Distribution of Pulmonary Capillary Transit Times

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The length of time that blood remains in the pulmonary capillary is an important variable in gas exchange. We have investigated the distribution of capillary transit times in isolated rabbit lungs perfused with a bicarbonate-free buffer. The time course of gas exchange was monitored by enclosing the lungs in a plethysmograph. A bolus of buffer containing dissolved acetylene was injected into the perfusion system. Exchange of this inert gas occurred as soon as the bolus reached the capillary bed, thereby describing the input function into the bed. A separate bolus injection of bicarbonate solution resulted in production and excretion of CO_2 as long as the bolus remained in the capillary bed. The rate of CO_2 production was adjusted by partial inhibition of endothelial carbonic anhydrase. The distribution of capillary transit times was computed from a model of CO_2 production in the capillary bed and the observed rates and volumes of acetylene and carbon dioxide excretion. The recovered distributions indicate that there is a fairly wide distribution of capillary transit times (relative dispersion, 0.45) around the mean value of 1.71 s (± 0.53 [SD]). Only 10% of capillary transit times are less than one half of mean transit time. It is likely that gas exchange reaches equilibrium in the capillary bed except possibly during strenuous exercise or exposure to high altitude or in disease. **Klocke RA, Schünemann HJ, and Grant BJB. Distribution of pulmonary capillary transit times.**

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During heavy exercise in humans, blood may leave the pulmonary capillary bed without attaining equilibrium with alveolar oxygen (1, 2). However, estimates of mean capillary transit time during exercise are in the range of 0.4 to 0.5 s (3, 4), a period sufficient to permit oxygen exchange to reach completion (5). These apparently conflicting observations could be reconciled if there were sufficient dispersion of capillary transit times around the mean value so that the time for equilibration was less than the mean transit time in portions of the vascular bed.

Measurements of pulmonary capillary transit times have been made in subpleural capillaries in the intact dog (6, 7) and rabbit (8). These estimates indicate that subpleural capillary transit time can vary substantially under different physiologic conditions. Using a combination of physiologic and morphologic techniques, Hogg and associates (9) measured the distribution of capillary transit times, i.e., the capillary transport function, in intact dogs studied at rest under anesthesia. Even in this circumstance of minimal stress, transit times as short as 0.18 s were present. Recently Audi and associates (10) described the capillary transport function in the isolated, perfused lobe of the dog using multiple injections of diazepam. By varying the albumin concentration in the perfusion buffer, they were able to alter the distribution of indicator between the extravascular and vascular compartments and the transport function was extracted from the resulting indicator-dilution curves.

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These previous estimates of capillary transit times have been achieved using markers that are confined to the vasculature (6–9) or enter lung water (10). These indicators may not provide representative data for the time available for respiratory gas exchange, especially if gas exchange occurs in vessels larger than capillaries (11). We have used the respiratory gas CO_2 as an indicator to determine the capillary transport function in isolated rabbit lungs perfused with a nonbicarbonate buffer. A bolus of bicarbonate and CO_2 in equilibrium is injected into the circulation, and the dissolved CO_2 is excreted when the bolus reaches the capillary bed. The loss of CO_2 disturbs chemical equilibrium, leading to production and further excretion of CO_2 during capillary transit. The rate and volume of CO_2 production is modulated by graded inhibition of endothelial carbonic anhydrase with acetazolamide. We modeled CO_2 excretion from the initial conditions and a distribution of capillary transit times described by a lagged normal density function. Parameters of the function were optimized to fit calculated CO_2 excretion to the experimental observations. The recovered lagged normal density function described the distribution of capillary transit times.

METHODS

The time course of gas exchange was determined with a plethysmographic technique (12). New Zealand White rabbits (0.83 ± 0.13 kg [SD]) were anesthetized with intravenous pentobarbital (65 mg/kg) and heparinized (1,000 U). After tracheostomy, the animals were ventilated (tidal volume, 7 ml; frequency, 12 breaths/min) with room air. The chest was opened, the pulmonary artery and left atrium were cannulated, and the lungs were flushed clear of blood. The perfusion buffer consisted of 131.5 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, 1.08 mM $MgCl_2$, 10.0 mM Tris buffer, and 40 gm/L bovine serum albumin with pH adjusted to 7.4. The buffer contained either 0.01 or 0.001 mM sodium acetazolamide. The lungs were removed from the animal, placed in the plethysmograph, and ventilated with room air until chamber temperature stabilized at 37° C. Ventilation was stopped, the plethysmograph was closed

to the atmosphere, the lungs were distended with 8 cm H_2O positive pressure, and perfusate flow was gradually increased to 100 ml/min/kg body weight. After vascular and plethysmographic pressures stabilized (1 to 2 min), two serial bolus injections were made. The two injections served as a reference (perfusate equilibrated with 80% acetylene-20% oxygen) or test (oxygenated buffer containing bicarbonate). In the latter buffer, NaCl was replaced in equal molarity with 30 or 60 mM $NaHCO_3$ and pH adjusted to ~7.4. Both bolus injections contained acetazolamide in the same concentration as the perfusate. The two bolus injections, 0.883 ml in volume, were made ~40 s apart in varying order in the experiments. After the reference injection, acetylene (C_2H_2) was released into the alveolar space essentially instantaneously as the bolus entered the capillary bed (5). After the test injection, dissolved CO_2 was excreted immediately (13) and subsequent CO_2 production and excretion proceeded at a slower rate. The quantity and timing of gas excretion after the injections were determined from the plethysmographic pressure recording as described previously (12).

