Annals of Biomedical Engineering, Vol. 29, pp. 731–739, 2001
Printed in the USA. All rights reserved.

0090-6964/2001/29(9)/731/9/$15.00

Impact of Volume-Dependent Alveolar Diffusing Capacity on Exhaled Nitric Oxide Concentration

NIKOLAOS M. TSOUKIAS[1] and STEVEN C. GEORGE[1,2]

[1]Department of Chemical and Biochemical Engineering and Materials Science, [2]Center for Biomedical Engineering, University of California, Irvine, Irvine, CA

(Received 21 November 2000; accepted 25 May 2001)

Abstract—Exhaled endogenous nitric oxide (NO) holds promise as a potential biomarker of pulmonary inflammation. Previous experimental and theoretical work has concluded that the alveolar concentration approaches a constant steady state value at end exhalation due to both a constant maximum flux or release of NO ($J_{max,alv}$) and a constant diffusing capacity ($D_{NO,alv}$) in the alveolar region. We have recently demonstrated that $D_{NO,alv}$ is not constant, but increases with alveolar volume (V_A) given by the following average relationship: $D_{NO,alv} = 48^* V_A^{2/3}$ ml/min/mmHg (where V_A is expressed in liters, STPD). We investigated the potential impact of a variable $D_{NO,alv}$ on exhaled concentration by incorporating the volume dependence into the currently accepted two-compartment model for NO exchange dynamics. Our results suggest that the mechanism underlying the plateau in exhaled concentration is a constant ratio $J_{max,alv}/D_{NO,alv}$. This constant ratio requires a volume dependence of $J_{max,alv}$ similar to $D_{NO,alv}$, and is likely due to a decreasing alveolar surface area during exhalation. © *2001 Biomedical Engineering Society.*
[DOI: 10.1114/1.1397786]

Keywords—NO, Exhalation, Endogenous, Normal human subjects.

INTRODUCTION

Nitric oxide (NO) was first reported in the exhaled breath of children with bronchial asthma in 1993,[1] and since that time there has been intense interest in the possible clinical use of this biological signal as a noninvasive index of pulmonary inflammation, disease severity, or disease progression.[9,17] The interpretation of the exhaled NO concentration (C_{exh}) is complicated by its dependence on many factors including the presence of inflammation in the lungs[10,19] and the exhalation flow rate ($\dot{V}_E$).[14,22] A complete understanding of NO exchange dynamics is needed to accurately interpret C_{exh}.

A combination of experimental and theoretical work has demonstrated three key features of NO exchange dynamics: (1) C_{exh} reaches nearly a plateau (slight negative phase III slope) concentration when $\dot{V}_E$ is held constant,[22] (2) C_{exh} is inversely related to $\dot{V}_E$,[12,14,22] and (3) the elimination rate ($E_{NO} = C^*_{exh}\dot{V}_E$) of NO is a positive function of $\dot{V}_E$.[15,21] These observations are attributed to a production of NO in both the airway and alveolar regions of the lungs. This unusual feature of NO exchange, combined with the fact that lung diseases can affect different regions of the lungs, creates a need to characterize both airway and alveolar contributions to C_{exh}.

An explicit assumption in previous work has been that the alveolar concentration approaches a constant steady state value at end exhalation due to both a constant maximum flux or release of NO ($J_{max,alv}$, moles/s) and a constant diffusing capacity ($D_{NO,alv}$) in the alveolar region. However, we have recently demonstrated that $D_{NO,alv}$ has a strong positive dependence on alveolar volume (V_{alv}) given by the following average relationship: $D_{NO,alv} = 6.37^*(V_{alv})^{2/3}$ ml/s (where V_{alv} is expressed in ml, STPD) or $D_{NO,alv} = 48^*(V_{alv})^{2/3}$ ml/min/mmHg (where V_{alv} is expressed in liters, STPD).[20,23] The steady state alveolar concentration of NO during expiration ($C_{alv,ss}$) is equal to the ratio $J_{max,alv}/D_{NO,alv}$. Thus, as $D_{NO,alv}$ decreases during exhalation, $C_{alv,ss}$ should increase (if $J_{max,alv}$ is constant) suggesting a time or volume dependence. This poses several important questions: (1) if C_{alv} increases during exhalation, then why does C_{exh} not increase?, and (2) how does one characterize the alveolar contribution to C_{exh}, if C_{alv} depends on V_{alv}?

We have previously described a two-compartment model to characterize NO exchange dynamics.[21] Since our initial description, several others groups have confirmed our description,[12,15] and the two-compartment model is the currently accepted description of NO exchange. Thus, this manuscript incorporates our new findings on the dependence of $D_{NO,alv}$ on V_{alv} into the two-compartment model and investigates the impact on the interpretation of C_{exh}. We demonstrate that a possible

Address correspondence to Steven C. George, MD, PhD, Department of Chemical and Biochemical Engineering and Materials Science, University of California, Irvine, Irvine, CA 92697-2575. Electronic mail: scgeorge@uci.edu

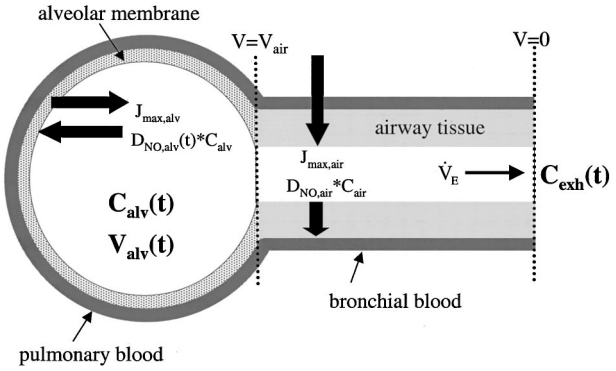

FIGURE 1. Schematic of the two-compartment model used to simulate endogenous NO exchange dynamics. Both the alveolar and airway compartments are characterized by a NO production or release (J) and a diffusing capacity (D).

mechanism underlying the experimentally observed shape of C_{exh} during exhalation is a constant ratio $J_{\mathrm{max,alv}}/D_{\mathrm{NO,alv}}$. This constant ratio implies a constant concentration of NO in the alveolar membrane which requires a volume dependence of $J_{\mathrm{max,alv}}$ similar to $D_{\mathrm{NO,alv}}$. Thus, C_{alv} is at or near a steady state value at end exhalation and may be used to characterize the alveolar contribution to C_{exh}.

