Rate of Disappearance of Labeled Carbon Dioxide from the Lungs of Humans during Breath Holding: a Method for Studying the Dynamics of Pulmonary CO_2 Exchange

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ABSTRACT The dynamics of CO_2 exchange in the lungs of man was studied by observing the rate of disappearance of a stable isotope of CO_2 ($^{13}CO_2$) from the alveolar gas during breath holding. Over 50% of the inspired isotope disappeared within the first 3 sec followed by a moderately rapid logarithmic decline in which one-half of the remaining $^{13}CO_2$ disappeared every 10 sec.

The large initial disappearance of $^{13}CO_2$ indicated that alveolar $^{13}CO_2$ equilibrated in less than 3 sec with the CO_2 stored in the pulmonary tissues and capillary blood. The volume of CO_2 in the pulmonary tissues calculated from this initial disappearance was 200 ml or 0.33 ml of CO_2 per milliliter of pulmonary tissue volume.

The alveolar to end-capillary gradient for $^{13}CO_2$ was calculated by comparing the simultaneous disappearance rates of $^{13}CO_2$ and acetylene. At rest and during exercise this gradient for $^{13}CO_2$ was either very small or not discernible, and diffusing capacity for CO_2 ($D_{L_{CO_2}}$) exceeded 200 ml/(min × mm Hg).

After the administration of a carbonic anhydrase inhibitor the rate of disappearance of $^{13}CO_2$ decreased markedly. $D_{L_{CO_2}}$ fell to 42 ml/(min × mm Hg) and at least 70% of the exchange of $^{13}CO_2$ with the CO_2 stores in the pulmonary tissues and blood was blocked by the inhibitor. These changes were attributed to impairment of exchange of $^{13}CO_2$ with the bicarbonate in the pulmonary tissues and blood.

The pH of the pulmonary tissues (V_{tis}) was determined by a method based on the premise that the CO_2 space in the pulmonary tissues blocked by the inhibitor represented total bicarbonate content. At an alveolar P_{CO_2} of 40 mm Hg pH of V_{tis} equalled 6.97 ± 0.09.

INTRODUCTION

Carbon dioxide is present in the blood and tissues of the lungs in the form of physically dissolved CO_2, bicarbonate, carbonic acid, and protein complexes (2). Chinard, Enns, and Nolan studied the interaction of these forms of CO_2 and the alveolar gas in the dog by injecting radioactive bicarbonate ($H^{14}CO_3^-$) and carbon dioxide ($^{14}CO_2$) into the pulmonary artery (3, 4). They concluded that the interconversion of the various forms of

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CO_2 is so rapid that the expired CO_2 may be considered to come from one large common CO_2 pool. Similar conclusions were reached by Feisal, Sackner, and DuBois who injected bicarbonate into the pulmonary artery of the dog and measured the evolution of CO_2 in the alveoli with a sensitive body plethysmograph (5). These investigators as well as others have found that inhibition of the enzyme carbonic anhydrase modified the exchange of CO_2 between blood, pulmonary tissues, and alveolar gas sufficiently to produce a significant alveolar to end-capillary CO_2 gradient (A-c CO_2 gradient) (6–8).

By measuring the rate of uptake of a stable carbon dioxide isotope ($^{13}CO_2$) from the alveolar gas during breath holding, we have developed a new technique for studying the interrelationships of the various forms of CO_2 and the A-c CO_2 gradient in the lungs of man. Measurements of the uptake of isotopic CO_2 have the advantage that they can provide the greatest possible A-c CO_2 gradient for a given total alveolar CO_2 tension, pulmonary capillary blood flow, and diffusing capacity. From the data it was possible to estimate the A-c CO_2 gradient and the diffusing capacity of the lungs for CO_2, and to determine the size of the CO_2 spaces in the lungs, the CO_2 dissociation curve of the lung tissues, the mean hydrogen ion concentration of the pulmonary tissues, and the relative contribution of bicarbonate to total CO_2 in the lungs.

METHODS

If a subject inspires a breath of a gas mixture containing CO_2 which has been enriched with the stable carbon isotope of mass 13 ($^{13}CO_2$), the distribution of the isotope within the lungs is likely to be governed by factors similar to those reported to influence the disappearance of soluble inert gases such as nitrous oxide and acetylene (9). There should be a rapid disappearance of the isotope initially because of its movement into the pulmonary parenchymal tissue volume (V_{tis}) and pulmonary capillary blood volume (Vc), followed by a more gradual disappearance as it is carried away by the blood flowing through the pulmonary capillaries. If the highly insoluble inert gas, neon, is added to the inspired mixture, the effect of dilution of the isotope in the lung's residual air can be taken into account. If acetylene (C_2H_2), whose rate of disappearance during breath holding is a function of pulmonary capillary blood flow ($\dot{Q}c$), is included in the inspired mixture, it is possible to evaluate the influence of $\dot{Q}c$ on the rate of disappearance of $^{13}CO_2$ (9). The CO_2 content of the pulmonary parenchymal tissue (V_{tis}) and Vc can be determined from the initial rapid disappearance of $^{13}CO_2$. The alveolar to end-capillary gradient for CO_2 can be calculated by comparing the rates of disappearance of C_2H_2 and $^{13}CO_2$. Once this gradient is known, the diffusing capacity for CO_2 ($D_{L_{CO_2}}$) can be determined. The relative contribution of bicarbonate to total CO_2 transport can be evaluated by making measurements before and after the administration of a carbonic anhydrase inhibitor.

Experimental procedure. $^{13}CO_2$ was prepared by adding hydrochloric acid to barium carbonate enriched 10-fold or more with carbon atoms of mass 13.[1] The experiments were conducted in the following manner: a rapidly responding mass spectrometer was tuned to the mass 44 peak in order to measure $^{12}CO_2$, and the instrument's inlet tube was attached to a mouthpiece through which the subject first blew out to residual volume. He then began rebreathing for three to six breaths from an unheated rubber anesthesia bag containing approximately 2 liters of 7–12% CO_2, 0–12% oxygen, and balance nitrogen. In the early experiments the per cent of O_2 in the rebreathing bag and inspired mixture was 21%. Because under these conditions the O_2 saturation of the blood increased from about 75–98% on entering the pulmonary capillaries, the P_{CO_2} would be expected to be slightly higher in the capillary blood than in the mixed venous blood (Haldane effect). In order to minimize this difference between mixed venous P_{CO_2} and capillary blood P_{CO_2} in the later experiments, we made the capillary O_2 saturation similar to the mixed venous level by using no O_2 in the rebreathing bag and by reducing the O_2 concentration in the inspired mixture to 5%.

The concentration and volume of CO_2 in the rebreathing bag was chosen so that after several breaths, the output of the mass spectrometer became constant indicating that the partial pressure of CO_2 (P_{CO_2}) in the subject's alveolar volume (VA) and the rebreathing bag were at the virtual mixed venous P_{CO_2} (10) (Fig. 1). The subject then exhaled to his residual volume and maximally inspired a gas mixture containing a total P_{CO_2} approximately equal to the virtual mixed venous P_{CO_2}, 0.6–1.0% $^{13}CO_2$, 0.4% CO, 0.5% neon, 0.75% C_2H_2, 5 or 21% O_2, and balance nitrogen. After breath holding for about 10 sec, he forcefully expired. Note that the concentration of CO_2 in the rebreathing bag and the inspired mixture were chosen so that during breath holding the P_{CO_2} in the blood entering the capillaries was similar to the P_{CO_2} in VA. As a result the total P_{CO_2} and CO_2 content in the pulmonary capillaries and alveoli remained relatively constant during the breath holding period. The first liter of the expirate was discarded in order to wash out the respiratory dead space, and the remaining gas was collected for analysis. The procedure was repeated after intervals of an hour for breath holding periods of approximately 3, 7, and 14 sec. Breath holding periods longer than 14 sec were avoided because of the possibility of recirculation of the isotope.

Calculating rate of disappearance of labeled CO_2. The per cent of total CO_2 in the form $^{13}CO_2$ in the inspired and expired gases was measured on a mass spectrometer by recording the output of either mass 12 and mass 13 or mass

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[1] Obtainable from the Isomet Corp., Palisades Park, N. J.

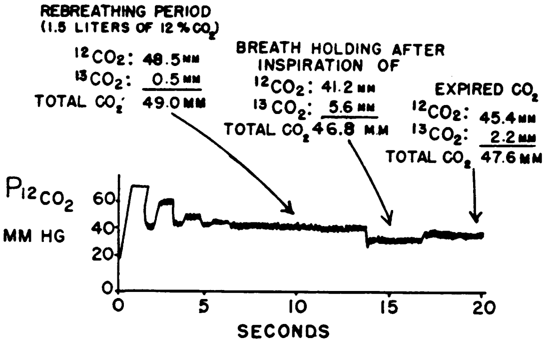

FIGURE 1 Tracing of a mass spectrometer record monitoring partial pressure of mass 44 ($P^{12}CO_2$) during measurement of rate of disappearance of $^{13}CO_2$ in subject RWH. Time scale commences when the subject began rebreathing from a 1.5 liter bag containing 12% CO_2 and balance air. $P^{12}CO_2$ became constant at 48.5 mm Hg and during the 14th sec he inspired the test gas mixture enriched with $^{13}CO_2$. He held his breath for 3 sec and then exhaled delivering an alveolar sample. Note that total P_{CO_2} is kept almost constant at 48 mm Hg, whereas $P^{13}CO_2$ falls to less than half of the value originally present in the inspired mixture.

44 and mass 45 (Table I). At the start of the experiment the natural occurrence of the isotope, defined as the concentration of the isotope divided by the total concentration of CO_2 in the specimen, was determined from a sample of the subject's expired air. Total [CO_2], [C_2H_2], [Neon], and (carbon monoxide] of the gas samples were measured on a gas chromatograph. Alveolar P_{CO_2} during breath holding was determined from either the continuous mass spectrometer record of expired breath or by analyzing the contents of the rebreathing bag. Alveolar volume (V_A) during breath holding was measured by adding the inspired volume recorded on a spirometer to the residual volume which had been determined previously by the closed circuit helium method (12). The disappearance from the alveoli of C_2H_2, carbon monoxide (CO), and $^{13}CO_2$ in excess of virtual mixed venous $^{13}CO_2$ ($^*CO_2$) were plotted on semilogarithmic graph paper against time (Fig. 2). From the C_2H_2 disappearance, pulmonary capillary blood flow ($\dot{Q}c$) and pulmonary parenchymal tissue volume (V_{tis}) were determined (9). Carbon monoxide diffusing capacity ($D_{L_{CO}}$) was calculated from the disappearance of CO (13).

