149–1156.

Cotton, D.J., Graham, B.L., 1980. Effect of ventilation and diffusion nonuniformity on DLCO exhaled in a lung model. J. Appl. Physiol. 48, 648–656.

Davidson, M.R., Fitzgerald, J.M., 1974. Transport of O_2 along a model pathway through the respiratory region of the lung. Bull. Math. Biol. 36, 275–303.

Dollfuss, R.E., Milic-Emili, J., Bates, D.V., 1967. Regional ventilation of the lung studied with boluses of ^{133}xenon. Respir. Physiol. 2, 234–246.

Engel, L.A., Paiva, M., 1981. Analyses of sequential filling and emptying of the lung. Respir. Physiol. 45, 309–321.

Fukuchi, Y., Cosio, M., Murphy, B., Engel, L.A., 1980. Intraregional basis for sequential filling and emptying of the lung. Respir. Physiol. 41, 253–266.

Graham, B.L., Dosman, J.A., Cotton, D.J., 1980. A theoretical analysis of the single breath diffusing capacity for carbon monoxide. IEEE Trans. Biomed. Eng. 27, 221–227.

Graham, B.L., Mink, J.T., Cotton, D.J., 1981. Improved accuracy and precision of single-breath CO diffusing capacity measurements. J. Appl. Physiol. 51, 1306–1313.

Graham, B.L., Mink, J.T., Cotton, D.J., 1983. Dynamic measurements of CO diffusing capacity using discrete samples of alveolar gas. J. Appl. Physiol. 54, 73–79.

Graham, B.L., Mink, J.T., Cotton, D.J., 1984. Overestimation of the single-breath carbon monoxide diffusing capacity in patients with air-flow obstruction. Am. Rev. Resp. Dis. 129, 403–408.

Guenard, H., Varene, N., Vaida, P., 1987. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respir. Physiol. 70, 113–120.

Gurtner, G.H., Fowler, W.S., 1971. Interrelationships of factors affecting pulmonary diffusing capacity. J. Appl. Physiol. 30, 619–624.

Jones, R.S., Meade, F., 1961. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q. J. Exp. Physiol. 46, 131–143.

Krogh, M., 1915. The diffusion of gases through the lungs of man. J. Physiol. Lond. 49, 271–300.

Martonen, T.B., Wilson, A.F., 1982. Theoretical basis of single breath gas absorption tests. J. Math. Biol. 14, 203–220.

Meyer, M., Hook, C., Rieke, H., Piiper, J., 1983. Gas mixing in dog lungs studied by single-breath washout of He and SF6. J. Appl. Physiol. 55, 1795–1802.

Miller, J.M., Johnson, J.R.L., 1966. Effect of lung inflation on pulmonary diffusing capacity at rest and during exercise. J. Clin. Invest. 45, 493–500.

Newth, C.J., Cotton, D.J., Nadel, J.A., 1977. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. J. Appl. Physiol. 43, 617–625.

Ogilvie, C.M., Forster, R.E., Blakemore, W.S., Morton, J.W., 1957. A standarized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. Clin. Invest. 36, 1–17.

Paiva, M., Engel, L.A., 1981. The anatomical basis for the sloping N2 plateau. Respir. Physiol. 44, 325–337.

Rose, G.L., Cassidy, S.S., Johnson, R.L., 1979. Diffusing capacity at different lung volumes during breath holding and rebreathing. J. Appl. Physiol. 47, 32–37.

Saumon, G., Loiseau, A., Loiseau, P., Georges, R., 1984. Lung transfer factor for carbon monoxide measured during a slow single breath without breath-holding and during slow exhalation. Bull. Eur. Physiopath. Resp. 20, 353–360.

Scheid, P., Hlastala, M.P., Piiper, J., 1981. Inert gas elimination from lungs with stratified inhomogeneity: theory. Respir. Physiol. 44, 299–309.

Six, D.P., de Vries, W.R., Luijendijk, S.C., 1991. Sloping alveolar plateaus of He and SF6 measured in excised cat lungs ventilated at constant volume by pressure changes. Respir Physiol 83, 277–293.

Staub, N.C., 1969. Respiration. Annu. Rev. Physiol. 31, 173–202.

Stokes, D.L., MacIntyre, N.R., Nadel, J.A., 1981. Nonlinear increases in diffusing capacity during exercise by seated and supine subjects. J. Appl. Physiol. 51, 858–863.

Tsoukias, N.M., 1999. Characterization of Nitric Oxide exchange in human lungs. Ph.D. Dissertation. University of California, Irvine.

Tsoukias, N.M., Dabdub, D., Wilson, A.F., George, S.C., 2000. Effect of alveolar volume and sequential filling on the diffusing capacity of the lungs: II. Experiment. Respir. Physiol. 120, 251–271.

Weibel, E.R., Untersee, P., Gil, J., Zulauf, M., 1973. Morphometric estimation of pulmonary diffusion capacity. VI. Effect of varying positive pressure inflation of air spaces. Respir. Physiol. 18, 285–308.