METHODS

Two-Compartment Model

The two-compartment model for nitric oxide exchange in the lungs has previously been described in detail[21] and is shown in Fig. 1 with minor modifications that reflect changes in the alveolar region. Briefly, the model consists of two compartments. First, a rigid tube surrounded by a homogeneous layer of tissue representing the airways. The axial gas phase transport is characterized by bulk convection (advection). The tissue phase produces NO uniformly in position, and at a constant rate, and consumes NO in a first order fashion. The outer boundary of the tissue is assumed to be blood. Since the reaction of NO with hemoglobin within the red cell is very rapid, and there are abundant protein-thiols (i.e., albumin) in the plasma, the concentration of free NO in the blood is assumed to be zero. The transport between the tissue and gas phase is described with Fick's First Law of Diffusion. The concentration profile in the tissue has been shown to rapidly ($<$0.6 s) reach a steady state;[21] thus, the mass balance in the airway compartment retains an analytical solution. The second compartment represents the alveolar regions of the lungs, and is thus expansile and considered to have a uniform concentration spatially (C_{alv}). The alveolar compartment is also surrounded by a thin layer of tissue separating the gas and blood (pulmonary circulation) phases which produces NO uniformly throughout the tissue. The model is meant to simulate the oral exhalation profile, and thus does not consider nasal contributions to exhaled NO.

Details of the mathematical development have been previously presented.[21] We will present only the salient features including the changes to the alveolar region (variable diffusing capacity). Mass balances in the airway and alveolar compartments produce the following differential equations:

Airway compartment:

$$\frac{\partial C_{\mathrm{air}}}{\partial t} = -\dot{\mathrm{V}}\frac{\partial C_{\mathrm{air}}}{\partial V} + \frac{J_{t:g,\mathrm{air}}}{V_{\mathrm{air}}}. \tag{1}$$

Alveolar compartment:

$$\frac{dC_{\mathrm{alv}}}{dt} = \frac{\dot{V}_I(C_{\mathrm{air}}(V_{\mathrm{air}}) - C_{\mathrm{alv}})}{V_{\mathrm{alv}}(t)} + \frac{J_{t:g,\mathrm{alv}}}{V_{\mathrm{alv}}(t)}, \tag{2}$$

where $\dot{V}_I = 0$ during expiration, C_{alv} and C_{air} are the concentration (mol/cm^3) of NO in the alveolar and airway compartments, respectively; $J_{t:g,\mathrm{alv}}$ and $J_{t:g,\mathrm{air}}$ are the molar fluxes (mol$\cdot$s^{-1}) of NO between the tissue and gas phases in the alveolar and airway compartment, respectively; $\dot{V}$ is the volumetric flow rate of air during expiration ($\dot{V}_E$) or inspiration ($-\dot{V}_I$), and is assumed constant in each phase; $V_{\mathrm{alv}}(t)$ is the alveolar volume given by $V_{\mathrm{alv}}(0) - \dot{V}t$; V_{air} is the volume of the airway compartment, and V is the axial (or longitudinal) position from the mouth in units of cumulative volume.

It can be shown through a mass balance on the tissue layers, that the flux of NO from the tissue phase to the gas phase can be described as linear function of the gas phase concentration:[21]

$$J_{t:g,i} = J_{\mathrm{max},i} - D_{\mathrm{NO},i}C_i, \tag{3}$$

where the subscript i represents either the airway compartment (''air'') or alveolar compartment (''alv''). $D_{\mathrm{NO},i}$ (ml/s) can be interpreted as the conductance for mass transfer between the tissue and gas phases, and is a function of several parameters including the thickness of the tissue layer, $L_{\mathrm{tiss},i}$, the rate of chemical consumption as characterized by a first order rate constant, k, the surface area available for diffusion A_i, and the diffusivity of NO in the tissue $\mathcal{D}_{\mathrm{NO,tiss}}$. Note that the term $D_{\mathrm{NO},i}{}^*C_i$ represents loss of NO due to diffusion into the blood. $D_{\mathrm{NO,air}}$ is the diffusing capacity of the airways, and is assumed to be constant with respect to time and axial position. $J_{\mathrm{max,air}}$ and $J_{\mathrm{max,alv}}$ represent the maximum fluxes or release rates (moles/s) of NO into the airway and alveolar compartments (i.e., the flux if NO concentration in bulk gas phase were zero), respectively. They depend on the same parameters as $D_{\mathrm{NO},i}$, but also de-

pend on the production rate of NO per unit volume of tissue, $S_{\text{tiss},i}$. The functional dependence of $J_{\max,i}$ and $D_{\text{NO},i}$ on these parameters is described in greater detail in the Discussion section.

$D_{\text{NO,alv}}$ (ml/s) is assumed to depend on the alveolar volume as recently described:[20,23]

$$D_{\text{NO,alv}} = \alpha^* (V_{\text{alv}})^{\beta} \quad (V_{\text{alv}} \text{ expressed in ml}), \quad (4)$$

where α and β are equal to 6.37 and 2/3, respectively, for an average adult. This dependence was determined experimentally by measuring the time rate of change of the exhaled NO concentration following rapid inhalation of a 50 ppm bolus.[20,23] $J_{\max,\text{alv}}$ may also depend on alveolar volume, however experimental determination of this dependence is not available. In the current study, we investigate two alternative scenarios for the dependence of $J_{\max,\text{alv}}$ on V_{alv}. For Case I, $J_{\max,\text{alv}}$ changes similarly to $D_{\text{NO,alv}}$ such as the ratio of the two remains constant. In Case II, $J_{\max,\text{alv}}$ remains constant independent of changes in V_{alv}. The physical and physiological meaning behind these assumptions is described in the Discussion section.