In practice it was found to be convenient to calculate the per cent disappearance of $^*CO_2$ by the following formula whose derivation is given in detail elsewhere (14, Appendix I):

$$\text{Fraction of } {}^*CO_2 \text{ remaining} = \frac{(PI_{neon})(PE_{CO_2})}{(PE_{neon})(PI_{CO_2})} \times \left[\frac{\dfrac{PE_{^{13}CO_2}}{PE_{CO_2}} - \text{natural occurrence of } {}^{13}CO_2}{\dfrac{PI_{^{13}CO_2}}{PI_{CO_2}} - \text{natural occurrence of } {}^{13}CO_2} \right] \quad (1)$$

PI_{neon} and PI_{CO_2} are the partial pressures of neon and CO_2, respectively, in the test gas mixture which is to be inspired, PE_{neon} and PE_{CO_2} are the partial pressures of neon and CO_2, respectively, in the expired gas sample, $PI_{^{13}CO_2}$ and $PE_{^{13}CO_2}$ are the partial pressures of $^{13}CO_2$ in the inspired test gas mixture and the expired gas mixture, and the natural occurrence of $^{13}CO_2$ is the fraction [$^{13}CO_2$]/[total CO_2] measured from the subject's expired air before inhaling gas mixtures enriched with $^{13}CO_2$.

Calculation of the CO_2 content of the lung's tissues (V_{tis}) from the rate of disappearance of labeled CO_2. Theoretical analyses indicate that a soluble inert gas equilibrates extremely rapidly (in less than 1 sec) with the finer parenchymal tissues of the lung (9), and that the initial disappearance of such a gas can be used to calculate the pulmonary parenchymal tissue volume (V_{tis}). Experimentally the logarithm of alveolar inert gas concentration is plotted against time of breath holding and the curve extrapolated to time zero (see line for C_2H_2 in Fig. 2). Any rapid initial solution of inert gas in the lung tissue will be seen as a depression of the intercept with respect to the zero time axis, and will depend on the relative volumes of lung tissue and alveolar gas, as well as the partition coefficient of the inert gas. In the case of $^*CO_2$, while a small amount will physically dissolve in the lung tissues (V_{tis}) a larger amount will exchange with the bicarbonate contained in V_{is} and the pulmonary capillary blood volume (Vc) (3). An additional

TABLE I

*Natural Occurrence of Stable Isotopes of Carbon Dioxide**

Mass	Occurrence of mass as per cent of total CO_2	Molecular structure	Occurrence of molecular structure as per cent of total CO_2
44	98.415	$^{12}C^{16}O^{16}O$	98.415
45	1.177	$^{13}C^{16}O^{16}O$ $^{12}C^{16}O^{17}O$	1.103 0.074
46	0.403	$^{13}C^{16}O^{17}O$ $^{12}C^{16}O^{18}O$, $^{12}C^{17}O^{17}O$	8.3×10^{-4}
47	0.0045	$^{13}C^{16}O^{18}O$, $^{13}C^{17}O^{17}O$ $^{12}C^{17}O^{18}O$	0.0045 1.5×10^{-5}
48	4.2×10^{-5}	$^{13}C^{17}O^{18}O$ $^{12}C^{18}O^{18}O$	1.7×10^{-7} 4.2×10^{-5}
49	4.6×10^{-7}	$^{13}C^{18}O^{18}O$	4.6×10^{-7}

* Calculated by law of probabilities from data of Nier (11) who reported occurrence of carbon-12 as 98.892% of total CO_2, carbon-13 as 1.108% of total CO_2, oxygen-16 as 99.758% of total O_2, oxygen-17 as 0.0373% of total O_2, and oxygen-18 as 0.2039% of total O_2. In the earlier experiments analysis of the labeled CO_2 was performed by first absorbing the CO_2 in the expired gas in a precipitate free solution of barium and sodium hydroxide, and then regenerating the CO_2 by the addition of hydrochloric acid. This processing increased the CO_2 concentration to 80% or higher which gave sufficient output on the mass spectrometer to measure mass 13 (carbon-13 fraction of $^{13}CO_2$ and mass 12 (carbon-12 fraction of $^{12}CO_2$). For the later experiments a different mass spectrometer became available which had sufficient separation of masses so that mass 44 ($^{12}C^{16}O^{16}O$) and mass 45 ($^{13}C^{16}O^{16}O$ and $^{12}C^{16}O^{17}O$) could be measured directly obviating the need of concentrating the CO_2 in the gas samples.

amount of $^*CO_2$ may exchange with CO_2 bound to hemoglobin as carbamino-hemoglobin or with other protein molecules (2). If it is assumed that the movement of $^*CO_2$ into V_{tis} and Vc is complete in 3 sec (our shortest period of breath holding), then the amount of depression of the intercept at time zero of the curve for $^*CO_2$ (Fig. 2) is a function of the amount of $^*CO_2$ which is taken up by V_{tis} and Vc. The total amount of $^*CO_2$ inspired must equal the amount of $^*CO_2$ which is present in V_{tis}, Vc, and the alveolar volume just after inspiration and before a significant amount of $^*CO_2$ is carried away by the pulmonary capillary blood flow ($\dot{Q}c$) or;

$$\frac{(V_A)(P^*co_{2_{t-a}})}{P_B - P_{H_2O}} = \frac{(V_A)(P^*co_{2_{t-b}})}{P_B - P_{H_2O}} + \frac{(Vc)(\alpha_b)(P^*co_{2_{t-b}})}{760} + \frac{(V_{tis})(\alpha_{tis})(P^*co_{2_{t-b}})}{760} \quad (2)$$

where V_A equals the alveolar volume in ml STPD; $P^*co_{2_{t-a}}$ is the P^*co_2 in mm Hg in V_A just after inspiration but before any P^*co_2 has moved into Vc and V_{tis}; Vc is the pulmonary capillary blood volume in ml; V_{tis} is the pulmonary parenchymal tissue volume in ml; $P^*co_{2_{t-b}}$ is the partial pressure of P^*co_2 in mm Hg in V_A, Vc, and V_{tis} just after the movement of P^*co_2 into Vc and V_{tis}; α_b is the effective solubility coefficient for all forms of CO_2 carried in the blood (i.e. physically dissolved CO_2, H_2CO_3, CO_2 in bicarbonate, CO_2 in the form of carbamino-hemoglobin, and other CO_2-protein complexes) in ml of CO_2 STPD per ml of blood per standard atmosphere.[2] α_{tis} is the effective solubility coefficient for all forms of CO_2 present in V_{tis} in ml of CO_2 STPD per ml of V_{tis} per standard atmosphere, P_B is the barometric pressure in mm Hg, and P_{H_2O} is the vapor pressure of water at the subject's body temperature.

Note that the fraction $P^*co_{2_{t-b}} \div P^*co_{2_{t-a}}$ equals the amount of $^*CO_2$ present in the alveoli, determined by extrapolating the rate of disappearance of $^*CO_2$ during breath holding back to time zero, divided by the amount of $^*CO_2$ predicted to be present from the neon dilution (Fig. 2). This fraction therefore equals $^*CO_2$ intercept in per cent $\div$ 100 and equation 2 can be rewritten in the following manner:

$$\frac{760\ V_A}{P_B - P_{H_2O}}\left[\frac{100}{^*CO_2 \text{ intercept in per cent}} - 1\right] = V_{tis}(\alpha_{tis}) + Vc(\alpha_b) \quad (3)$$

Since $V_{tis}(\alpha_{tis}) + Vc(\alpha_b)$ equals the CO_2 content in ml STPD of the lungs at a partial pressure of CO_2 of 760 mm Hg, the CO_2 content at the alveolar P_{CO_2} present during breath holding ($P_{A_{CO_2}}$) can be calculated by multiplying the left-hand term of equation 3 by $P_{CO_2} \div 760$ or:

CO_2 content of $(V_{tis} + Vc)$ at $P_{A_{CO_2}}$ =

$$\frac{(P_{A_{CO_2}})(V_A)}{P_B - P_{H_2O}}\left[\frac{100}{^*CO_2 \text{ intercept in per cent}} - 1\right]. \quad (4)$$

If the CO_2 content of V_{tis} alone is desired, it can be determined by measuring Vc using carbon monoxide (15) and the CO_2 content of the subject's blood at $P_{A_{CO_2}}$ (see below), and then subtracting the product of these two terms from the CO_2 content of V_{tis} + Vc. In these experiments the CO_2 content of the pulmonary capillary blood during breath holding was determined indirectly from measurements of P_{CO_2} and P_{O_2} in the rebreathing bag or the expired sample. These values were then used to obtain the CO_2 content from a standard CO_2 dissociation curve (16). In two of the subjects the CO_2 content and P_{CO_2} of a blood specimen drawn from the antecubital vein were measured by the Astrup technique (17) and this value was then adjusted to the CO_2 content at $P_{A_{CO_2}}$ by using the nomograms published by Bartels and coworkers (18).

Calculation of pulmonary capillary blood flow ($\dot{Q}c$) from the rate of disappearance of $^*CO_2$. If there is no alveolar to end-capillary gradient (A-c CO_2 gradient) for CO_2, the rate of disappearance of $^*CO_2$ from the alveolar gas during breath holding will be flow limited. Then $\dot{Q}c$ calculated from the disappearance of $^*CO_2$ should be equal to values obtained with an inert gas such as C_2H_2. As a means of verifying the presence or absence of a significant A-c CO_2 gradient, we compared in our subjects $\dot{Q}c$ measured by the C_2H_2 breath holding method with $\dot{Q}c$ determined from the rate of disappearance of $^*CO_2$. $\dot{Q}c$ using $^*CO_2$ was determined in the following manner: the instantaneous rate of change of the concentration of $^*CO_2$ in the lungs after inspiration of the test gas and after equilibration of $^*CO_2$ with lung tissue and capillary blood must equal the amount which is being removed by $\dot{Q}c$ or:

$$\frac{d}{dt}\left[\frac{P_A{}^*co_2}{760}\right]\left[V_A\frac{760}{P_B - P_{H_2O}} + \alpha_{tis}(V_{tis}) + \alpha_b(Vc)\right] = (\dot{Q}c)(\alpha_b)\left[\frac{P_A{}^*co_2}{760}\right] \quad (5)$$

where $\dot{Q}c$ is in milliliter per minute and $P_A{}^*co_2$ is the partial pressure of $^*CO_2$ in the alevoli at any instant. The terms $\alpha_{tis}(V_{tis}) + \alpha_b(Vc)$ can be eliminated by substitution (see equation 3). Integration over the interval of breath holding gives:

$$\dot{Q}c = \frac{V_A\left[\frac{760}{P_B - P_{H_2O}}\right]\left[\frac{100}{CO_2 \text{ intercept in per cent}}\right]}{\alpha_b(t_{BH})} \times \ln\left[\frac{P_A{}^*co_{2_{t-b}}}{P_A{}^*co_{2_t}}\right] \quad (6)$$

where $P_A{}^*co_{2_t}$ equals the $P_A{}^*co_2$ in mm Hg at the end of breath holding and t_{BH} is the breath holding time in minutes.