Lung volumes were measured by helium dilution and were 40.9 ± 3.6 ml BTPS. Lung water was determined as the difference in weight before and after drying the lungs under vacuum in a desiccator. Mean wet/dry ratio was 5.72 ± 0.63, similar to that in other studies (13, 14). These ratios could not be corrected for fluid present in the pulmonary vasculature because no vascular indicator was present. To avoid interference of edema with gas exchange, experiments were discarded before data analysis if the wet/dry ratio was > 6.5. Mean left atrial pressure was −1.1 ± 1.6 cm H_2O, and alveolar pressure was held at 8 cm H_2O. Therefore, the lungs were in Zone 2 conditions.

Vascular pressures were recorded with Model P23Db transducers (Gould Inc., Valleyview, OH). Plethysmographic pressure was monitored with a differential pressure transducer (Model MP-45-871; Validyne Engineering, Northridge, CA) with the most sensitive diaphragm available (± 2 cm H_2O). Transducer outputs were amplified and recorded with analog-to-digital circuitry (Model DT2801; Data Translation, Marlboro, MA). The plethysmographic signal was processed off-line to convert the pressure signal to volume (12). Data were sampled and processed with a 0.005-s sampling interval. To reduce computational time when the theoretical model was fitted to the experimental data, consecutive pairs of data points were replaced by their average and analysis was performed using a 0.01-s interval.

Data are expressed as the mean ± SD. Statistical comparisons were made with ANOVA using SPSS. Post hoc testing for significant differences was performed with Tukey's protected *t* test using the harmonic mean of the sample size. Parameter values of the lagged normal density function and the acceleration factor for CO_2-bicarbonate reactions were estimated by the Levenburg-Marquardt method (15). This method requires a vector of the first derivatives of the fitted function. The required derivatives were calculated numerically during the search procedure from the change of the residual sum of squares due to 10% changes from the prevailing parameter value with a second degree Lagrangian polynomial.

THEORY

The transport function of a vascular bed can be assessed by indicator-dilution experiments if the profile of the indicator concentration with time can be monitored directly at the entrance and exit of the bed. Neither measurement is feasible in the case of a capillary bed. The input function to the capillary bed can be inferred from the excretion pattern of an inert gas since these gases are excreted almost instantaneously in the capillary (5). The output function cannot be measured directly or indirectly. However, if a reaction proceeds at a constant rate during capillary transit and the product of that reaction is excreted, the quantity of excreted material is a function of the time spent in the capillary bed. CO_2 is an excellent choice for such an indicator. CO_2 and bicarbonate in equilibrium are injected into the pulmonary circulation. When the injectate reaches the capillary bed, dissolved CO_2 is excreted instantaneously (13), leading to further production and excretion of CO_2 until the injectate leaves the capillary bed. Bicarbonate and buffers in the injectate provide a large reservoir to maintain the reaction in a quasi-linear state. Furthermore, the rate of the reaction itself can be modulated with graded inhibition of the enzyme carbonic anhydrase, which is endogenous to the pulmonary vasculature (13).

The distribution of vascular transit times has been described successfully with a lagged normal density function (16). This mathematical form was utilized to describe the pulmonary capillary transport function (h_i) at any time interval ($i\Delta t$)

$$h_i = \sum_{k=1}^{k=i} \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{k\Delta t - t_c}{\sigma}\right)^2\right] \cdot \frac{1}{\tau} \exp\left[\frac{-(i-k+1)\Delta t}{\tau}\right] \quad (1)$$

where t_c and σ are the central time and standard deviation of a normal density function. τ is the time constant of the superimposed exponential and Δt is the interval of numerical integration. The area under the transport curve is normalized to a value of unity. Likewise, the transport function of the vascular system from the point of injection to the entrance to the capillary bed can be represented by another lagged normal density curve, g_i. The appearance of the injected bolus at the entrance to the capillary, defined as the portion of the vasculature that exchanges gas, is heralded by the excretion of acetylene. Excretion over time is cumulative, so total C_2H_2 exchange, G_i, is

$$G_i = \sum_{i=1} g_i \quad (2)$$

at any time interval ($i\Delta t$). Constants of the lagged normal density function (g_i) were optimized to fit G_i to observed C_2H_2 excretion over time. Knowing the initial conditions in the bolus, the concentrations of reactants entering the capillary bed at any point in time can be calculated from g_i.

