Measurement of O₂ Diffusing Capacity of the Lungs with a Stable O₂ Isotope*

R. W. HYDE,† R. E. FORSTER, G. G. POWER,‡ J. NAIRN,§ AND R. RYNES

(From the Department of Physiology, Division of Graduate Medicine, and the Department of Medicine, University of Pennsylvania, Philadelphia, Pa.)

In 1946 Lilienthal and his colleagues (1) described an ingenious technique for determining the O₂ diffusing capacity of the lungs. Their method required the measurement of mixed venous and arterial oxygen pressure (P_{O₂}) while the subject was breathing air as well as breathing 12 to 14% O₂ and assumed that the diffusing capacity, cardiac output, and venous admixture were constant during the studies.

The great difficulty in this method of measuring pulmonary diffusing capacity lay in the fact that the capillary blood becomes almost completely equilibrated with alveolar gas by the time it reaches the end of the capillary, rendering the calculated value of diffusing capacity exquisitely sensitive to the measured alveolar-arterial O₂ gradient (2, 3). A method based on the uptake of isotopic O₂ provides the greatest possible alveolar-end capillary O₂ gradient for a given total alveolar P_{O₂} and diffusing capacity, since the mixed venous blood enters the capillaries in effect at a much lower O₂ tension than is possible for the total mixed venous P_{O₂} to attain in life.

The rate of disappearance of radioactive O₂ from the lungs has been measured previously (4), but to our knowledge this information has not been used to calculate the O₂ diffusing capacity of the lungs. This paper describes a technique for determining the O₂ diffusing capacity from the rate of disappearance of a stable O₂ isotope,

³⁴O₂, from the lungs during breath holding. Many of the assumptions required for the older method are circumvented. The results with the newer technique are similar to those obtained by Lilienthal and associates (1). An additional finding was a considerable decrease of O₂ diffusing capacity (D_{LO₂}) with increasing O₂ tension.

Methods

Determination of D_{LO₂} at low alveolar P_{O₂} (approximately 42 mm Hg). The stable O₂ isotope of mass 34 was used in these studies (see Table I). This isotope can be produced inexpensively in the laboratory by the electrolysis of deuterium oxide (D₂O) enriched eightfold over the natural abundance with ¹⁸oxygen.¹ The actual experimental method consisted of having the seated subject rebreathe from a bag holding 2 to 3 L of a mixture containing approximately 8% CO₂ and 92% nitrogen for four to six breaths so that the P_{O₂} and P_{CO₂}, monitored by a rapidly responding mass spectrometer sampling at the mouthpiece, reached a plateau indicating that the P_{O₂} and P_{CO₂} in the bag and the alveoli were in equilibrium with the mixed venous blood (Figure 1). He then expired to residual volume and maximally inspired a gas mixture containing approximately 6.5% ³²O₂, 0.2% ³⁴O₂, 7% CO₂, 1% acetylene (C₂H₂), 0.5% neon (Ne), 0.4% carbon monoxide (CO), and the balance nitrogen. The partial pressures of O₂ and CO₂ in this mixture were chosen to equal those in the mixed venous blood. After breath holding for about 10 seconds, the subject forcefully expired. Note that the rebreathing maneuver and the inspired mixture made capillary P_{O₂} and P_{CO₂} constant at their respective mixed venous values during breath holding. As a result, capillary O₂ saturation is also constant along the capillary obviating the need for a Bohr integration procedure. Whereas the normal alveolar-capillary gradient for total P_{O₂} has been abolished, an alveolar-capillary gradient for ³⁴O₂ has been produced. The first liter of the expirate was discarded to wash out the respiratory dead space, and the remaining gas was collected for analysis. The P³²O₂ and P³⁴O₂ of the expired sample were measured on a mass spectrometer, and the other gases were measured on a gas chromatograph. The mixed venous P³²O₂, P³⁴O₂, and P_{CO₂} were determined either from the continuous mass spectrometer record of expired breath

* Submitted for publication October 18, 1965; accepted March 31, 1966.

This work was supported in part by a grant from the Life Insurance Research Fund.

† Deland Fellow of the American Philosophical Society. Address requests for reprints to Dr. R. W. Hyde, Dept. of Physiology, Division of Graduate Medicine, University of Pennsylvania, Philadelphia, Pa. 19104.

‡ This investigation was carried out during the tenure of a National Heart Institute postdoctoral fellowship.

§ Isaac Ott Research Fellow and Fellow of the William McCann Research Trust.

¹ ¹⁸Oxygen-enriched D₂O purchased from the Oak Ridge National Laboratory, Oak Ridge, Tenn.