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[2] α_b varies with the partial pressure of CO_2 present because of the alinearity of the CO_2 dissociation curve. Therefore, in the experiments in this paper α_b was always defined for the particular alveolar P_{CO_2} present during breath holding ($P_{A_{CO_2}}$) and was calculated by the following formula:

$$\alpha_b = \frac{[CO_2]\ 760}{P_{A_{CO_2}}}$$

where $[CO_2]$ is the CO_2 content of the subject's blood at $P_{A_{CO_2}}$ in milliliter STPD per milliliter of blood. See text for technique of determining $[CO_2]$.

Determination of the slope of the CO_2 dissociation curve of the pulmonary tissues. Techniques for the determination of the CO_2 dissociation curve of the lung tissues have included a plethysmographic method (19), direct measurement of the CO_2 content in vitro after equilibration with known concentrations of CO_2 (20), and methods based on the collection of alveolar gas samples after varying breath holding periods (21, 22). In this study a modification of the breath holding methods described by DuBois (21) and Fenn and Dejours (22) was used. The method is described in Appendix I.

Calculation of the diffusing capacity of lungs for CO_2—($D_{L_{CO_2}}$). While normally it is believed there is little or no end-capillary gradient for CO_2 between the alveolar gas, the plasma, and the red blood cells in the pulmonary end-capillary blood, Chinard and coworkers (3) and Soni, Feisal, and DuBois (23) have shown that after the administration of a carbonic anhydrase inhibitor, a significant gradient between alveolar P_{CO_2} and the P_{CO_2} in the end-capillary blood is present due to the slower conversion of bicarbonate to molecular CO_2. If a measureable gradient is produced, the diffusing capacity of the lungs for CO_2 ($D_{L_{CO_2}}$) could be calculated from the rate of disappearance of $*CO_2$ during breath holding by a method identical with that recently described for O_2 using a stable O_2 isotope (14). Constantine, Craw, and Forster (24) have reported values for θ_{CO_2}, (defined as the rate at which blood takes up CO_2 expressed in ml of CO_2 STPD per minute per mm Hg per milliliter of blood) before and after the addition of a carbonic anhydrase inhibitor to the blood. These values for θ_{CO_2}, the calculated value of $D_{L_{CO_2}}$ (See above), and the diffusing capacity of the alveolar-capillary membrane ($D_{M_{C'}}$) should be related to each other by the following equation (15):

$$\frac{1}{D_{L_{CO_2}}} = \frac{1}{D_{M_{CO_2}}} + \frac{1}{(V_c)(\theta_{CO_2})} \qquad (7)$$

$D_{L_{CO_2}}$ and $D_{M_{CO_2}}$ are expressed in milliliter of CO_2 STPD per minute per mm Hg, and Vc is the pulmonary capillary blood volume in milliliters determined by measuring the carbon monoxide diffusing capacity at different alveolar O_2 tensions (15).

RESULTS

Measurement of rate of disappearance of $*CO_2$ in five resting subjects. Analysis of the gas samples collected after the shortest period of breath holding, which was about 2.5 sec, showed that over half of the $*CO_2$ had disappeared from the alveolar gas (Tables II and III, and Fig. 2). Thereafter the remaining $*CO_2$ decreased at approximately 10%/sec. Comparable disappearance rates have been reported by West and Dollery using radioactive CO_2 ($^{11}CO_2$) (25). Pulmonary capillary blood flow calculated from the disappearance of C_2H_2 ($\dot{Q}_{C_2H_2}$) and the value obtained from the rate of disappearance of $*CO_2$ ($\dot{Q}*_{CO_2}$), differed by no more than 0.4 liter/min ($r = +0.99$, $P > 0.9$). Mean values for $\dot{Q}_{C_2H_2}$ and $\dot{Q}*_{CO_2}$ were 7.24 liters/min and 7.23 liters/min, respectively. This close agreement indicates that on the average there was no significant alveolar to end-capillary gradient for $*CO_2$ because if a gradient was present, $\dot{Q}*_{CO_2}$ would have been significantly smaller than $\dot{Q}_{C_2H_2}$ (Table III).

TABLE II

Physical Characteristics of Experimental Subjects

Subject	Age	Sex	Height	Weight	Body surface area
	yr		*in.*	*lb.*	m^2
RWH	32*	M	71	160	1.94
WFR	23	M	72	180	2.02
PBK	24	M	69	155	1.84
RJMP	30	M	68	170	1.90
MAF	27	F	67	118	1.62

* 36 yr old at time of exercise measurements.

CO_2 content of lung tissue. The mean amount of CO_2 present in the lungs of the five subjects determined from the initial rapid loss of $*CO_2$ from the alveoli was 244 ml STPD (Table IV). Of this volume approximately 50 ml represented CO_2 in the capillary blood (Vc) and 194 ml represented CO_2 in the lung tissue (V_{tis}). The CO_2 concentration of the sum of V_{tis} and Vc was 0.36 ml STPD per ml and the CO_2 content of V_{tis} alone was 0.33 ml STPD per ml. If one assumes that the amount of physically dissolved CO_2 is the same as in water, then approximately 0.04 ml/ml is in the form of physically dissolved CO_2 [3] and 0.29 ml/ml must be present in other forms such as bicarbonate. The concentration of CO_2 in V_{tis} was about 35% less than its concentration in the pulmonary capillary blood, about equal to the values reported for muscle and brain (16, 26), and in good agreement with the measurements for lung tissue published by DuBois, Fenn, and Britt (20). They found that at an alveolar P_{CO_2} of 50 mm Hg dog lungs perfused with Ringer's solution contained approximately 0.35 ml of CO_2 per ml of tissue.

CO_2 dissociation curve of the lungs. The CO_2

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[3] At $P_{MV_{CO_2}}$ equal to 50 mm Hg the total physically dissolved CO_2 would be: $0.57 \times 50/760$ or 0.04-ml STPD per ml of V_{tis} where 0.57 is the solubility coefficient of CO_2 in water at 37°C in milliliter STPD per atmosphere.

TABLE III

*Alveolar Volume (VA), Disappearance Rates of C_2H_2 and *CO_2, Pulmonary Capillary Blood Flow ($\dot{Q}c$), Alveolar Po_2 (PA_{O_2}), Alveolar Pco_2 (PA_{CO_2}), and Diffusing Capacity for CO (DL_{CO}) and CO_2 (DL_{CO_2}) in Five Human Subjects*

Subject	Body position	VA ml STPD	% *CO_2[*] remaining at extrapolated zero time	% C_2H_2[*] remaining at extrapolated zero time	$K^*_{CO_2}$[‡]	$K_{C_2H_2}$[‡]	$\dot{Q}^*_{CO_2}$[§]	$\dot{Q}_{C_2H_2}$[§]	PA_{CO_2}	PA_{O_2}	DL_{CO}	DL_{CO_2}
					sec^{-1}	*sec^{-1}*	*liter/min*	*liter/min*	*mm Hg*	*mm Hg*	*ml/(min × mm Hg)*	
A. At rest												
RWH	Sitting	5675	55.0	90.2	0.078	0.0123	6.42	6.66	50.0	130	33.1	223
WFR	Sitting	6080	60.8	89.4	0.104	0.0127	7.91	7.51	47.6	127	39.2	i[‖]
PBK	Sitting	5300	58.8	93.2	0.111	0.0165	7.79	8.12	48.0	131	33.5	224
RJMP	Supine	4100	56.7	90.5	0.128	0.0202	7.72	7.84	50.5	44	42.2	317
MAF	Supine	3960	59.7	90.2	0.115	0.0160	6.32	6.08	43.3	36	28.8	i[‖]
Mean		5025	58.2	90.7	0.107	0.0155	7.23	7.24	47.9	—	—	—
B. During exercise												
RWH	Supine	5230	55.7	90.6	0.149	0.025	12.0	12.6	54.1	32	60.0	263
C. After acetazolamide												
RWH	Sitting	5770	82.7	89.6	0.062	0.0195	3.01	10.9	40.5	137	40.5	42

[*] Calculated by extending the line of least mean squares drawn from the plots of the per cent of *CO_2 or C_2H_2 remaining in the alveolar gas samples back to the point on the time axis representing the start of breath holding (Figs. 2, 5, and 6).

[‡] Calculated from the line of least mean squares drawn from the plots of per cent *CO_2 or C_2H_2 remaining at different breath holding times using the following formula: $^*CO_{2_0}/^*CO_{2_t}$ or $C_2H_{2_0}/C_2H_{2_t} = e^{Kt}$ where the zero and t subscripts are the per cent of the gas remaining at the start and the end of a time interval and t equals the time interval in seconds.

[§] Pulmonary capillary blood flow determined from the rate of disappearance of *CO_2($\dot{Q}^*_{CO_2}$) and C_2H_2($\dot{Q}_{C_2H_2}$).

[‖] Calculated DL_{CO_2} in this subject was an imaginary number because $\dot{Q}$*CO_2 was greater than $\dot{Q}_{C_2H_2}$.

dissociation curve of the lungs ($V_{tis} + Vc$) is shown in Fig. 3. The middle point on the curve was determined at the alveolar Pco_2 present during breath holding after inspiring *CO_2. Two additional points were obtained by multiple breath holding maneuvers after inspiring air or 12–15% CO_2 (Table V). Fig. 4 shows the CO_2 dissociation curve of V_{tis} alone. The data for this figure was determined by subtracting the volume of CO_2 calculated to be in Vc from the volume in V_{tis} + Vc. Above virtual mixed venous Pco_2 an increase in alveolar Pco_2 of 1 mm Hg resulted in an average movement of 1.5 ml of CO_2 into V_{tis} + Vc or 1.2 ml into V_{tis} in the five subjects. Below virtual mixed venous Pco_2 a decrease in alveolar Pco_2 of 1 mm Hg was accompanied by a loss of 2.2 ml of CO_2 from V_{tis} + Vc or 1.7 ml from V_{tis} (Tables VI and VII).