When an element of an injected bolus enters the capillary bed, the entire element participates in gas exchange. However, the fraction of that element which can exchange CO_2 decreases as portions of the element traverse shorter capillary pathways and leave the capillary bed. The fraction of the element which has left the capillary bed at any instant is provided by the integral of the area under the capillary transport function to that point in time. The fraction remaining in the bed is described by the residue function (R) and is equal to unity minus the integral of the capillary transport function (17). Thus, the residue function varies from unity to zero with time as the area under the transport function inversely increases from zero to unity. The fraction (R) of element j remaining in the bed at interval i is

$$R_{ij} = 1 - \sum_{k=1} h_k \quad (3)$$

In this analysis, we will assume random coupling of g_i and h_i, i.e., all elements of the injected bolus are randomly distributed throughout the capillary bed. We can then calculate v_{ij}, the quantity of CO_2 excreted in each time interval by the fractions of the elements of the bolus remaining in the capillary bed

$$= R_{ij} V_B \alpha S (P_i \quad P_{\prime} \quad (4)$$

where R_{ij} is the residue function for element j in time interval i, V_B is the volume of each element, α is the solubility of CO_2 in the perfusate (mM/mm Hg), S is the fractional equilibration of CO_2 partial pressures across the alveolar-capillary membrane in the computational time increment, P_{ij} is the P_{CO_2} in element j at time i and P_{Ai} is alveolar P_{CO_2} at time interval i. S was calculated assuming that equilibration of CO_2 occurs as a first-order process with a half-time of 0.003 s (18). With excretion of CO_2, the concentration of CO_2 in each element falls by the amount v_{ij} and CO_2 concentration is altered to reflect this decrease. The cumulative volume of CO_2 excreted to time interval i is

$$V_i = v_m \sum_{i=1}^{i} \sum_{j=1}^{j=i} v_{ij} \quad (5)$$

where v_m is the molar volume of CO_2.

With loss of CO_2 from each element during the time interval, chemical equilibrium is disturbed and CO_2 is produced by dehydration of carbonic acid. The concentration of CO_2 increases by the amount

$$\Delta[CO_2]_{ij} = (1+a)\left(\frac{k_{H_2} \quad \left[HCO_3^-\right] \quad [H^+]_{ij}}{K_1} - k_{co_2}[CO_2]_{ij}\right) \quad (6)$$

where a is the acceleration of CO_2-bicarbonate reactions by endothelial carbonic anhydrase, K_1 is the first acidic dissociation constant of carbonic acid, and $k_{H_2CO_3}$ and k_{CO_2} are the kinetic constants of dehydra-

tion and hydration, respectively (19). Concentrations of HCO_3^- and buffer components are altered to reflect the production of CO_2. The resulting change in pH is

$$\Delta pH_i \quad \Delta[CO_2]_{ij}/\beta_i \tag{7}$$

where β is calculated from the slope of the Tris buffer curve at the concentrations present in the element and the concentration and buffering power of albumin (0.12 mM H^+/g albumin/pH [20]). Alveolar P_{CO_2} at the end of the time interval i is

$$P_{A_i} = \frac{(P_B - 47)\,V_i}{0.826\,(V_L + V'_w)} \tag{8}$$

where 0.826 converts volume from STPD to BTPS, V_L is lung volume (BTPS), and V'_w is the equivalent gas volume of lung water, calculated from measured lung water, the partition coefficient of CO_2 (0.55), and lung tissue pH (6.8), assuming equilibrium between bicarbonate and CO_2. Calculation of alveolar P_{CO_2} and the volume of CO_2 entering the alveoli included the effect of solution of CO_2 in lung tissue because this small fraction was not measured with the plethysmographic technique.

Using the initial conditions in the bolus as it entered the capillary bed (described by Equation 2), the excretion of CO_2 with time was calculated iteratively from Equations 3 through 8. Values were assumed for a, the acceleration factor for catalysis by endothelial carbonic anhydrase, and the three constants required to define h_i. These four constants were varied to optimize the fit of V_i to the experimental data of CO_2 excretion.

RESULTS

The exchange of C_2H_2 and CO_2 in an experiment with a total CO_2 content of 60 mM in the test bolus and 0.01 mM acetazolamide in all solutions is shown in Figure 1. Both experimental curves of C_2H_2 and CO_2 excretion are plotted with the scales normalized for each curve to the total quantity of the gas excreted. The difference in mean transit times between the two excretion curves is 0.32 s. The integrated lagged normal density function fitted to C_2H_2 excretion is also plotted in the figure but cannot be discerned from the experimental C_2H_2 curve. The time course of CO_2 excretion was calculated from the acetylene data and the model of CO_2 exchange described above, varying the assumed distribution of capillary transit times and acceleration factor to achieve the best fit to the experimental data for CO_2 excretion.