Incorporating the two potential options for describing $J_{t:g,\text{alv}}$ into Eq. (3) gives rise to two different models for the alveolar compartment:

$$\text{Case I: } J_{t:g,\text{alv}} = \alpha (V_{\text{alv}})^{\beta} (\overline{C}_{\text{tiss,alv}} - C_{\text{alv}}), \quad (5)$$

$$\text{Case II: } J_{t:g,\text{alv}} = J_{\max,\text{alv}} - \alpha (V_{\text{alv}})^{\beta} C_{\text{alv}}, \quad (6)$$

where $\overline{C}_{\text{tiss,alv}}$ is defined as the constant ratio of $J_{t:g,\text{alv}}$ and $D_{\text{NO,alv}}$, has units of concentration, and represents the steady state alveolar concentration or equivalently the alveolar tissue concentration. For Case I, $J_{\max,\text{alv}}$ is equal to the product $\alpha (V_{\text{alv}})^{\beta} \overline{C}_{\text{tiss,alv}}$, and thus retains the same functional dependence on V_{alv} as $D_{\text{NO,alv}}$.

Inserting Eq. (5) or (6) into Eq. (2), results in the following expressions for the alveolar concentration during exhalation, $C_{\text{alv}}(t)$:

$$\text{Case I: } C_{\text{alv}}(t) = \overline{C}_{\text{tiss,alv}} + [C_{\text{alv}}(0) - \overline{C}_{\text{tiss,alv}}] \times e^{[\varphi([V_{\text{alv}}(t)]^{\beta} - [V_{\text{alv}}(0)]^{\beta})]}, \quad (7)$$

$$\text{Case II: } C_{\text{alv}}(t) = \left[C_{\text{alv}}(0) - \frac{J_{\max,\text{alv}}}{\dot{V}_E \beta} e^{(\phi V_{\text{alv}}(0)^b)} \times [E_1[\varphi V_{\text{alv}}(0)^{\beta}] - E_1[\varphi V_{\text{alv}}(t)^{\beta}]] \right] \times e^{[\varphi V_{\text{alv}}(t)^{\beta} - \varphi V_{\text{alv}}(0)^{\beta}]}, \quad (8)$$

TABLE 1. Parameter values for two-compartment model.

Parameter	Case I	Case II	Units
Airway			
V_{air}	200	200	cm^3
$D_{\text{NO,air}}$	3.2	3.2	cm^3/s
$J_{\max,\text{air}}$	18.0	18.0	pmol/s
Alveoli			
V_{alv}	2300–6500	2300–6500	cm^3
$D_{\text{NO,alv}}$	$\alpha (V_{\text{alv}})^{\beta}$	$\alpha (V_{\text{alv}})^{\beta}$	cm^3/s
α	6.37	6.37	$cm^{3-\beta}/s$
β	0.67	0.67	…
$J_{\max,\text{alv}}$	$\alpha (V_{\text{alv}})^{\beta} * C_{\text{tiss,alv}}$	300	pmol/s
$C_{\text{tiss,alv}}$	0.20 (5)	…	pmol/cm^3 (ppb)
$C_{\text{alv}}(0)$	0.10 (2.58)	0.12 (2.35)	pmol/cm^3 (ppb)

where $V_{\text{alv}}(t) = V_{\text{alv}}(0) - \dot{V}_E t$, $\varphi = \alpha / \beta \dot{V}_E$, and E_1 is the exponential integral (see the Appendix). $C_{\text{alv}}(0)$ is determined by solving the governing equations during inspiration (see the Appendix). Then, combining either Eq. (7) or (8) with the solution of Eq. (1) results in the following expression for the exhaled concentration:

$$C_{\text{exh}}(t + \tau_{\text{res}}) = \left(C_{\text{alv}}(t) - \frac{J_{\max,\text{air}}}{D_{\text{NO,air}}} \right) \exp\left(- \frac{D_{\text{NO,air}}}{\dot{V}_E} \right) + \frac{J_{\max,\text{air}}}{D_{\text{NO,air}}}, \quad (9)$$

where τ_{res} is the residence time of a differential gas bolus in the airway compartment and is equal to the ratio of the airway compartment volume to $\dot{V}_E$. In other words, the exhaled concentration at time $t + \tau_{\text{res}}$ needs C_{alv} at the time in which the bolus of air left the alveolar compartment and entered the airway compartment.

Parameter values for the simulations are given in Table 1. In general, we have chosen values that are representative of a normal adult human lung. Key parameters include $\overline{C}_{\text{tiss,alv}}$ of 5 ppb (for Case I) which corresponds to a steady state C_{alv} of 5 ppb,[15,22] and a steady state concentration in the airway compartment ($J_{\max,\text{air}} / D_{\text{NO,air}}$) of ~150 ppb.[4,15] We chose two values for $\dot{V}_E$ (50 and 250 ml/s), which correspond to the recommended values of the American Thoracic Society and the European Respiratory Society, respectively.[9,17]

RESULTS

Figures 2 and 3 demonstrate C_{exh} and C_{alv} at an exhalation flow rate of 50 and 250 ml/s, respectively. NO concentration is plotted as a function of exhaled volume. Note that for Case I, C_{exh} and C_{alv} reach a plateau. The volume at which the plateau is reached depends on $\dot{V}_E$

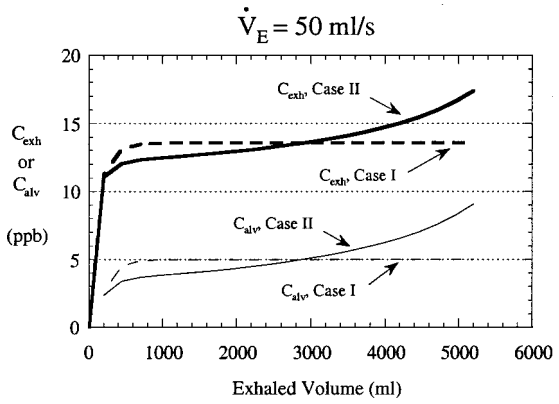

FIGURE 2. Model simulations of the exhalation and alveolar concentrations of NO for the two different cases at an exhalation flow rate of 50 ml/s (ATS guidelines).

(600 and 3000 ml for $\dot{V}_E = 50$ and 250 ml/s, respectively), but the *time* at which the plateau is reached is a constant equal to ~10 s. C_{alv} plateaus at a value of 5 ppb which corresponds to the steady state value or $\overline{C}_{tiss,alv}$. C_{exh} plateaus at different values, 13.6 and 6.8 ppb, respectively, for $\dot{V}_E = 50$ and 250 ml/s due to different contributions from the airway compartment. These contributions are ~63% and 25% of C_{exh}, respectively, for $\dot{V}_E = 50$ and 250 ml/s.