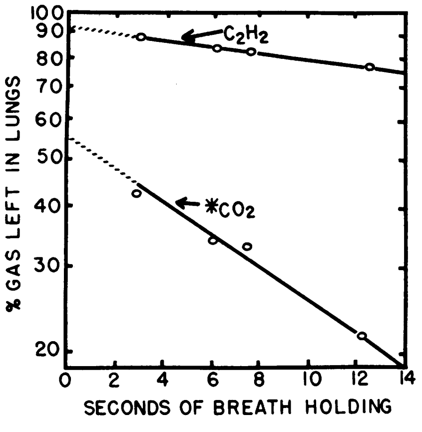

FIGURE 2 Graph showing the disappearance of C_2H_2 and *CO_2 from the alveolar gas during breath holding at an alveolar Pco_2 of approximately 50 mm Hg in subject RWH. The line of least squares for each gas was determined from the four points resulting from four separate breath holding periods. The extension of the lines from the 3 sec points to time zero is an extrapolation. The depressed intercepts at time zero result from the initial uptake of the gases by the pulmonary capillary blood (Vc) and pulmonary tissues (V_{tis}) (see text).

If the movement of CO_2 into and out of V_{tis} was limited to CO_2 in physical solution, only about 0.45 ml of CO_2 would be expected to leave or enter V_{tis} for each mm Hg change in alveolar Pco_2.[4] Since the mean figure for our subjects is two to

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[4] Assuming that the CO_2 solubility in V_{tis} is the same as in water (0.57-ml STPD per ml per atmosphere), the change in CO_2 content for each millimeter change in Pco_2 in a subject with V_{tis} equal to 600 ml would be: (0.57 × 600)/760 or 0.45 ml/mm Hg.

TABLE IV

CO_2 Content of Pulmonary Tissue Volume (V_{tis}) and Pulmonary Capillary Blood Volume (Vc) in Five Human Subjects at Virtual Mixed Venous P_{CO_2}

Subject	Alveolar P_{CO_2}	Volume of Vc $+V_{tis}$	CO_2 in Vc+ $+V_{tis}$	CO_2/ Vc $+V_{tis}$	Volume of Vc*	CO_2/Vc	Volume of V_{tis}	CO_2 in V_{tis}	CO_2/ V_{tis}
	mm Hg	*ml*	*ml*	*ml/ml*	*ml*	*ml/ml*	*ml*	*ml*	*ml/ml*
A. At rest									
RWH	50.0	865	326	0.39	112	0.53‡	753	255	0.34
WFR	47.6	900	262	0.29	110	0.52‡	790	205	0.26
PBK	48.0	542	250	0.46	78	0.52‡	464	209	0.45
RJMP	50.5	597	219	0.37	94	0.50§	503	172	0.34
MAF	43.3	604	161	0.27	71	0.42§	533	151	0.25
Mean:	47.9	702	244	0.36	93	0.50	608	198	0.33
B. During exercise									
RWH	54.1	760	318	0.42	—	0.53§	—	—	—
C. After acetazolamide									
RWH	40.5	942	66	0.07	—	0.48‡	—	—	—

* Determined from measurements of the carbon monoxide diffusing capacity (15).

‡ Estimated from mixed venous P_{CO_2} and P_{O_2} (see text).

§ Estimated from the CO_2 content, P_{CO_2}, and P_{O_2} of a peripheral venous blood corrected to mixed venous P_{CO_2} and P_{O_2} with standard monograms (see text).

three times greater than this figure, CO_2 in forms such as bicarbonate or carbamates must make a major contribution to the steepness of the CO_2 dissociation curve.

The slope of the dissociation curve of V_{tis} observed in the five subjects of this study is similar to the values reported by Sackner, Feisal, and DuBois using a plethysmographic technique (19). In their five subjects mean slope of V_{tis} was 1.32 ml of CO_2 per mm Hg compared to 1.41 ml of CO_2 per mm Hg in the present study. In addition, they made calculations from previous breath hold-

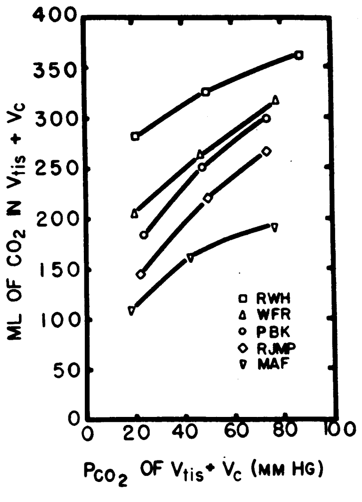

FIGURE 3 CO_2 dissociation curve of V_{tis} + Vc (pulmonary parenchyma tissue volume plus pulmonary capillary blood volume). The middle point on the curve for each subject was obtained by breath holding with $^{13}CO_2$. The upper and lower points were determined from analysis of alveolar gas samples after inhaling 12–15% CO_2 or 0% CO_2.

TABLE V

Changes in Volume of CO_2 in the Pulmonary Tissues (V_{tis}) and Pulmonary Capillary Blood (Vc) after the Inspiration of Air or 12–15% CO_2

Subject	RV	IV	Inspired P_{CO_2}	Change in alveolar P_{CO_2}*	Change in volume of CO_2 in V_{tis}+Vc
	ml	*ml*	*mm Hg*	*mm Hg*	*ml*
RWH	1860	4350	0	51–21	−45
	1630	4310	109	49–88	+37
WFR	1860	4840	0	48–20	−57
	1860	4840	99	48–79	+57
PBK	1450	4000	0	51–24	−74
	1450	4000	98	48–76	+49
RJMP	1000	3500	0	47–22	−64
	1000	3500	96	45–74	+61
MAF	880	3080	0	42–19	−50
	880	3080	100	43–78	+30

Abbreviations: RV, residual volume in ml STPD; IV, the inspired volume in ml STPD.

* The first figure is the alveolar P_{CO_2} in RV just before inhaling IV. It was determined by the rebreathing method. The second figure is $P_{equilCO_2}$ which is defined as the P_{CO_2} in the alveolar gas, V_{tis}, and Vc immediately after inspiration. See Appendix I for method of measurement.

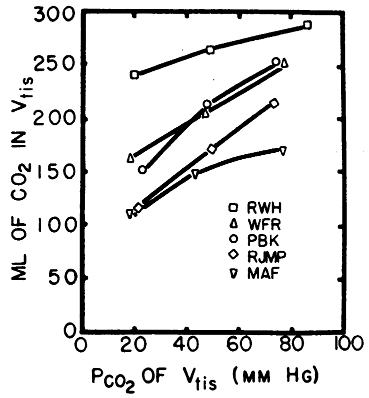

FIGURE 4 CO_2 dissociation curve of the pulmonary parenchyma (V_{tis}). The data for these curves were obtained by subtracting from the total CO_2 in V_{tis} + Vc, the amount of CO_2 calculated to be in Vc. CO_2 contained in Vc was determined from the product of Vc measured by the carbon monoxide method (15) and the CO content of the capillary blood (see text).

ing data collected by DuBois and reported by Fenn and Dejours (22). Their technique, which is similar to the breath holding method described in this report, gave an average value of 1.63 ml/mm Hg in nine subjects for the slope at an alveolar P_{CO_2} slightly below virtual mixed venous P_{CO_2}. In our subjects the slope for V_{tis} below virtual mixed venous P_{CO_2} was 1.67 ml/mm Hg.

Measurements during exercise. In one subject the rate of uptake of $^*CO_2$ and C_2H_2 was determined in the supine position while exercising on a bicycle ergometer. Measurements were made during the 6th min of exercise at a work load of 90 w at which time pulse rate was 120 beats/min, O_2 consumption was 1250 ml/min, and the respiratory quotient was 0.91. Compared to resting measurements in the same subject the initial rapid disappearance of $^*CO_2$ and C_2H_2 showed little change, but the fall in the concentration of these gases during subsequent breath holding doubled (Tables III B and IV B and Fig. 5). $\dot{Q}$c calculated from the rate of disappearance of the two gases was 12.0 and 12.6 liters/min for $^*CO_2$ and C_2H_2, respectively. This similarity in blood flows suggested that even a doubling of the cardiac output is not associated with an alveolar to end-capillary CO_2 gradient of any significance.

Effect of body position. Because of the possibility that uneven distribution of ventilation and volume with respect to perfusion (uneven $\dot{V}$A/$\dot{Q}$c and uneven VA/$\dot{Q}$c) might influence the rate of disappearance of $^*CO_2$, measurements were made in both the sitting and supine positions as well as during exercise (Table III). Neither body position nor exercise were found to produce any consistent differences in the calculated CO_2 content of the lung tissues, relative disappearance rates of $^*CO_2$ and C_2H_2, or other parameters. Therefore, the different degrees of uneven $\dot{V}$A/$\dot{Q}$c and VA/$\dot{Q}$c very likely produced by exercise and by varying body position did not appear to alter appreciably the factors determining the rate of disappearance of $^*CO_2$ from the alveoli.

Measurements after the administration of a carbonic anhydrase inhibitor (acetazolamide). One subject (RWH), over a 15 min interval, received an intravenous infusion of 500 ml of 5% glucose and water containing 7 g of sodium aceta-

TABLE VI

Slope of the CO_2 Dissociation Curve of the Pulmonary Parenchymal Tissues (V_{tis}) Plus the Pulmonary Capillary Blood (Vc)

Subject	ml/mm Hg	ml/mm Hg	ml/mm Hg	ml/mm Hg per m² of surface area	ml/mm Hg per 100 ml of V_{tis}+Vc
	(20–50)*	(50–80)*	(20–80)*	(20–80)*	(20–80)*
RWH	1.52	0.95	1.20	0.62	0.14
WFR	2.06	1.84	1.95	0.97	0.22
PBK	2.79	1.75	2.23	1.21	0.41
RJMP	2.54	2.11	2.33	1.23	0.39
MAF	2.18	0.87	1.41	0.87	0.23
Mean	2.22	1.50	1.82	0.98	0.28

* Approximate lower and upper values of P_{CO_2} in mm Hg used for the calculation of the slope of the CO_2 dissociation curve.