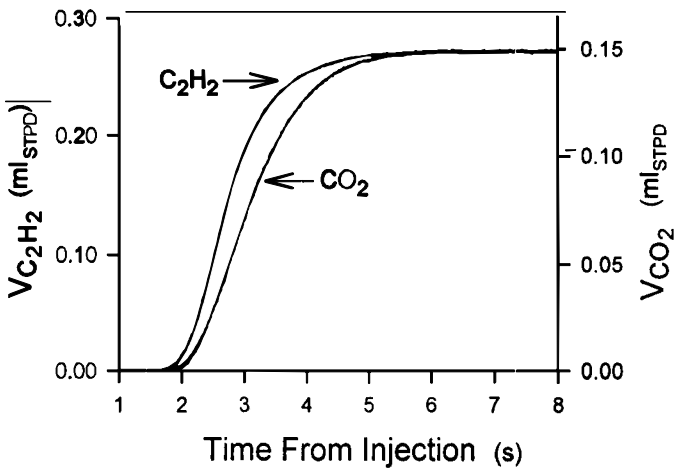

Figure 1. Exchange of CO_2 and acetylene as a function of time after injection of each bolus. Scales on the ordinates are adjusted so that total CO_2 (0.271 ml) and C_2H_2 (0.149 ml) output are equal. Both experimental exchange and computed exchange for each gas are plotted in the figure, but the differences are barely discernible. The coefficients of variation between the experimental and calculated curves for C_2H_2 and CO_2 are 0.003 and 0.005, respectively.

Both the experimental and computed CO_2 excretions are plotted in Figure 1. The coefficients of variation for the differences between the experimental and computed curves for C_2H_2 and CO_2 for all 23 experiments were 0.005 ± 0.002 and 0.010 ± 0.006, respectively.

The summary of data from the four series of experiments is included in Table 1. As expected, CO_2 excretion differed significantly between all series except A and D ($p < 0.01$). Mean acetylene excretion was 0.284 ± 0.021 ml STPD for all experiments and did not differ among the different series. The calculated acceleration factor was significantly greater in Group A than in all other groups ($p < 0.05$).

The distribution of capillary transit times and the corresponding residue function calculated from the excretion curves in Figure 1 are plotted in Figure 2. The mean transit time of the transport function (first moment) is 1.44 s with a variance (second moment) of 0.31 s^2. The transport functions obtained from the 12 experiments conducted in the presence of 0.01 mM acetazolamide, normalized to the mean transit time of each function, are plotted in Figure 3. The shape of the functions are quite similar; all experiments were conducted under Zone 2 conditions of perfusion. The mean transit time for all 23 experiments was 1.71 ± 0.53 s. The variance was 0.63 ± 0.49 s^2. The curves were skewed moderately in time. The skewness coefficient, a dimensionless constant calculated from the second and third moments, was 1.37 ± 0.45 (21). As shown in Figure 3, the curves were sharply peaked. The dimensionless kurtosis coefficient, calculated from the second and fourth moments, was 5.96 ± 1.28 (21). There were no significant differences among the moments or skewness and kurtosis coefficients from the four series of experiments. The relative dispersion (standard deviation of the transport function divided by the mean transit time) averaged 0.45 ± 0.17 for all data sets.

The residue function from each experiment was normalized by the mean transit time of the experiment. The mean normalized residue function (± 1 SD) from all experiments is plotted in Figure 4. This curve indicates that only 10% of capillary pathways have transit times shorter than one half of the mean transit time. However, the curve drops off sharply thereafter, and at the mean transit time only 40% of the injected bolus remains in the capillary bed. By 1.5 mean transit times, only 10% of the capillary pathways are still involved in gas exchange.

DISCUSSION

These experiments provide an estimate of the capillary transport function which is based on excretion of a respiratory gas and therefore should be directly applicable to gas exchange. The recovered distributions were obtained from the entire capillary bed rather than a portion of bed. Additionally, the measurements involve completely different technology and assumptions than those used previously. We will address the assumptions required to measure the capillary transport function, followed by a comparison of these findings to other reported data. The factors determining the distribution of transit times in the capillary bed then will be discussed. Finally, the dispersion of transit times in the entire pulmonary circulation will be addressed.