For Case II, C_{exh} and C_{alv} do not reach a plateau, but monotonically increase during exhalation. The difference between C_{exh} and C_{alv} is nearly constant during exhalation as evidenced by the similar shape of C_{alv} and C_{exh}. Early in the exhalation, C_{exh} and C_{alv} for Case II are less than the corresponding values of Case I, but are larger at end exhalation. The end-exhaled C_{exh} and C_{alv} are 17.4 and 9.1 ppb, and 9.7 and 8.0 ppb, respectively, for $\dot{V}_E = 50$ and 250 ml/s.

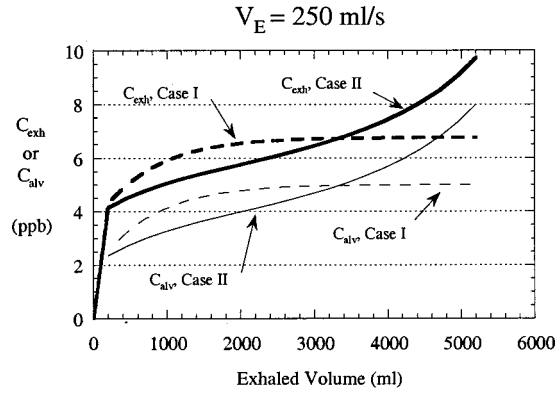

FIGURE 3. Model simulations of the exhalation and alveolar concentrations of NO for the two different cases at an exhalation flow rate of 250 ml/s (ERS guidelines).

DISCUSSION

We investigated two alternative scenarios for the dependence of $J_{t:g,alv}$ on V_{alv}. According to Case I the ratio $J_{max,alv}/D_{NO,alv}$ is held constant. The implicit assumption behind such a scenario is that the alveolar tissue concentration $\overline{C}_{tiss,alv}$ remains constant. Equilibrium kinetics of NO in the tissue layer can produce such a behavior. However, based on the current knowledge of NO kinetics in the tissue, a continuous constant NO production per unit tissue volume ($S_{tiss,alv}$) is likely. As discussed below, under such a condition, changes in the surface area but not in the thickness of the alveolar membrane during exhalation will result in a constant ratio of $J_{max,alv}/D_{NO,alv}$. Thus, such a scenario is consistent with a continuous recruitment/derecruitment of alveoli during inspiration/expiration to account for the increase/decrease of the surface area of the alveolar membrane without significant stretching.

In Case II, the implicit assumption is that the tissue volume participating in gas exchange is constant during exhalation. Thus, the surface area and thickness of the alveolar membrane are changing during expiration (or inspiration) in an inverse fashion to preserve a constant tissue volume. Under these circumstances, the total amount of NO produced, remains constant (assuming that $S_{tiss,alv}$ is constant). Thus, when C_{alv} is zero, equal and constant amounts of NO are lost to the blood and the gas phase on either side of the tissue, and thus, $J_{max,alv}$ remains constant and equal to half of the total NO production.

Figure 4 depicts the two cases schematically. In Fig. 4(A) (Case I) the surface area available for diffusion between the gas phase and the blood, A_{alv}, changes, but not $L_{tiss,alv}$. As a result, $\overline{C}_{tiss,alv}$ remains constant and $J_{max,alv}$ decreases during exhalation in proportion to $D_{NO,alv}$. In Fig. 4(B) (Case II) A_{alv} decreases and $L_{tiss,alv}$ increases to preserve a constant tissue volume. $\overline{C}_{tiss,alv}$ increases (as well as the concentration gradient at the interface) during exhalation, and $J_{max,alv}$ (the product of the gradient at the interface with A_{alv}) remains constant.

The plateau observed in Case I for C_{exh} (Figs. 2 and 3) more closely resembles the experimentally observed exhalation profiles for NO which have a slightly negative phase III slope, even after prolonged breathhold.[22] The plateau of both C_{exh} and C_{alv} can be attributed to the constant ratio of $J_{max,alv}$ and $D_{NO,alv}$. It has previously been shown that C_{alv} will approach a steady state value when $J_{t:g,alv}$ is zero.[8,21] When a steady state exists, it is evident from Eq. (5) that C_{alv} is equal to the ratio $J_{max,alv}/D_{NO,alv}$. Even though both vary with alveolar volume, Case I assumes that both have the same functional dependence, and that the ratio remains constant. During inspiration, C_{alv} is perturbed from its steady state