TABLE VII

Slope of the CO_2 Dissociation Curve of the Pulmonary Parenchymal Tissues (V_{tis})

Subject	ml/mm Hg	ml/mm Hg	ml/mm Hg	ml/mm Hg per m² of surface area	ml/mm Hg per 100 ml of V_{tis}
	(20–50)*	(20–80)*	(20–80)*	(20–80)*	(20–80)*
RWH	0.86	0.61	0.72	0.37	0.10
WFR	1.45	1.52	1.49	0.74	0.19
PBK	2.38	1.50	1.90	1.03	0.41
RJMP	2.01	1.79	1.91	1.01	0.38
MAF	1.65	0.64	1.05	0.64	0.21
Mean	1.67	1.21	1.41	0.76	0.26

* Approximate lower and upper values of P_{CO_2} in mm Hg used for the calculation of the slope of the CO_2 dissociation curve.

zolamide (100 mg/kg).[5] At the time of the breath holding measurements 4 hr later end-tidal P_{CO_2} was 14 mm Hg and virtual mixed venous P_{CO_2} determined by the rebreathing method was 40.5 mm Hg. The subject complained of marked intoxication, difficulty in performing simple arithmetic calculations, severe headache, ataxia, and occasional transient episodes of red, blurred vision lasting 5–15 sec. This severe reaction is in striking contrast to the minor symptomatology reported by Janowitz, Dreiling, Rolbin, and Hollander who gave humans doses as high as 154 mg/kg (27). The difference in symptoms may be related to the rapid infusion of the drug within 15 min in the present study compared to the administration over a 1–8 hr period in the experiments reported by Janowitz and coworkers.

During intoxication with acetazolamide $\dot{Q}_{C_2H_2}$ was 10.9 liters/min and $*CO_2$ disappeared at a rate of 6.3%/sec so that $\dot{Q}*_{CO_2}$ was only 3.01 liters/min. The CO_2 stores in $V_{tis} + V_c$ calculated from the initial disappearance of the isotope were only 69 ml (Tables III C and IV C and Fig. 6). Without enzyme inhibition at this $\dot{Q}_{C_2H_2}$ the subject would be expected to have a $*CO_2$ disappearance rate of 23%/sec and CO_2 stores in $V_{tis} + V_c$ equal to 313 ml. Since the lower values observed after the administration of acetazolamide are most likely due to the decreased ability of $*CO_2$ to exchange with the bicarbonate in V_{tis} and Vc, the difference between the above observed and predicted values of CO_2 in $V_{tis} + V_c$ must represent CO_2 in the form of bicarbonate. In this subject the fraction of total CO_2 in the form of bicarbonate calculated by this method was 79% for V_{tis} and 73% for Vc (Table VIII). Because only the conversion of $*CO_2$ to bicarbonate inhibited by acetazolamide was taken into account by this calculation, these percentages are minimal values. The above findings support the view that the carbonic anhydrase in lung tissues and blood is necessary in order to have equilibration of the CO_2 stores in V_{tis}, Vc, and alveolar gas during the time it takes blood to traverse the pulmonary capillaries (3, 5).

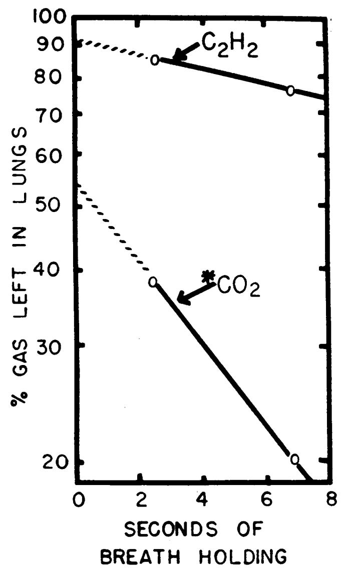

FIGURE 5 Graph showing the rate of disappearance of C_2H_2 and $*CO_2$ from the lungs of subject RWH during exercise. Compared to resting values (Fig. 2) there was little change in the zero intercepts but subsequent disappearance was twice as fast.

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[5] Kindly supplied by Dr. R. N. Fallon, Lederle Laboratories as the sodium salt of Diamox acetazolamide.

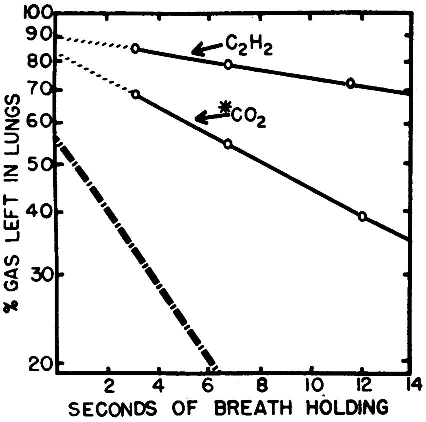

FIGURE 6 Graph showing the rate of disappearance of C_2H_2 and $^*CO_2$ from the lungs of subject RWH after the administration of the carbonic anhydrase inhibitor acetazolamide. The heavy dashed line represents the rate of disappearance of $^*CO_2$ predicted on the basis that there was no alveolar to end-capillary gradient or alveolar to pulmonary tissue gradient for $^*CO_2$. Since the experimental line had a higher value at zero time and a less steep slope, equilibration between CO_2 in blood, lung tissue, and alveolar gas was not present during enzyme inhibition.

Calculation of hydrogen ion concentration (H^+) in V_{tis}. If the concentration of bicarbonate ($^-HCO_3$) and CO_2 in physical solution (αP_{CO_2}) in V_{tis} are known, its hydrogen ion concentration $[H^+]$ can be calculated from the relationship $pH = pK' + \log([^-HCO_3]/\alpha P_{CO_2})$. $[^-HCO_3]$ and total CO_2 in V_{tis} was measured in subject RWH (see above) and in the other subjects $[^-HCO_3]$ was assumed to be 80% of the total CO_2 in V_{tis} at the alveolar P_{CO_2} present during breath holding. αP_{CO_2} was calculated from the formula $\alpha P_{CO_2} =$ (alveolar P_{CO_2}) (V_{tis}) (0.57)/760 where 0.57 is the Bunsen solubility coefficient for CO_2 in water at 37°C. pK' was assumed to equal 6.03 (28). If the amount of CO_2 which cannot be assigned to $^-HCO_3$ or αP_{CO_2} is assumed not to change with alterations in P_{CO_2}, $[H^+]$ of lung tissue for any CO_2 tensions above and below virtual mixed venous P_{CO_2} can be calculated.[6] Fig. 7 shows the results for the five subjects. At P_{CO_2} of 25 mm Hg average pH was 7.14 (SD = 0.06), at P_{CO_2} of 40 mm Hg average pH was 6.92 (SD = 0.09), and at P_{CO_2} of 70 mm Hg equalled 6.74 (SD = 0.11). The pH at P_{CO_2} of 25 and 40 mm Hg was similar to the values recently reported for intracellular muscle tissue (29), but the value at 70 mm Hg for muscle was 6.91 which is 0.17 pH units higher than the value determined for V_{tis}. This discrepancy is probably not significant in view of the

TABLE VIII

CO_2 Transport in Subject RWH at Virtual Mixed Venous P_{CO_2} = 40.5 mm Hg

	CO_2 content of $V_{tis}+Vc$*		CO_2 content of Vc*		CO_2 content of V_{tis}*	
	ml	*%*	*ml*	*%*	*ml*	*%*
Total CO_2						
Control	313‡	100	54§	100	259	100
After acetazolamide	66‖	21	15¶	27	51	20
Bicarbonate**	247	79	39	73	208	80
CO_2 in physical solution‡‡	26	8	3	6	23	9
Undesignated CO_2§§	40	13	12	21	28	11

* V_{tis}, pulmonary parenchymal tissue volume; Vc, pulmonary capillary blood volume.

‡ Control CO_2 content of $V_{tis}+Vc$ was determined from the initial rapid disappearance of $^*CO_2$ during breath holding.

§ Control CO_2 content of Vc was determined from a measurement of Vc by the carbon monoxide method and an estimate of the CO_2 content of the pulmonary capillary blood (see text).

‖ Apparent CO_2 content of $V_{tis}+Vc$ calculated from the initial rapid disappearance of $^*CO_2$ after acetazolamide administration.

¶ The CO_2 content of Vc after acetazolamide administration was assumed to be reduced from control values in proportion to the decrease in the apparent Bunsen solubility coefficient for total CO_2 in the blood determined from the rate of disappearance of $^*CO_2$ and C_2H_2 during breath holding.

** The bicarbonate fraction of the CO_2 content was considered to equal the difference in CO_2 content between measurements made before and after acetazolamide administration.

‡‡ Calculated assuming a solubility coefficient of 0.57 ml of CO_2 STPD per ml per atmosphere.

§§ Volume of CO_2 not assignable to bicarbonate or CO_2 in physical solution.

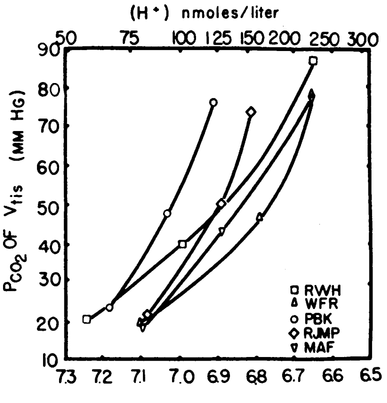

FIGURE 7 Relationship between P_{CO_2} and pH of the pulmonary parenchymal tissues (V_{tis}).

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[6] According to data reported by Roughton (2), CO_2 transported in the form of carbaminohemoglobin does not show a great change with variation in P_{CO_2}. We have assumed that the CO_2-protein complexes in lung tissue behave in the same manner.

variation in the individual values, the fact that the calculations include both intra- and extracellular spaces, and that the change in P_{CO_2} in V_{tis} took place in a few seconds while, in the muscle experiments, the tissues were incubated at constant P_{CO_2} for 4–6 hr.