In the present experiments, the capillary is defined functionally by the ability to exchange gas. Oxygen in high concentrations or a rapidly diffusible gas such as hydrogen can be detected in pulmonary arteries shortly after inhalation (11). The quantity of gas that could exchange before reaching the anatomically defined capillary is uncertain. The endothelial area of the arterial tree in the cat is estimated to be 14 to 25% of the capillary surface area (22), raising the possibility of exchange in small arterial vessels. The ratio of endothelial surface area to arterial wall

TABLE 1

EXPERIMENTAL CONDITIONS IN TEST BOLUS AND CO_2 EXCRETION

Series	n	pH	P_{CO_2} (mm Hg)	HCO_3^- (mM)	Actz* (mM)	V_{CO_2}† (ml)	a‡
A	5	7.442 ± 0.014	41.6 ± 1.4	28.4 ± 0.4	0.001	0.094 ± 0.012	43.9 ± 15.7
B	6	7.435 ± 0.001	42.4 ± 0.2	28.4 ± 0.2	0.01	0.068 ± 0.014	11.9 ± 3.2
C	6	7.378 ± 0.036	96.6 ± 7.6	56.7 ± 0.6	0.001	0.160 ± 0.014	25.2 ± 11.3
D	6	7.450 ± 0.020	82.5 ± 3.9	57.2 ± 0.7	0.01	0.106 ± 0.013	10.1 ± 2.9

* Acetazolamide concentration.
† Volume of CO_2 (STPD) excreted.
‡ Acceleration factor calculated for CO_2-bicarbonate reactions.

thickness which governs diffusion out of vessels increases dramatically in the distal arterial bed. However, this ratio should be much less than that in the capillaries because arterioles supplying the capillary bed are relatively large (15 to 20 μm in diameter) and do not have intermediate size vessels prior to supplying the capillary bed (6). In any event, the physiologic variable of interest is the time available for gas exchange, regardless of the anatomical site. Although it is theoretically possible that C_2H_2 diffuses out of arteries more rapidly than CO_2 because its slightly smaller size and greater solubility, we have demonstrated previously that the two gases are exchanged with identical kinetics in the pulmonary vascular bed (13).

Determination of the capillary transfer function with indicator-dilution methodology requires several assumptions. First, the permeability of the endothelium to the indicator must either be infinite or uniformly distributed throughout the bed. If not uniformly distributed, areas with decreased permeability will lead to underestimates of transit times in those portions of the bed. Second, the indicator should leave the vasculature and enter a large, well-mixed sink. These two caveats present no difficulty to CO_2 since the gas is exchanged essentially instantaneously (13) and the alveolar space is orders of magnitude larger than the capillary bed. Also, large diffusion gradients are unlikely in the gas phase as opposed to a liquid phase. These requirements may be more problematic for a less diffusible indicator that enters lung water rather than the alveoli (10). The small quantities of CO_2 and C_2H_2 entering lung water in the present experiments were included in the model of exchange by Equation 8 and an analogous equation for C_2H_2. Both gases have similar diffusibility, and the kinetics of their movement into lung water was assumed to be identical. Finally, if the indicator is produced in the capillary, the rate of production must be relatively constant. In our experiments, this required the assumption of uniform distribution of carbonic anhydrase throughout the capillary bed. In addition, the reaction cannot approach equilibrium during capillary transit. Otherwise, the transit time in a lengthy pathway will be underestimated as the rate of production of the indicator decreases with time. We circumvented this latter problem with several approaches. The perfusate had sufficient buffer to prevent large changes in pH. Because small quantities of CO_2 were excreted, alveolar P_{CO_2} remained < 4 mm Hg; minimal back pressure of CO_2 in the alveoli was present to impede diffusion of CO_2 out of the capillary. Most importantly, catalysis of bicarbonate dehydration was partially inhibited to prevent the reaction from reaching completion. Previously, we have measured CO_2 excretion under similar circumstances in the presence of complete carbonic anhydrase inhibition or in the absence of any enzyme inhibition (13). Comparison of these data to the present measurements of CO_2 excretion (Figure 5) indicate that bicarbonate dehydration to CO_2 reached only 36 to 66% of its equilibrium values in these experiments. The validity of the assumption of constant CO_2 production is underscored by the lack of significant differences between the moments of the transport function when determined at substantially different rates of CO_2 production due to variations in bicarbonate and acetazolamide concentrations.

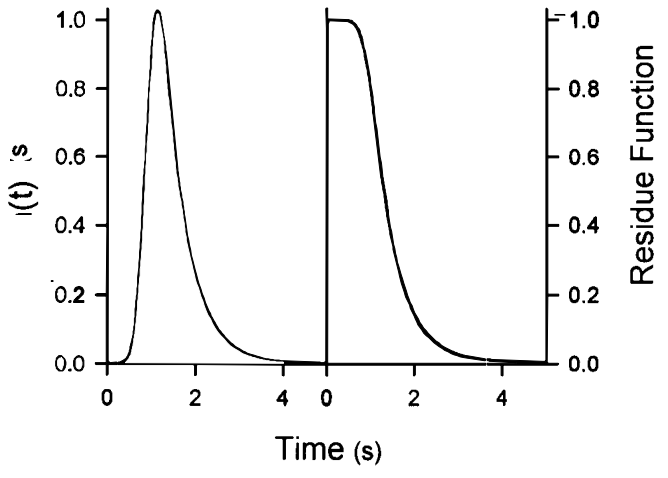

Figure 2. Left panel: Capillary transport function showing distribution of capillary transit times recovered from data presented in Figure 1. *Right panel*: Residue function calculated from the transport function in the adjacent panel illustrating the fraction of the bolus remaining in the capillary bed as a function of time.