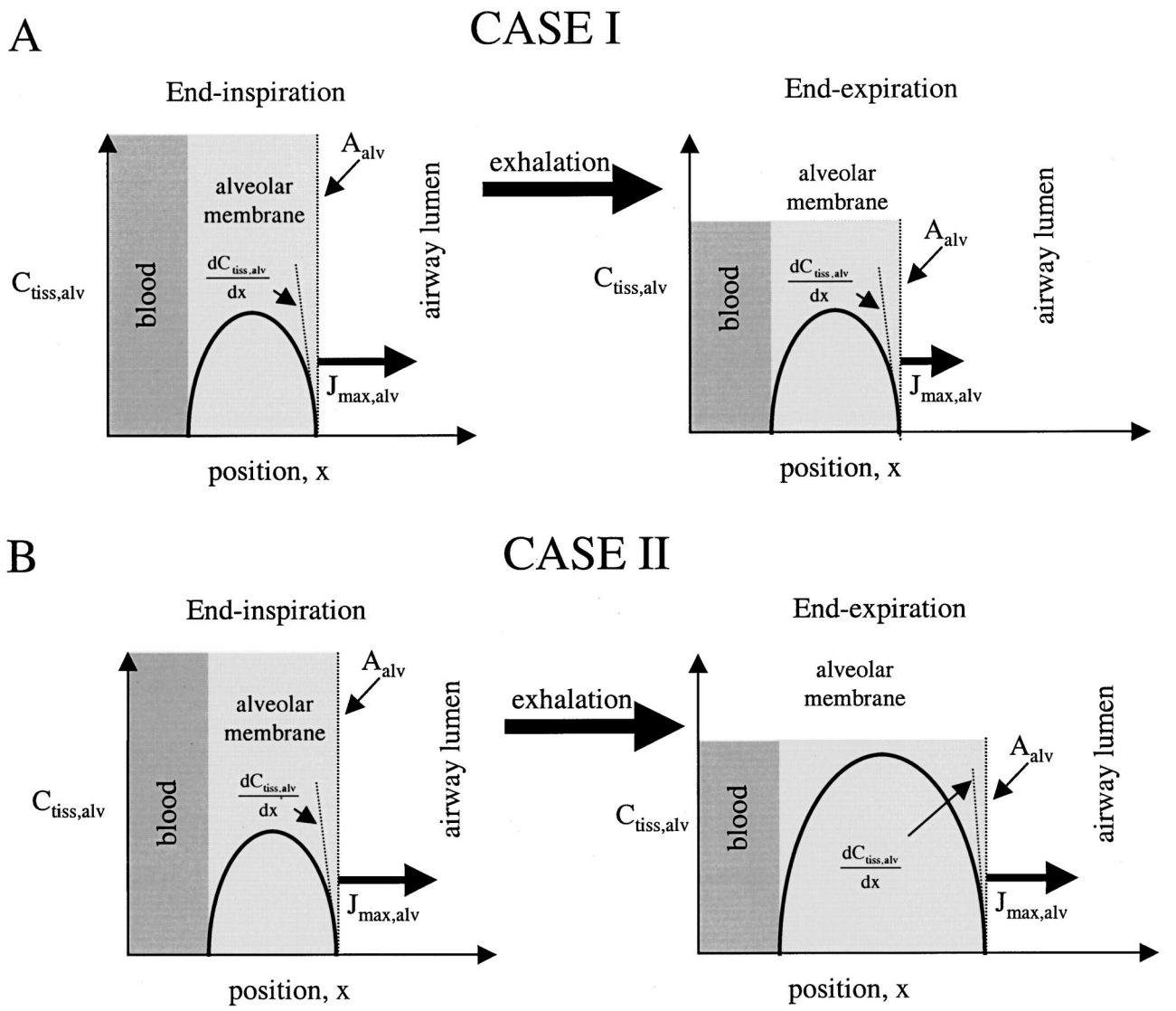

FIGURE 4. Schematic of the two possible cases to describe $J_{max,alv}$ from the tissue to the gas phase demonstrating the tissue concentration as a function of x, and the flux as proportional to product of the concentration gradient at the tissue–gas interface and the surface area. (A) In Case I, surface area, but not tissue thickness, changes during exhalation. $J_{max,alv}$ decreases during exhalation as the mean concentration in the tissue and concentration gradient at the interface are held constant. (B) In Case II, surface area decreases and tissue thickness increases to preserve a constant tissue volume. $J_{max,alv}$ is held constant as the increase in the mean concentration and concentration gradient offset the decrease in surface area.

value because the concentration of NO in the inspired air is assumed to be zero according to ATS guidelines. The inspired air absorbs NO from the airway compartment and enters the alveolar compartment with a concentration that is larger than zero, but smaller than $\overline{C}_{tiss,alv}$. These concepts can be easily generalized to a case where inspired concentration is greater than $\overline{C}_{tiss,alv}$ whereby the perturbation to C_{alv} is positive. A reduced contribution from the airway compartment at higher flow rates is due to a reduced residence time in the airway compartment and is consistent with previous theoretical and experimental observations.[14,21]

For Case II, the monotonically increasing values of C_{exh} are not consistent with experimental observations.[22] In addition, the increase (~6 ppb) in C_{exh} predicted over the course of phase III could easily be detected by current analytical techniques, such as chemiluminescence, whose resolution is ~1 ppb. C_{exh} increases during exhalation due to the fact that $D_{NO,alv}$ is decreasing during exhalation while $J_{max,alv}$ is held constant. Thus, the ratio $J_{max,alv}/D_{NO,alv}$ is changing with exhaled volume which causes $C_{tiss,alv}$ to increase [Eq. (7)]. Hence, C_{alv} never reaches a steady state value. Figure 5 depicts C_{alv} and the steady state value of C_{alv} at each exhaled volume,

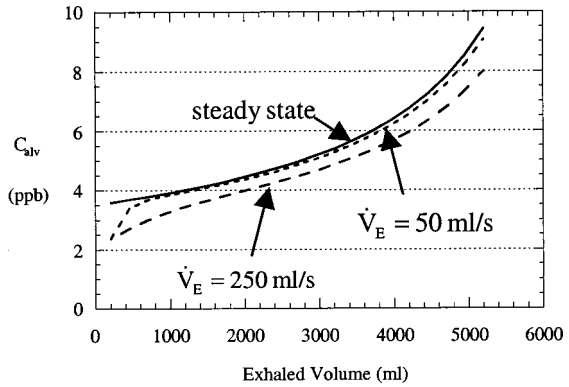

FIGURE 5. Model simulation of the alveolar concentrations at an exhalation flow rate of 50 and 250 ml/s compared to the steady state concentration in the alveoli at each exhaled (or alveolar) volume.

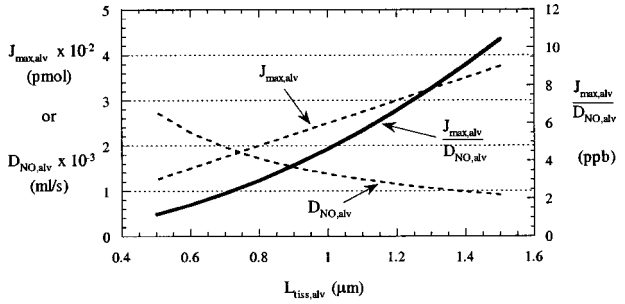

FIGURE 6. Predicted dependence of $J_{max,alv}$ and $D_{NO,alv}$ on alveolar membrane thickness ($L_{tiss,alv}$) based on Eqs. (10) and (11). The following representative values for the key parameters in these equations are as follows: $D_{NO,tiss}=3.3\times10^{-5}$ cm^2/s, $k=0.173$ s^{-1}, $A_{alv}=1\times10^5$ cm^2, and $S_{tiss,alv}=50$ pM/s. These values are described in detail in our previous description of the two-compartment model, or provide reasonable values for $J_{max,alv}$ and $D_{NO,alv}$ consistent with Table 1 and experimental values. The absolute values are not as critical as the dependence of $J_{max,alv}$ and $D_{NO,alv}$ on $L_{tiss,alv}$.