The diffusing capacity for CO_2 (DL_{CO_2}). In the presence of active carbonic anhydrase, the pulmonary capillary blood flows calculated from the rate of disappearance of $*CO_2$ and C_2H_2 were almost identical. This finding indicates that on the average there is an extremely small alveolar end-capillary gradient for $*CO_2$ so that DL_{CO_2} cannot be measured accurately. For example in two subjects (WFR and MAF) the solution for DL_{CO_2} produced an imaginary number because $\dot{Q}*_{CO_2}$ was actually greater than $\dot{Q}_{C_2H_2}$. In the other subjects the value at rest was over 200 ml/(min × mm Hg) but slight errors in the rate of disappearance of $*CO_2$ or C_2H_2 would produce large changes in DL_{CO_2}.

After the administration of acetazolamide, end-capillary $P*_{CO_2}$ calculated by previously described methods (14) was less than one-third of the alveolar $P*_{CO_2}$ and the resulting DL_{CO_2} was only 42 ml/(min × mm Hg). Because of the large alveolar to end-capillary gradient DL_{CO_2} in this special circumstance can be measured with reasonable precision. For example, a 3% error in $\dot{Q}_{C_2H_2}$ produces less than a 1% change in DL_{CO_2}. The reduction in DL_{CO_2} during carbonic anhydrase inhibition is most likely due to a fall in θ_{CO_2}, the diffusing capacity of the blood for CO_2, which has been shown in vitro to decrease markedly after the administration of acetazolamide. According to this hypothesis, the $P*_{CO_2}$ in the blood in the capillary would most certainly have equilibrated with that in the alveolar gas long before the end of the capillary, but would not be in equilibrium with the blood bicarbonate, either within or without the red cell. Using equation 7 the value for θ_{CO_2} which would account for the DL_{CO_2} of 42 ml/(min × mm Hg) in this subject can be calculated in the following manner: according to equation 7: $1/42 = 1/DM_{CO_2} + 1/(\theta \times 130)$ where 130 represents an estimate of Vc during carbonic anhydrase inhibition in this subject in ml (Tables III and IV). According to Table III DL_{CO_2}, which must be less than DM_{CO_2}, is at least five times greater than 42, if indeed it is not so large it cannot be measured by this technique at all. Therefore, the term $1/DM_{CO_2}$ becomes so small it can be neglected, giving $\theta = 0.32$ ml/(min × mm Hg × ml). This in vivo value is considerably smaller than the in vitro value of 0.6 ml (min × mm) reported by Constantine, Craw, and Forster (24).

This discrepancy may be related to the fact that a significant part of the initial reaction these authors measured during carbonic anhydrase inhibition was due to the formation of carbamate superimposed on the slower formation of bicarcarbonate. If θ_{CO_2} is calculated from their data collected after the first 100 msec of the reaction, at which time the carbamate reaction has likely gone to completion, θ_{CO_2} is only 0.24 ml/(min × mm Hg) per ml, a figure in better agreement with the value measured in vivo. However it is likely that during carbonic anhydrase inhibition θ_{CO_2} varies along the length of the capillary. Large values would be expected at times when carbamate concentrations are changing and small values when only bicarbonate levels are varying. Because θ_{CO_2} may at times be a variable quantity, the present in vivo value as well as the reported in vitro values measured during carbonic anhydrase inhibition should be considered as approximations.

DISCUSSION

Estimate of size of DL_{CO_2} and the alveolar-capillary CO_2 gradients due to diffusion in normal subjects. In these experiments at the start of the capillary after inspiring $^{13}CO_2$, the alveolar $P^{13}_{CO_2}$ was 5–10–fold greater than the $P^{13}_{CO_2}$ in the virtual mixed venous blood. In contrast during resting ventilation the difference between unlabeled alveolar P_{CO_2} and mixed venous P_{CO_2} is only about 20%. Despite this ability of isotopic CO_2 to magnify any difference between alveolar and end-capillary P_{CO_2}, in our subjects no consistent gradient was detected and DL_{CO_2} could not be calculated accurately. Since we estimate that the analytical methods permit the detection of a difference of 5% between alveolar and end-capillary $P^{13}_{CO_2}$, only a minimal value for DL_{CO_2} can be calculated based on the assumption of an alveolar to end-capillary gradient of this size. In the five subjects at rest this minimal value for DL_{CO_2} was 220 (SD = 30) ml/(min × mm Hg). The minimal value for the diffusing capacity of the pulmonary membrane (DM_{CO_2}) determined from the minimal value of $\mathrm{DL}_{\mathrm{CO}_2}$, a value of $\theta\mathrm{CO}_2$ equal to 5.1 ml/(min × mm Hg × ml) (24), and equation 7 was 464 (SD = 165) ml/(min × mm Hg). This value is approximately one-third the value for $\mathrm{DM}_{\mathrm{CO}_2}$ predicted on the basis of measurements of the diffusing capacity of the pulmonary membrane for CO ($\mathrm{DM}_{\mathrm{CO}}$), the molecular weights of CO and $^*\mathrm{CO}_2$, and the relative solubilities of CO and CO_2 in water.[7]

Because the technique for calculating a minimal value for $\mathrm{DL}_{\mathrm{CO}_2}$ required that total alveolar $\mathrm{P}_{\mathrm{CO}_2}$ ($\mathrm{PA}_{\mathrm{CO}_2}$) equal virtual mixed venous $\mathrm{P}_{\mathrm{CO}_2}$ ($\mathrm{PMV}_{\mathrm{CO}_2}$), this value of $\mathrm{DL}_{\mathrm{CO}_2}$ does not directly describe the usual events that take place along the capillary during normal ventilation where $\mathrm{PA}_{\mathrm{CO}_2}$ is about 7 mm Hg less than $\mathrm{PMV}_{\mathrm{CO}_2}$. However, once an estimate of $\mathrm{DL}_{\mathrm{CO}_2}$ has been obtained, the rate of change in capillary $\mathrm{P}_{\mathrm{CO}_2}$ as blood traverses the pulmonary capillaries during normal physiological conditions can be calculated (Appendix II). This calculation is considerably simpler than is the case for O_2 because the CO_2 dissociation curve for blood is, for all practical purposes, linear over the range of values between $\mathrm{PMV}_{\mathrm{CO}_2}$ and arterial $\mathrm{P}_{\mathrm{CO}_2}$, and $\theta\mathrm{CO}_2$ can be assumed to be constant over this interval. The slight inaccuracy in this calculation resulting from the difference in diffusion coefficient for CO_2 of mass 45 as against mass 44 is likely to be small compared to errors which may arise from uneven distribution of diffusing capacity and blood flow, the use of a minimal value for $\mathrm{DL}_{\mathrm{CO}_2}$, and the Haldane effect which, according to recent in vivo measurements, may modify the rate of change of capillary $\mathrm{P}_{\mathrm{CO}_2}$ presumably because of variations in $\theta\mathrm{CO}_2$ along the capillary (30).

The unbroken curve in Fig. 8, curve A, was calculated using a minimal value for $\mathrm{DL}_{\mathrm{CO}_2}$ for a normal subject at rest. This curve, therefore, represents the slowest rate of change in capillary $\mathrm{P}_{\mathrm{CO}_2}$ compatible with the data. According to this minimal value $\mathrm{Pc}_{\mathrm{CO}_2}$ would fall half way to the alveolar $\mathrm{P}_{\mathrm{CO}_2}$ in 0.9 sec and 90% of the distance in 0.3 sec.

The dashed curve below curve A represents the change in $\mathrm{Pc}_{\mathrm{CO}_2}$ calculated from a value of $\mathrm{DL}_{\mathrm{CO}_2}$

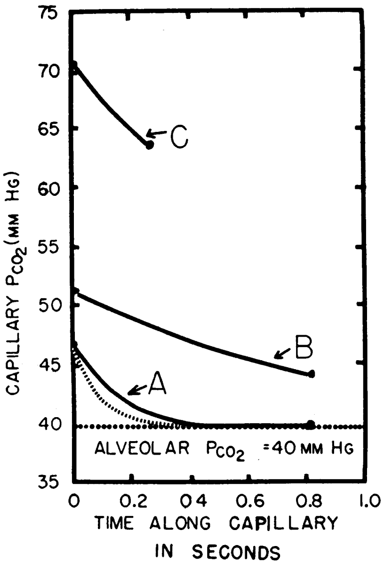

FIGURE 8 Time course of the fall in capillary $\mathrm{P}_{\mathrm{CO}_2}$ as blood traverses the pulmonary capillaries. Curve A is a plot for a normal subject at rest calculated using a minimal valve for $\mathrm{DL}_{\mathrm{CO}_2}$ derived from the rate of disappearance of $^*\mathrm{CO}_2$ from the alveolar gas. The broken curve below curve A was calculated using a value for $\mathrm{DL}_{\mathrm{CO}_2}$ derived from the size of the carbon monoxide diffusing capacity (see text). Alveolar and capillary $\mathrm{P}_{\mathrm{CO}_2}$ are essentially in equilibrium in 0.4 sec. Curve B is a plot for a normal subject after the administration of acetazolamide calculated on the basis that the inhibitor reduced the diffusing capacity for CO_2 from 250 to 40 ml/(min × mm Hg) and alveolar $\mathrm{P}_{\mathrm{CO}_2}$ remained at 40 mm Hg. Under these conditions an alveolar to end-capillary gradient for CO_2 of 4 mm Hg would be present. Curve C describes the time course along the capillary during carbonic anhydrase inhibition predicted to take place in a subject with a pulmonary disease which had reduced the capillary blood volume and diffusing capacity of the pulmonary membrane to one-third of normal and in addition prevented him from decreasing his alveolar $\mathrm{P}_{\mathrm{CO}_2}$ below 40 mm Hg. Under these conditions $\mathrm{DL}_{\mathrm{CO}_2}$ would be only 10.8 ml/(min × mm Hg) and in order to have a sufficient alveolar-capillary CO_2 gradient for elimination of the body's CO_2 production, mixed venous $\mathrm{P}_{\mathrm{CO}_2}$ would have to increase to 70.5 mm Hg. The alveolar to end-capillary CO_2 gradient would be 23.5 mm Hg. The following dimensions of the capillary bed and lungs representative for our subjects were used in these calculations, namely: alveolar $\mathrm{P}_{\mathrm{CO}_2}$ = 40 mm Hg; CO_2 production = 288-ml STPD per minute; $\dot{Q}c$ = 7200 ml/min; slope of CO_2 dissociation curve = 0.0057 ml STPD per milliliter per mm Hg; minimal $\mathrm{DL}_{\mathrm{CO}_2}$ = 250 ml/(min × mm Hg); $\mathrm{DL}_{\mathrm{CO}_2}$ calculated from measurements of $\mathrm{DL}_{\mathrm{CO}}$ = 435 ml/(min × mm Hg); and Vc = 100 ml. $\theta\mathrm{CO}_2$ was assumed normally to be 5.1 ml/(min × mm Hg) per ml and 0.35 ml/(min × mm Hg) per ml during carbonic anhydrase inhibition. Capillary transit time calculated from the relationship Vc ÷ $\dot{Q}c$, equalled 0.83 sec in the normal subject and 0.28 sec in the hypothetical subject with pulmonary disease.