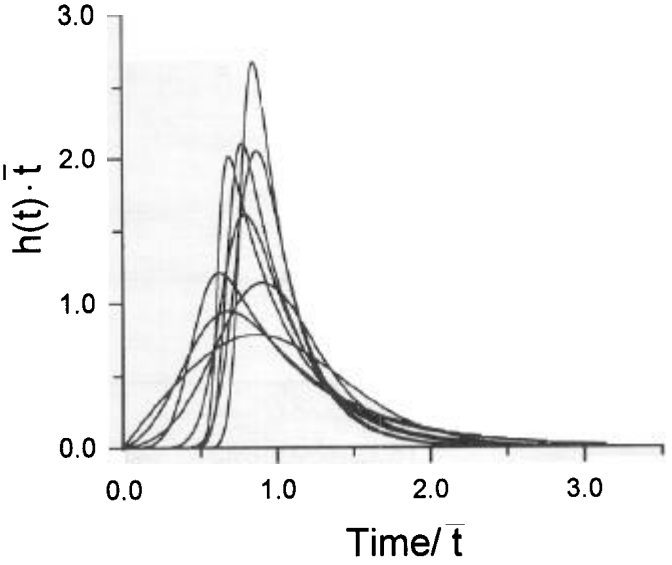

Figure 3. Capillary transport functions recovered in the 12 experiments with 0.01 mM acetazolamide (Groups B and D in Table 1). The transport functions have been normalized by the mean transit time ($\bar{t}$).

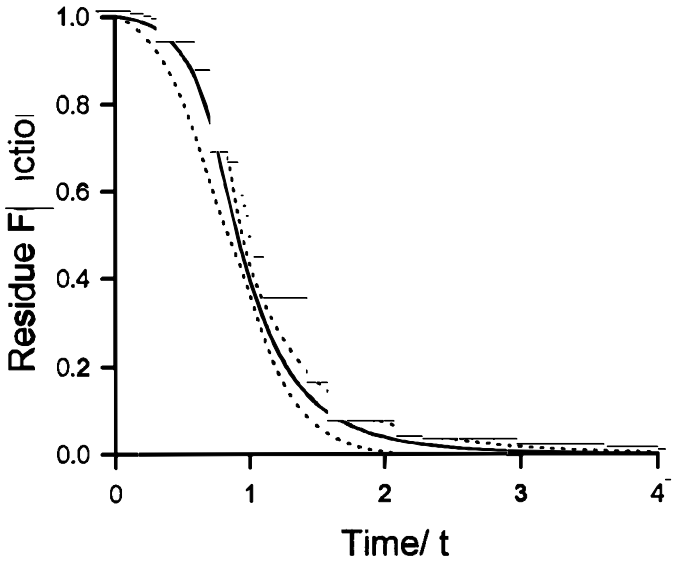

Figure 4. Mean value of the residue functions (*solid line*) recovered from all 23 experiments. Time has been normalized to the mean transit time ($\bar{t}$). The dashed lines indicate the variability (± 1 SD) around the mean of the functions.

This method of estimating the transport function also assumes that each element of the capillary bed receives the same input profile of reactants. Despite the ability to provide a highly reproducible injection, there is some dispersion of the bolus in the perfusion system (12) as well as in the arterial portion of the bed (23). This results in variation of reactant concentrations with time when the bolus enters the capillary bed. Calculation of the initial conditions in each capillary element over time from the C_2H_2 data implicitly assumes that dispersion and delay of the bolus in the arterial tree is independent of the transit time through individual capillaries (16). In their recent estimates of the pulmonary capillary transport function, Audi and associates (10) provided evidence that input to the capillary bed is indeed random and there is no coupling of arterial pathways to elements of the capillary bed which have a distribution of transit time different than the overall bed.

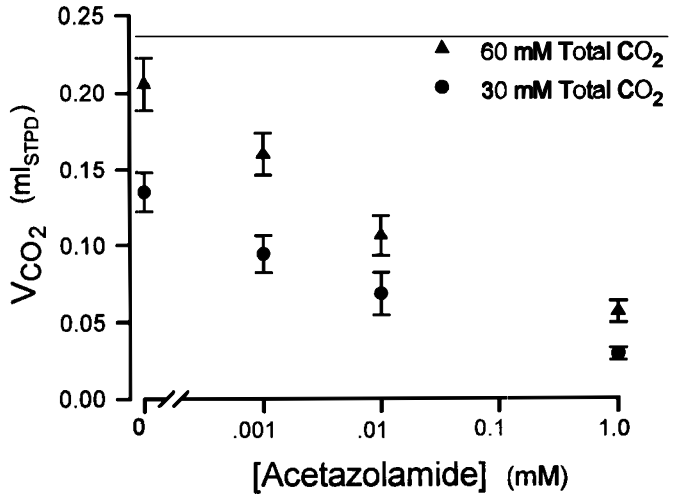

Figure 5. Effect of inhibition of endothelial carbonic anhydrase by acetazolamide on CO_2 excretion (± SD). The values for acetazolamide concentrations of 0.0 and 1.0 mM are taken from reference 13. Even in the presence of complete inhibition (1.0 mM acetazolamide), CO_2 excretion occurs because dissolved CO_2 is present in the bolus and bicarbonate dehydration occurs at its natural, uncatalyzed rate.