$C_{alv,ss}(V_{alv})$ (that is, if exhalation were stopped at each lung volume and C_{alv} allowed to reach a steady state). It can be seen that C_{alv} never reaches its steady state value, and that the difference between C_{alv} and $C_{alv,ss}(V_{alv})$ is exaggerated as exhalation flow rate increases.

The fact that Case I produces a plateau C_{exh}, which more closely resembles experimental observations, raises several interesting questions regarding alveolar exchange dynamics. Case I assumes that $\overline{C}_{tiss,alv}$ is constant. This possibility might suggest an equilibrium among competing sources and sinks for NO such that alveolar membrane ''seeks'' a constant concentration. For example, if a small net positive flux of NO from the alveolar membrane to the gas phase was induced (as might be the case when exhalation begins), this might shift the equilibrium to the right of the auto-oxidation of NO to maintain a constant NO concentration.[7] There are several other reactions of NO that involve substrates such as glutathione and other protein-thiols whose equilibrium might be altered in such a way to favor a constant tissue-phase concentration.[5,6] This possibility, of course, depends on the *rate* of reaction in these schemes, and needs further analysis beyond the scope of this manuscript.

In our previous detailed description of the two-compartment model,[21] we presented an analytical description of $J_{max,i}$ and $D_{NO,i}$ given by the following expressions:

$$J_{max,i}=A_i S_{tiss,i}\sqrt{\frac{D_{NO,tiss}}{k}}\times\{\coth(\xi)-[\coth(\xi)+1]\exp(-\xi)\}, \quad (10)$$

$$D_{NO,i}=A_i\lambda_{tiss,air}\sqrt{D_{NO,tiss}k}[\coth(\xi)], \quad (11)$$

where $\lambda_{tiss,air}$ is the tissue:air partition coefficient of NO, and $\xi=L_{tiss,i}/k\sqrt{D_{NO,tiss}/k}$. This description is not able to simulate the potential volume dependence of these parameters; however, these expressions do provide some insight into the potential relationship between $J_{max,alv}$ and $D_{NO,alv}$. For example, there is experimental evidence to support a thickening of the alveolar membrane (i.e., $L_{tiss,alv}$)[3,18] and/or a reduction in surface area (i.e., A_{alv}) during exhalation.[24] It is evident from Eqs. (10) and (11) that a change in A_{alv} would have the same impact on $J_{max,alv}$ and $D_{NO,alv}$; thus, their ratio would be constant during exhalation. However, if the tissue thickness changes during exhalation alone, the result is not so apparent. In this case we assume that surface area is constant, but thickness increases. This would obviously *increase* the tissue volume participating in gas exchange during exhalation, and represents an extreme case relative to Case I and Case II. Figure 6 plots $J_{max,alv}$ and $D_{NO,alv}$ (using representative values for the parameters, see figure legend) as a function of $L_{tiss,alv}$. Note that as $L_{tiss,alv}$ increases from 0.5 to 1.5 μm (as the trend would be during exhalation), $D_{NO,alv}$ decreases, but $J_{max,alv}$ *increases* because tissue volume increases while holding the NO production rate per unit volume constant. This causes the ratio $J_{max,alv}/D_{NO,alv}$ to be a strong positive function of $L_{tiss,alv}$. This result, perhaps, supports the concept that surface area changes during exhalation, and not membrane thickness. A changing surface area creates a constant ratio $J_{max,alv}/D_{NO,alv}$, and thus a constant C_{alv} and a constant C_{exh}, which is consistent with experimental observations.

Another possibility is that the production rate of NO from nitric oxide synthase (NOS) is altered due to the mechanical strain in the alveolar membrane during exhalation. There is some indirect evidence that stretch en-

hances NO production or release.[2] These studies, however, cannot distinguish between a change in NO production from NOS and a change in NO release due a shift in the chemical equilibrium. In any event, we cannot rule out the possibility that during exhalation, the production rate of NO from NOS is decreased. This effect would tend to maintain a constant tissue concentration.

An important point to consider is the functional dependence of $D_{NO,alv}$ on V_{alv}. The dependence used in these simulations was determined experimentally in normal subjects;[20,23] however, the range of V_{alv} for which the dependence was determined is approximately 2.5–5 liters; thus, use of this correlation at very small or large V_{alv} represents an extrapolation. In our simulations, this corresponds to exhaled volumes of 1.7–4.2 liters. It is evident from Figs. 2 and 3 that the impact of a variable $D_{NO,alv}$ has a progressively greater impact as exhalation proceeds and that a relatively steep slope occurs after exhaled volume of 4.2 liters. It is quite possible that $D_{NO,alv}$ reaches a lower limit for $V_{alv}<2.5$ liters. It is also important to note, however, that even if this were the case, the slope of the alveolar plateau would still be positive, albeit smaller, which is still not observed experimentally.

Another possibility to consider is the fact that the conducting airways are not truly rigid, but do expand and contract slightly during inspiration and expiration, respectively. Our model governing equations assume that the airways are rigid; however, it can easily be demonstrated that even if the airways were expansile (i.e., decreasing radius during expiration), the governing equations would not change (assuming a constant $D_{NO,air}$, mathematical proof not shown). The model equations describe the concentration of NO in the exhaled breath. Thus, if the airway diameter were to decrease during exhalation, the smaller volume would increase the concentration of NO; however, the smaller volume would also decrease the residence time of the gas in the airways and therefore decrease the exhaled concentration (i.e., decrease the uptake of NO from the airways).