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[7] If it is assumed that $\mathrm{DM}_{\mathrm{CO}}$ equals 60 ml/(min × mm Hg), $\mathrm{DM}_{\mathrm{CO}_2}$ would equal 60 × (0.592/0.0185)($\sqrt{28/45}$) or 1500 ml/(min × mm Hg). 28 and 45 are the mol wt of CO and $^*\mathrm{CO}_2$, and 0.529 and 0.0185 are their respective solubilities in water.

determined on the basis of the following two assumptions. First, according to the molecular weights of CO and $*CO_2$ and their relative solubilities in water, $D_{M_{CO_2}}$ is 25-fold greater than $D_{M_{CO}}$. Second, Vc for CO_2 may be larger than Vc for CO but recent measurements of O_2 vs. ether exchanges between the pulmonary vessels and the alveolar gas imply that this difference can be at the most 20% (31). Using these assumptions according to equation 7: $1/D_{L_{CO_2}} = 1/(25 \times 60) + 1/(5.1)(100 + 20)$ or $D_{L_{CO_2}} = 435$ ml/(min × mm Hg) where 60 is a representative value for $D_{M_{CO}}$ in normal subjects (32), 5.1 is the value for θ_{CO_2} (24), and 100 is a representative figure for Vc in our subjects measured with the CO technique. According to this estimate of $D_{L_{CO}}$ $P_{C_{CO_2}}$ would fall half way to the arterial P_{CO_2} in 0.06 sec and 90% of the distance in 0.18 sec. Since the mean capillary transit time for the pulmonary capillaries is approximately 0.8 sec, this data indicates that normally using either method of estimating $D_{L_{CO_2}}$, $P_{A_{CO_2}}$, and $P_{C_{CO_2}}$ are in equilibrium by the time the blood reaches the end of the capillaries. However, if some of the capillaries have shorter transit times due to uneven distribution of blood flow, or if the mean transit time is decreased by severe exercise or lung disease, it is likely that an alveolar to end-capillary CO_2 gradient of approximately 1–2 mm Hg would result. Such a gradient would be difficult to detect with present methods of measuring alveolar and arterial P_{CO_2} and, moreover, would not cause hypercapnea because even a slight increase in alveolar ventilation can readily compensate for so small a rise in end-capillary P_{CO_2}.

After the administration of acetazolamide, carbon dioxide exchange along the capillary was dramatically altered and because a large alveolar to end-capillary $*CO_2$ gradient developed, $D_{L_{CO_2}}$ could be measured with reasonable precision (see above) (Fig. 9).

The subject compensated for the decrease in $D_{L_{CO_2}}$ by vigorous hyperventilation so that the alveolar P_{CO_2} fell to approximately 14 mm Hg and the gradient between $P_{MV_{CO_2}}$ and $P_{A_{CO_2}}$ increased from 7 to 26 mm Hg. The calculated decrease in the capillary P_{CO_2} as blood traverses the capillaries, assuming chemical equilibrium between the dissolved CO_2 and bicarbonate, was 12 mm Hg. The difference between alveolar P_{CO_2} and equilibrated end-capillary P_{CO_2} was 14 mm Hg. The hyperventilation was so intense that, despite the development of a much less efficient alveolar-capillary CO_2 exchange, virtual mixed venous P_{CO_2} fell from 50 to 40 mm Hg. This hyperventilation was probably secondary to intracellular hypercarbia and acidosis in the cells of the respiratory centers (33).

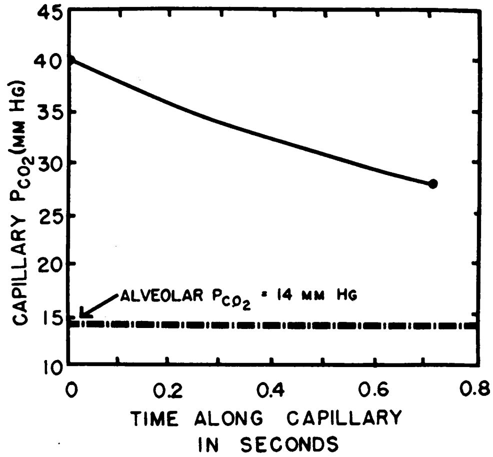

FIGURE 9 Time course of the fall in capillary P_{CO_2} as blood traverses the pulmonary capillaries during carbonic anhydrase inhibition in subject RWH at which time his $D_{L_{CO}}$ had fallen to 42 ml/(min × mm Hg). Note the large alveolar to end-capillary CO_2 gradient of 14 mm Hg. The P_{CO_2} of 40 mm Hg at the start of the capillary was measured with the rebreathing technique (10). Alveolar P_{CO_2} of 14 mm Hg is an approximation on the basis of an observed end-tidal P_{CO_2} varying between 11 and 15 mm Hg. Slope of the CO_2 dissociation curve was assumed to be 0.006-ml STPD per mm Hg (16), and Vc was assumed to have increased from the resting value of 112–130 ml on the basis of the measured increase in carbon monoxide diffusing capacity during carbonic anhydrase inhibition. Pulmonary capillary transit calculated from the relationship $V_c \div \dot{Q}_c$ equalled 0.715 sec.

Figure 8 curve B shows the changes in capillary P_{CO_2} which would have taken place in a normal subject if acetazolamide administration had not been accompanied by a decrease in alveolar P_{CO_2}. In order to have a sufficient alveolar-capillary pressure gradient to eliminate the body's production of CO_2, the arterial P_{CO_2} would have increased from 40 to 44 mm Hg and the end-capillary to alveolar CO_2 gradient would have been 4 mm Hg.

Effect of carbonic anhydrase inhibition on patients with compromised lung function. Carbonic anhydrase inhibitors have been advocated for use in patients with chronic obstructive lung disease in order to improve arterial blood oxygenation and lower the arterial P_{CO_2} (34, 35). The rationale of this therapy has been disputed (36). In order to evaluate the effect of carbonic anhydrase inhibition in CO_2 elimination in the presence of severe pulmonary disease, we calculated the capillary P_{CO_2} for a hypothetical subject who was unable to lower his alveolar P_{CO_2} below 40 mm Hg, and who, in addition, had values for $D_{M_{CO_2}}$ and Vc one-third of normal (Figure 8 curve C). Pulmonary capillary blood flow does not usually decrease strikingly in pulmonary disease so that the capillary transit time equal to $V_c/\dot{Q}_c$ is considerably reduced. Under these conditions during severe carbonic anhydrase inhibition a steady state for CO_2 elimination would not be reached until the end-capillary P_{CO_2} had risen from 40.3 to 63 mm Hg, a rise large enough to have significant physiological and clinical consequences. Since some patients with pulmonary disease cannot hyperventilate in order to compensate for the less efficient pulmonary elimination of CO_2 secondary to carbonic anhydrase inhibition, it is not surprising that occasionally symptoms suggestive of increased acidosis and clinical deterioration may accompany therapy with these drugs (35, case 10, 37). However, clinically much smaller doses of acetazolamide are administered than were used in this study so that the increase in the alveolar to end-capillary CO_2 gradient in patients is likely to be negligible or at least considerably smaller than the large gradient calculated to have developed in this hypothetical patient. While carbonic anhydrase inhibitors may cause hyperventilation and thereby lower the arterial and alveolar P_{CO_2} in many patients, even in normal subjects modest amounts of these agents are known to impair CO_2 elimination (38).

Validity of the measurement of hydrogen ion concentration in lung tissue. Intracellular electrodes, indicators, and the dissociation of weak bases and acids (39–41) have all been used to measure intracellular pH. In this study the lung pH was measured by the third technique using the bicarbonate and carbonic acid system. This method, which was first used by Warburg in 1922 (41) has given discordant values because of the difficulty in measuring intracellular bicarbonate levels. For example, in rat muscle the acid-labile CO_2 fraction is almost twice as large as the barium soluble fraction, which suggests that significant amounts of intracellular CO_2 may be present in nonbicarbonate fractions such as CO_2 protein complexes (42). In the present study we measured the total CO_2 in the lungs that exchanges with alveolar gas in a second or less before and after carbonic anhydrase inhibition and assumed that the difference must be the total bicarbonate in the lungs.

This method has several limitations and sources of error. First, the method is only applicable to a tissue such as the lungs where very rapid exchange of labeled CO_2 facilitated by carbonic anhydrase takes place (4). Second, if carbonic anhydrase inhibition is incomplete, the bicarbonate fraction will be underestimated. Fortunately in this study, 80% of the CO_2 exchange was blocked by the inhibitor, and since 9% of the CO_2 exchange could be assigned to dissolved CO_2, the error due to incomplete inhibition could not exceed 11% of the total CO_2 space. Third, carbonic anhydrase inhibition may produce a change in the bicarbonate and intracellular nonbicarbonate bound CO_2. We have no way to evaluate this source of error except to point out that several hours after the administration of a carbonic anhydrase inhibitor, the CO_2 content of blood changes only slightly (7). Fourth, the toxic effects during carbonic anhydrase inhibition were severe enough in one human to make us reluctant to administer large doses of the inhibitor to others, and, therefore, we have data on only one subject. Fifth, evidence has been recently presented in vitro that carbonic anhydrase, in addition to catalyzing the conversion of CO_2 to bicarbonate, directly facilitates the transport of molecular CO_2 (43, 44). If this action of carbonic anhydrase is of significance in vivo, the CO_2 exchange space in the lungs blocked by carbonic anhydrase inhibition might be greater than the bicarbonate space. This would produce an underestimation of the hydrogen ion concentration. However, diffusion distances in the lung parenchyma are generally so small, that a carrier enzyme could not appreciably increase the size of the CO_2 space. Sixth, the concentration of CO_2 in the lungs that is neither bicarbonate nor dissolved CO_2 may vary widely among individuals and may be influenced by acetazolamide.