The lagged normal density curve has been used successfully to model transport functions in different portions of the vasculature (10, 24, 25). This model fits a function with a single peak and smooth contour to the experimental data. If the true transport function were bimodal, as would occur if blood were diverted to a second capillary bed after leaving an initial gas exchange unit, a unimodal function would still be fit to the data. Methods that do not rely on a specific mathematical function to describe the distribution of transit times are available, but involve other compromises such as smoothing of the recovered function (26) or computational complexity (27). The lagged normal density function was helpful in these experiments because it added relatively little computational time to the model of CO_2 production and elimination. Its adequacy to describe the input function derived from the C_2H_2 data was excellent. The coefficient of variation between the computed C_2H_2 excretion and observed data was only 0.005 and calculated C_2H_2 excretion was virtually indistinguishable from experimental data (Figure 1). The fit of the lagged normal density function to the distribution of capillary transit times was tested by convolving the input function with the recovered capillary transport function and the model of CO_2 exchange. The coefficient of variation between experimental and computed CO_2 excretion was only 0.010. These variations in matching the experimental data are approximately 5 to 10 times smaller than other values reported in experiments in the pulmonary vasculature (10, 24, 28). The congruence between experimental and computed exchange, despite lack of smoothing of input data, is attributable to a number of factors, including constant flow, lack of recirculation, essentially continuous rather than discrete data collection, and a high signal-to-noise ratio (Figure 1). Furthermore, the experimental curves represent gas exchange integrated over time and these data will have substantially less variation than instantaneous gas exchange which is the differentiated form of the experimental curve. Recovering the transport function from integrated data is equally as valid as using differentiated measurements (27).

The majority of measurements of pulmonary capillary transit time have been accomplished by direct observation of the subpleural capillary network. The limitations inherent in this approach have been extensively discussed by the investigators who have used this technique (6–8, 28–30). The major concern is the assumption that subpleural capillary blood flow is representative of flow throughout the entire capillary bed. Estimations of subpleural capillary flow by laser-Doppler technology correlate well with total flow through the isolated, perfused dog lobe over a wide variation of flow and alveolar and vasculature pressures (31). Absolute quantitative flow cannot be measured with this technology, so correlations between the signal and total flow do not necessarily indicate that subpleural flow per gram of tissue is identical to that in the remainder of the lung. During vasoconstriction induced pharmacologically, marked discrepancies between the laser-Doppler signal and total flow have been observed (31). However, laser-Doppler signals are affected by more variables than flow, so it is difficult at this point to determine if subpleural flow does deviate from the pattern present in the rest of the lung or if the laser-Doppler signal is being altered by a variable other than flow. Recent modification of the direct observation of fluorescent indicators in the subpleural circulation have been accomplished to avoid background fluorescence, which can cause artifactual shortening of measured transit time (28).

It is reassuring that the present estimate of mean pulmonary capillary transit time (1.71 s) falls in the midrange of available estimates despite utilizing a significantly different experimental

approach. Other estimates in the literature were obtained under a variety of conditions with three different methods employed to estimate transit time. Using a combination of morphometric and physiologic observations, Hogg and colleagues (9, 32) reported mean transit times in anesthetized dogs and humans of 1.37 and 1.6 s, respectively. It is likely that the vasculature of the lungs in these experiments was in Zone 2 or 3 conditions, but these data were not reported. These two studies estimate transit time of erythrocytes. All other studies of the capillary transport function have utilized plasma indicators and may yield slightly greater transit times since red cells pass through capillaries slightly faster than plasma (the Fahraeus effect).

Audi and associates (10) obtained a mean transit time of 3.2 s from analysis of indicator-dilution curves after multiple, sequential injections of a diffusible indicator in an isolated, perfused dog lobe preparation. They estimated that blood flow in their experiments was only one-half that present in the resting animal, perhaps resulting in the prolonged transit time. Inspection of their data indicates that the experiments were conducted in Zone 2 and 3 conditions.