The above argument assumes a constant $D_{NO,air}$ during exhalation, which is unlikely, as $D_{NO,air}$ depends on A_{air} which depends on the radius. If $D_{NO,air}$ were to decrease during exhalation due to either a decreasing A_{air} or an increasing $L_{tiss,air}$, the result would be a progressively decreasing exhaled concentration or a negative slope in phase III of the exhalation profile. However, we have previously reported a slightly, but statistically significant, negative phase III slope of the NO exhalation profile following a prolonged breathhold and a constant exhalation flow rate.[22] This is consistent with our argument that Case I is a more appropriate model for the alveolar region. That is, the effects of the alveolar region generate a flat phase III due to a constant ratio $J_{max,alv}/D_{NO,alv}$, and the negative slope observed for the phase III slope of NO is due to a decreasing $D_{NO,air}$.

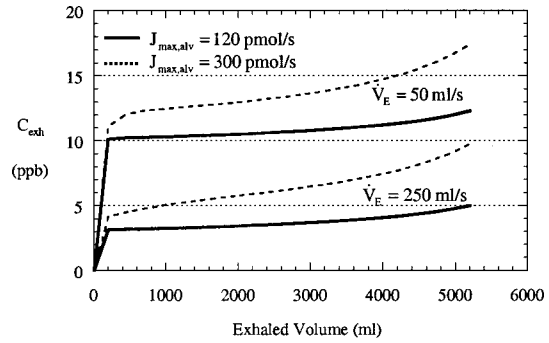

FIGURE 7. Model simulation of the exhaled NO concentration for Case II at an exhalation flow rate of 50 and 250 ml/s when $J_{max,alv}$ is reduced from 300 to 120 pmol/s. This reduction corresponds to reducing the steady state alveolar concentration from 5 to 2 ppb for Case I.

The simulations in this study were performed under expected values of the parameters for normal subjects. This provides a starting point to understand the basic physiological mechanisms underlying the NO exhalation profile. The choice for a steady state C_{alv} of 5 ppb is based on previously reported experimental values. However, other investigators have estimated values as small as 2 ppb.[12] Under these conditions, the relative contribution of the alveolar region to exhaled NO is smaller and thus the impact of a variable $D_{NO,alv}$ is less. This is demonstrated in Fig. 7 where we have lowered $J_{max,alv}$ from 300 to 120 pmol/s. This reduces $\overline{C}_{tiss,alv}$ to 2 ppb for Case I. We can then use this value of $J_{max,alv}$ to simulate Case II, and compare the result to that of Figs. 2 and 3. We can see that the positive slope is reduced, and it is very nearly flat for exhaled volume less than 4.2 liters. Thus, we cannot rule out the possibility that the slightly negative phase III slope for NO could be due to an alveolar region described by Case II combined with a significantly decreasing $D_{NO,air}$ during exhalation. Future work must focus on determining the functional relationship between V_{alv} and $D_{NO,air}$.

A final concept to consider is the impact of serial and parallel inhomogeneities on the shape of the exhalation profile. Parallel inhomogeneities result from parallel convective pathways in the lungs whose convective conductance is heterogeneous.[11] 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.[13,16] When convective flow is very slow (i.e., distal to the ~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 positively sloping alveolar plateau during a single breath washout experiment of an inert gas such as nitrogen or helium.[11] The exhalation profile of NO is similar to the single breath nitrogen washout in the sense that inspiration consists of air with zero concentration of the tracer gas (i.e., NO or N_2), then exhalation follows the evolution of the tracer gas from the alveolar region. Thus, the fact that a plateau or a slightly negative slope is observed experimentally for NO, would argue that an additional factor such as a variable $D_{NO,air}$ is acting to counteract inhomogeneities. Thus, inhomogeneities in gas mixing would only serve to create a more positive phase III slope. This fact points strongly to accepting Case I as the more realistic model of the alveolar region for NO, but does not provide any additional explanation for the mechanisms, which might create a constant $\overline{C}_{tiss,alv}$. In addition, we cannot rule out the possibility of spatial inhomogeneities in $J_{max,alv}$ which may serve to alter the slope of phase III of the exhalation profile.

In summary, we have presented two possible models for the alveolar region to explain the exchange dynamics of NO. These are based on the fact that we now know that $D_{NO,alv}$ depends strongly on V_{alv} which is contrary to assumptions made in previous models of NO exchange. Case I assumes that tissue-phase NO concentration in the alveolar region is constant during exhalation. This assumption causes $J_{max,alv}$ to depend on V_{alv}. In contrast, Case II assumes that $J_{max,alv}$ is constant. We have demonstrated that under normal conditions, Case I is able to reproduce a plateau in exhaled NO concentration which is more consistent with experimental observations. Thus, we conclude that $J_{max,alv}$ is also volume dependent such that the ratio of $J_{max,alv}/D_{NO,alv}$ remains nearly constant. The precise mechanism for maintaining a constant ratio is not known, but is likely due to a decreasing alveolar surface area during exhalation.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (BES-9619340) and the National Institutes of Health (R29 HL60636).

APPENDIX

Solution of Eq. (2) (mass balance in the alveolar region) during inspiration:

Case I:

$$C_{alv}(t)=\left(C_{alv}(0)+\frac{\alpha\overline{C}_{tiss,alv}}{V_{alv}(0)}\ \frac{e^{-\psi V_{alv}(0)^{\beta}}}{\dot{V}_I} \times \int_{V_{alv}(0)}^{V_{alv}(t)} V^{\beta}e^{\psi V^{\beta}}dV+\frac{\dot{V}_I C_{air}(V_{air})}{V_{alv}(0)}\ \frac{e^{-\psi V_{alv}(0)^{\beta}}}{\dot{V}_I} \times \int_{V_{alv}(0)}^{V_{alv}(t)} e^{\psi V^{\beta}}dV\right)\frac{V_{alv}(0)}{V_{alv}(t)} \times e^{(\psi\{[V_{alv}(0)]^{\beta}-[V_{alv}(t)]^{\beta}\})}.$$