Since none of the above limitations to this method of determining tissue pH appear insurmountable, we believe it deserves further evaluation and refinement in the experimental animal and isolated tissue preparations.

Dimensions and dynamics of CO_2 stores in the lungs of man. The data presented above support the findings of a number of investigators (3–5), that the various forms of carbon dioxide present in the lungs exchange so rapidly, that the alveolar Pco_2 can be considered to come from one common CO_2 pool. Representative figures for an adult human with a resting lung volume of 3000-ml STPD at an alveolar Pco_2 of 40 mm Hg are: 150 ml of CO_2 in the alveolar gas, 200 ml of CO_2 in the pulmonary tissue (V_{tis}), and 50 ml of CO_2 in the capillary blood (Vc). At least 70% of the CO_2 in Vc and V_{tis} is in the form of bicarbonate. In the subjects in this study a change in alveolar Pco_2 of 1 mm Hg was accompanied by the movement of about 2 ml of CO_2 into or out of V_{tis} and Vc.

Calculations made by DuBois, Britt, and Fenn (45) have demonstrated the considerable ability of the CO_2 stores in V_{tis} and Vc to buffer changes in alveolar Pco_2. They showed that this buffering action of V_{tis} and Vc reduces the predicted variation in alveolar Pco_2 during the respiratory cycle in resting man from about 3 to 2 mm Hg. The CO_2 stores in V_{tis} and Vc must also buffer oscillations in alveolar Pco_2 secondary to the pulsatile nature of the pulmonary capillary blood flow. We estimate that this change in our subjects is in the order of 0.13 mm Hg between diastole and systole. Without this buffering capacity of V_{tis} and Vc this figure would be 0.18 mm Hg. Bosman, Lee, and Marshall have also presented evidence for this buffering mechanism for CO_2 during the cardiac cycle from measurements of instantaneous carbon dioxide exchange in the lungs using a body plethysmograph (30).

Individual variation of the CO_2 content and shape of the CO_2 dissociation curve of lung tissue. The five subjects in this study showed considerable differences in the CO_2 content of their lung tissue and the slope of its CO_2 dissociation curve which could not be explained by error in the method or difference in body size. For example the CO_2 content of V_{tis} + Vc at an alveolar Pco_2 of 45 mm Hg varied from 104 ml/m^2 of surface area in subject MAF to 164 ml/m^2 in RWH. This is a determination with an experimental error which we believe is less than 20% of the measurement. Other investigators using different techniques have also noted similar variations in these quantities (19, 20). Therefore, the pulmonary intracellular CO_2 concentration most likely has a wider range of normal values than observed in plasma.

Validity of calculating CO_2 content of pulmonary capillary blood from alveolar PCO_2 during breath holding. Recently several investigators have reported that virtual mixed venous Pco_2 obtained by rebreathing is greater than the mixed venous Pco_2 measured from samples of pulmonary arterial blood even when pulmonary arterial and alveolar Po_2 are the same (47, 48). The effect of this interesting finding on the pulmonary capillary CO_2 content which was required in this study for the calculation of $\dot{Q}c_{CO_2}$ and the alveolar end-capillary CO_2 gradient, has not been clarified at the present time. Fortunately the shape of the CO_2 dissociation curve for blood is such that changes in Pco_2 produce proportionally less changes in CO_2 content. For example a 10% elevation in the rebreathing Pco_2, which is the approximate magnitude suggested by these reports, will at most produce an overestimation of capillary CO_2 content of 4% and would give the false impression that alveolar P*co_2 was 4% greater than end-capillary P*co_2. This source of error is therefore small enough not to alter the conclusions reached in this study.

APPENDIX I

Method for the determination of the slope of the CO_2 dissociation curve of the pulmonary tissues. The subject rebreathed several breaths from a 2 liter bag containing 8% CO_2 in order to make alveolar Pco_2 equal to virtual mixed venous Pco_2(PMV_{CO_2}). He then maximally inspired a breath of room air, held his breath for 3 sec, and then delivered an alveolar gas sample. Pco_2 of the expired gas sample was determined from the output of the continuously recording mass spectrometer. Every 15 min the procedure was repeated until three to six determinations of Pco_2 had been made with time of breath holding varying from 2 to 14 sec. During breath holding CO_2 will leave V_{tis} and Vc, enter the alveoli, and a new equilibrium between Pco_2 in the alveolar gas, Vc and V_{tis} will be reached at a Pco_2 lower than PMV_{CO_2} (21, 22). This equilibration takes place in less than 1–2 sec and the amount of CO_2 entering the alveoli will be a function of the CO_2 dissociation curves of V_{tis} and Vc (5, 19). The CO_2 contained in the residual volume (RV), V_{tis}, and Vc before inspiration plus any CO_2 contained in the inspired gas will equal the CO_2 contained in the alveolar volume, V_{tis} and Vc immediately after inspiration or

$$\frac{P_{MV_{CO_2}}}{P_B - P_{H_2O}}(RV) + CO_2 \text{ content of } Vc + V_{tis} \text{ at } P_{MV_{CO_2}} + \frac{P_{insp_{CO_2}}}{P_B - P_{H_2O}}(IV - DS) = \frac{P_{equil_{CO_2}}}{P_B - P_{H_2O}}(RV + IV - DS) + CO_2 \text{ Content of } Vc + V_{tis} \text{ at } P_{equil_{CO_2}}$$

where RV and IV are respectively the residual volume and the inspired volume in ml STPD, $P_{equil_{CO_2}}$ is the partial pressure of CO_2 in the alveoli just after inspiration in mm Hg, and $P_{insp_{CO_2}}$ is the partial pressure of CO_2 in mm Hg, if any, in IV and DS is the respiratory dead space in milliliters STPD. DS in milliliter BTPS was assumed to equal the subject's ideal weight in pounds (46). If $P_{equil_{CO_2}}$ can be measured, equation 7 can be solved for the CO_2 content of V_{tis} + Vc at $P_{equil_{CO_2}}$.

$P_{equil_{CO_2}}$ was determined in the following manner: the difference between the P_{CO_2} of the expired gas samples and mixed venous P_{CO_2} was plotted against time of breath holding on semilog paper and the line drawn through the points was extrapolated back to zero time. Plotting the points in this way should theoretically, and in practice did, produce a straight line function (21, 22) (Fig. 10). The P_{CO_2} at the extrapolated zero time under these conditions represented $P_{equil_{CO_2}}$. The same procedure was repeated for each subject using an inspired gas containing 12–15% CO_2 so that a point on the CO_2 dissociation curve of V_{tis} + Vc could be determined at a P_{CO_2} greater than $P_{MV_{CO_2}}$.

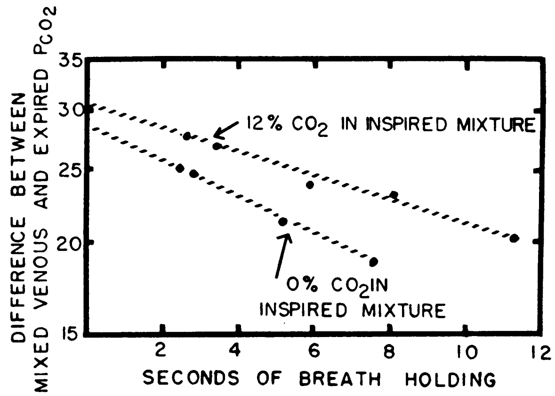

FIGURE 10 Graph of data used to determine $P_{equil_{CO_2}}$ from the rate of change of alveolar P_{CO_2} during breath holding after inhaling 12% CO_2 (upper line) and 0% CO_2 (lower line) in subject WFR. Alveolar gas samples were collected after breath holding periods varying from 2.4 to 11.4 sec. The difference between alveolar P_{CO_2} and $P_{MV_{CO_2}}$ was plotted against time of breath holding on semilog paper and a line drawn through the points was extrapolated back to the start of breath holding. In this subject after inspiring 12% CO_2 $P_{equil_{CO_2}}$ minus the $P_{MV_{CO_2}}$ of 48 mm Hg was 31 mm Hg so that $P_{equil_{CO_2}}$ equalled 48 plus 31 or 79 mm Hg. After inspiring air (lower line in the figure) the difference was 28 mm Hg so that $P_{equil_{CO_2}}$ equalled 48 minus 28 or 20 mm Hg.

APPENDIX II

Method for determining the changes in intracapillary P_{CO_2} as blood flows through the pulmonary gas exchange vessels. If it is assumed that: (*a*) alveolar P_{CO_2} ($P_{A_{CO_2}}$) is constant during the capillary transit time; (*b*) diffusing capacity for CO_2 ($D_{L_{CO_2}}$) is evenly distributed along the capillary; (*c*) $D_{L_{CO_2}}$ and pulmonary capillary blood flow are evenly distributed; and (*d*) the diffusing capacity for CO_2 within the blood (θ_{CO_2}) is constant, then:

$$(P_{C_{CO_2}} - P_{A_{CO_2}})D_{L_{CO_2}} = \frac{d}{dt}[CO_2]Vc \qquad (1)$$

where $P_{C_{CO_2}}$ equals the intracapillary P_{CO_2} at any instant in mm Hg, $[CO_2]$ is the CO_2 content of 1 ml of blood in milliliter STPD and Vc is the pulmonary capillary blood volume in milliliter. $D_{L_{CO_2}}$ is expressed in ml/(min × mm Hg). Since the CO_2 dissociation curve is almost linear over the interval between mixed venous P_{CO_2} and end-capillary P_{CO_2}, $[CO_2]$ can be said to equal $(K)P_{C_{CO_2}} + K_1$ where K is the slope of the CO_2 dissociation curve in milliliter of CO_2 per milliliter of blood per mm Hg change in $P_{C_{CO_2}}$ and K_1 is a constant which will disappear during differentiation. Substituting this expression into equation 1 and integrating gives:

$$\frac{P_{C_{CO_{2_0}}} - P_{A_{CO_2}}}{P_{C_{CO_{2_t}}} - P_{A_{CO_2}}} = e^{\frac{t\,D_{L_{CO_2}}}{(K)Vc(60)}} \qquad (2)$$

where t equals the time along the capillary in seconds, $P_{C_{CO_{2_0}}}$ equals the intracapillary P_{CO_2} at the beginning the capillary, and $P_{C_{CO_{2_t}}}$ equals the $P_{C_{CO_2}}$ at time t.

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