Based on direct fluorescent measurements in the subpleural vasculature in intact dogs, Wagner and coworkers reported an average capillary transit time of 1.6 s in dependent lung (6), but this increased substantially in mid-lung (3.1 s) and upper lung (12.3 s). Direct subpleural transit time measurements in the dependent lung are identical to those obtained indirectly from diffusing capacity of the whole lungs (7). Using the same technique in the rabbit, Wang and colleagues (8) reported a substantially shorter mean transit time of 0.6 s in the subpleural capillaries at normal functional residual capacity. This increased to 2.78 s with increased lung distension with 20 cm positive pressure. However, the majority of this increase in transit time resulted from a profound decrease in cardiac output. In fact, there is substantial evidence that blood flow is the primary determinant of pulmonary capillary transit time (28–30). As flow is increased moderately over resting values, Presson and associates (28) noted that transit time decreases and there is recruitment of previously nonperfused capillaries. With additional increase in flow, transit time continued to decrease without further recruitment of capillaries. Because the latter decrease in transit time was not inversely proportional to the increase in flow, these investigators suggested that there may have been further dilation of patent capillaries, thereby increasing capillary volume and lessening the impact of increasing flow on transit time. Transition from Zone 2 to Zone 3 results in a small increase in transit time as determined by direct observation of subpleural capillaries, but the rate of blood flow through the vasculature appears to be a much more dominant factor (30). Although our measurements were made with blood flow similar to that reported in intact anesthetized rabbits (8), cardiac output in the conscious animal has been reported to be more than twice as great (33). In view of the effect of blood flow on transit time, actual transit times in intact organisms without anesthesia could be substantially shorter.

In an extensive series of indicator-dilution experiments involving the entire pulmonary circulation, Knopp and Bassingthwaighte (24) reported a mean relative dispersion (standard deviation of the transport function divided by the mean transit time) of 0.46. This value was substantially greater than the relative dispersion of 0.18 obtained in studies of a single artery in the same laboratory (25). The investigators attributed this greater dispersion to behavior of the lung as a series of parallel pathways rather than as a single conduit such as an artery. Furthermore, relative dispersion of the transport function across the whole lung decreased with decreasing transit time associated with increased cardiac output. Maseri and colleagues (34) observed a similar relative dispersion for the pulmonary circulation which tended to decrease with increased flow. The decreasing relative dispersion reflects more homogeneous distribution of flow in the pulmonary circulation as cardiac output increases. Because the capillary bed is variably distended at low flow (28), this appears to be the most likely site for improvement in flow heterogeneity. Capen and colleagues (29) reported that the relative dispersion in the subpleural capillary vasculature *in vivo* remained unchanged despite a greater than threefold increase in cardiac output. In more recent work from the same laboratory, relative dispersion in the capillary bed of an isolated, perfused dog lobe fell from 0.54 to 0.44 with a doubling of a relatively low basal flow (28). When flow was doubled again, the relative dispersion decreased insignificantly to 0.41. These investigators felt that their previous inability to detect changes in the relative dispersion was the result of complete distension of the vascular bed at the flows used in those experiments. In the present experiments, we obtained a relative dispersion of 0.45, similar to that reported by Presson and associates (28) at moderate rates of flow. There was no correlation between the relative dispersion and mean transit time in our experiments, but flow was not varied and the transit times did not change appreciably.

The degrees of acceleration of bicarbonate dehydration obtained from fitting computed CO_2 excretion to the experimental data are similar to that reported previously with the same concentrations of inhibitor (35). Bidani and coworkers (35) reported a catalysis factor of 11.2 in the rat lung in the presence of 0.01 mM acetazolamide. This is essentially identical to our recovered values of 11.9 and 10.1 (Table 1). In the presence of 0.001 mM acetazolamide, the agreement between the two studies was less favorable. We observed values of 43.9 and 25.2 compared to their report of 53.3. However, there was considerable variation in both studies and the catalysis-inhibitor concentration relationship is quite steep at this concentration. Slight differences in available inhibitor can have substantial effects on observed catalysis. These investigators concluded that endothelial carbonic anhydrase, even in the absence of any inhibition, will have minimal effects on overall CO_2 exchange but will prevent disequilibrium between erythrocyte and plasma pH (35).

The recovered distributions of capillary transit times in these experiments have relatively few pathways that are sufficiently short from a temporal standpoint to prevent completion of gas exchange. These data suggest that disequilibrium between alveolar and capillary gas tensions would exist only with markedly shortened mean transit times occurring with strenuous exercise, with disease states in which the permeability characteristics of the alveolar-capillary membrane are altered, or at elevated altitude where the oxygen gradient for diffusion is decreased. However, extrapolation of these data to the intact organism must be tempered even though measurements were made in the intact pulmonary microcirculation. Our experiments were performed with perfusate free of cells. Erythrocytes traverse the microcirculation 10% more rapidly than plasma (36), and red cells could have a different transport function than plasma indicators. This has potential importance especially for oxygen exchange, but it is reassuring that our findings are similar to distributions obtained with labeled red cells in dogs (9) and humans (32) as well as with plasma markers in dogs (28, 29) and rabbits (30).

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