Case II:

$$C_{alv}(t)=\left(C_{alv}(0)+\frac{J_{max,alv}+\dot{V}_I C_{air}(V_{air})}{V_{alv}(0)}\ \frac{e^{-\psi V_{alv}(0)^{\beta}}}{\dot{V}_I} \times \int_{V_{alv}(0)}^{V_{alv}(t)} e^{\psi V^{\beta}}dV\right)\frac{V_{alv}(0)}{V_{alv}(t)} \times e^{(\psi\{[V_{alv}(0)]^{\beta}-[V_{alv}(t)]^{\beta}\})},$$

$$\psi=\frac{\alpha}{\beta\dot{V}_I},$$

$$\int_{V_{alv}(0)}^{V_{alv}(t)} V^{\beta}e^{\psi V^{\beta}}dV=\left(\frac{\psi V^{\beta}-1}{\psi^2}e^{\psi V^{\beta}}\right)_{V_{alv}(0)}^{V_{alv}(t)},$$

$$\int_{V_{alv}(0)}^{V_{alv}(t)} e^{\psi V^{\beta}}dV=\sum_{i=0}^{\infty}\frac{\psi^i[V_{alv}(t)^{\beta i+1}-V_{alv}(0)^{\beta i+1}]}{i!(\beta i+1)}.$$

Exponential integral:

$$E_1(z)=\int_z^{\infty}\frac{e^{-t}}{t}dt=-\gamma-\ln(z)-\sum_{n=1}^{\infty}\frac{(-1)^n z^n}{nn!}.$$

REFERENCES

[1] Alving, K., E. Weitzberg, and J. M. Lundberg. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur. Respir. J.* 6:1368–1370, 1993.

[2] Bannenberg, G. L., and L. E. Gustafsson. Stretch-induced stimulation of lower airway nitric oxide formation in the guinea-pig: Inhibition by gadolinium chloride. *Pharm. Toxicol.* 81:13–18, 1997.

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

[4] DuBois, A. B., P. M. Kelley, J. S. Douglas, and V. Mohsenin. Nitric oxide production and absorption in trachea, bronchi, bronchioles, and respiratory bronchioles of humans. *J. Appl. Physiol.* 86:159–167, 1999.

[5] Fukuto, J. M. Chemistry of nitric oxide: Biologically relevant aspects. In: Nitric Oxide: Biochemistry, Molecular Biology, and Therapeutic Implications, edited by Ignarro and Murad. San Diego: Academic, 1995, pp. 1–15.
[6] Gaston, B., J. M. Drazen, J. Loscalzo, and J. S. Stamler. The biology of nitrogen oxides in the airways. *Am. J. Respir. Crit. Care Med.* 149:538–551, 1994.
[7] Hung, J. F., K. Fang, R. Malik, A. Snyder, N. Malhotra, T. A. Platts-Mills, and B. Gaston. Endogenous airway acidification. Implications for asthma pathophysiology. *Am. J. Respir. Crit. Care Med.* 161:694–699, 2000.
[8] Hyde, R. W., E. J. Geigel, A. J. Olszowka, J. A. Krasney, R. E. Forster, M. J. Utell, and M. W. Frampton. Determination of production of nitric oxide by lower airways: Theory. *J. Appl. Physiol.* 82:1290–1296, 1997.
[9] Kharitonov, S., K. Alving, and P. J. Barnes. Exhaled and nasal nitric oxide measurements: Recommendations. The European Respiratory Society Task Force. *Eur. Respir. J.* 10:1683–1693, 1997.
[10] Moilanen, E., and H. Vapaatalo. Nitric oxide in inflammation and immune response. *Ann. Med.* 27:359–367, 1995.
[11] Paiva, M., and L. A. Engel. The anatomical basis for the sloping N_2 plateau. *Respir. Physiol.* 44:325–337, 1981.
[12] Pietropaoli, A. P., I. B. Perillo, A. Torres, P. T. Perkins, L. M. Frasier, M. J. Utell, M. W. Frampton, and R. W. Hyde. Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. *J. Appl. Physiol.* 87:1532–1542, 1999.
[13] Scheid, P., M. P. Hlastala, and J. Piiper. Inert gas elimination from lungs with stratified inhomogeneity: Theory. *Respir. Physiol.* 44:299–309, 1981.
[14] Silkoff, P. E., P. A. McClean, A. S. Slutsky, H. G. Furlott, E. Hoffstein, S. Wakita, K. R. Chapman, J. P. Szalai, and N. Zamel. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am. J. Respir. Crit. Care Med.* 155:260–267, 1997.
[15] Silkoff, P. E., J. T. Sylvester, N. Zamel, and S. Permutt. Airway nitric oxide diffusion in asthma. Role in pulmonary function and bronchial responsiveness. *Am. J. Respir. Crit. Care Med.* 161:1218–1228, 2000.
[16] Six, D. P., W. R. de Vries, and S. C. Luijendijk. Sloping alveolar plateaus of He and SF6 measured in excised cat lungs ventilated at constant volume by pressure changes. *Respir. Physiol.* 83:277–293, 1991.
[17] Slutsky, A. S., and J. M. Drazen. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children 1999. *Am. J. Respir. Crit. Care Med.* 160:2104–2117, 1999.
[18] Staub, N. C., Respiration. *Annu. Rev. Physiol.* 31:173–202, 1969.
[19] Stewart, T. E., F. Valenza, S. P. Reveiro, A. D. Wener, G. Volgyesi, J. Brendan, M. Mullen, and A. S. Slutksy. Increased nitric oxide in exhaled gas as an early marker of lung inflammation in a model of sepsis. *Am. J. Respir. Crit. Care Med.* 151:713–718, 1995.
[20] Tsoukias, N. M., D. Dabdub, A. F. Wilson, and S. C. George. Effect of alveolar volume and sequential filling on the diffusing capacity of the lungs. I. Theory. *Respir. Physiol.* 120:231–250, 2000.
[21] Tsoukias, N. M., and S. C. George. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J. Appl. Physiol.* 85:653–666, 1998.
[22] Tsoukias, N. M., Z. Tannous, A. F. Wilson, and S. C. George. Single-exhalation profiles of NO and CO_2 in humans: Effect of dynamically changing flow rate. *J. Appl. Physiol.* 85:642–652, 1998.
[23] Tsoukias, N. M., A. F. Wilson, and S. C. George. Effect of alveolar volume and sequential filling on the diffusing capacity of the lungs. II. Experiment. *Respir. Physiol.* 120:251–271, 2000.
[24] Weibel, E. R., P. Untersee, J. Gil, and M. Zulauf. Morphometric estimation of pulmonary diffusion capacity. VI. Effect of varying positive pressure inflation of air spaces. *Respir. Physiol.* 18:285–308, 1973.