TABLE I
Natural occurrence of stable isotopes of oxygen

Mass	Molecular structure	Occurrence as per cent of total oxygen*
32	$^{16}\text{O}-^{16}\text{O}$	99.518
33	$^{16}\text{O}-^{17}\text{O}$	0.075
34	$^{16}\text{O}-^{18}\text{O}$, $^{17}\text{O}-^{17}\text{O}$	0.407
35	$^{17}\text{O}-^{18}\text{O}$	0.000015
36	$^{18}\text{O}-^{18}\text{O}$	0.000042

* Calculated by law of probabilities from data of Nier (5), who reported occurrence of ^{16}O oxygen as 99.758% of total oxygen, ^{17}O oxygen as 0.0373% of total oxygen, and ^{18}O oxygen as 0.2039% of total O_2 . For these experiments O_2 of mass 34 was used as the tracer gas.

or by analyzing the contents of the rebreathing bag. The procedure was repeated after intervals of an hour for breath-holding periods of approximately 3, 7, or 14 seconds. The highly insoluble inert gas neon was used to correct for the initial dilution of the inspired gases in the residual volume (see Appendix I). Alveolar volume (V_A) was measured by adding the inspired volume to the residual volume, which had been determined previously by the closed-circuit helium method (6). The disappearance from the alveoli of acetylene, carbon monoxide, and $^{34}\text{O}_2$ in excess of mixed venous $^{34}\text{O}_2$ was plotted on semilogarithmic paper against time (Figure 2). From the C_2H_2 disappearance, pulmonary capillary blood flow ($\dot{Q}_c$) and pulmonary parenchymal tissue volume (V_t) were determined (7). Carbon monoxide diffusing capacity (DL_{CO}) was calculated from the CO disappearance (8).

FIG. 1. TRACING OF A MASS SPECTROMETER RECORD MONITORING MASS 32 O_2 DURING THE MEASUREMENT OF DL_{O_2} TIMED FROM THE START OF EXPIRATION. After the subject (RWH) had reached residual volume during the seventh second, he commenced rebreathing from a 3-L bag containing 92% N_2 and 8% CO_2 . The P_{O_2} became constant at about 42 mm Hg, and during the seventeenth second he inspired the test gas mixture enriched with $^{34}\text{O}_2$, held his breath for 6 seconds, and then exhaled delivering an alveolar sample. Note that the total P_{O_2} is kept almost constant at 42 mm Hg, whereas $P^{34}\text{O}_2$ falls to approximately one-half the value originally present in the inspired mixture. DL_{O_2} = lung O_2 diffusing capacity; P_{O_2} = oxygen pressure.

FIG. 2. GRAPH SHOWING THE DISAPPEARANCE OF C_2H_2 , LABELED O_2 ($PA^{34}\text{O}_2$ IN EXCESS OF MIXED VENOUS $P^{34}\text{O}_2$), AND CARBON MONOXIDE FROM THE ALVEOLAR GAS DURING BREATH HOLDING AT AN ALVEOLAR P_{O_2} OF 42 MM HG IN SUBJECT RWH. The line of least mean squares for each gas was determined from the four points resulting from four separate breath-holding periods. DL_{O_2} was calculated from the C_2H_2 and labeled O_2 lines, DL_{CO} from the CO line. The extension of the lines from the 3-second points to time zero is an extrapolation. The depressed intercept at time zero for acetylene is considered to be caused by the rapid uptake of C_2H_2 by the pulmonary parenchyma (7). The depressed intercept for labeled O_2 is considered to be caused by the initial uptake of the isotope by the blood in the pulmonary capillaries (see text). The gradual disappearance rate over 15 seconds is flow limited in the case of C_2H_2 , diffusion limited in the case of CO, and a combination of both flow and diffusion limited in the case of labeled O_2 . $PA^{34}\text{O}_2$ = alveolar $^{34}\text{O}_2$ pressure.

The decrease in $P^{34}\text{O}_2$ in the alveolar gas can be described by the following differential equation. The term on the left is the change in alveolar $^{34}\text{O}_2$ and those on the right the net exchange in the blood.²

$$\left(\frac{V_A}{P_B - P_{H_2O}} + \frac{V_t(\alpha_{O_2})}{760} \right) \frac{dPA^{34}\text{O}_2}{dt} = \frac{\dot{Q}_c(\alpha_{O_2} + \alpha_{O_2}) P_{mv}^{34}\text{O}_2}{760} - \frac{\dot{Q}_c(\alpha_{O_2} + \alpha_{O_2}) P_{c^{34}\text{O}_2} T_L}{760}, \quad [1]$$

where V_A equals the alveolar volume in milliliters STPD (standard temperature and pressure, dry) during breath holding. V_t equals the volume of the pulmonary tissues, α_{O_2} equals the Bunsen solubility coefficient for O_2 in V_t ³

² This equation does not take into account the fact that after the isotope leaves the alveoli it is temporarily stored in the pulmonary capillary blood before leaving the lungs. See Appendix II for a mathematical analysis that takes this factor into account.

³ We assumed that the solubility of O_2 in lung tissue (α_{O_2}) and the solubility of O_2 physically dissolved in blood (α_{O_2}) were the same, namely 0.0236 ml O_2 STPD per standard atmosphere per ml.

in milliliters STPD per milliliter of tissue per standard atmosphere, P_B is the barometric pressure in millimeters Hg, P_{H_2O} is the vapor pressure of water at body temperature in millimeters Hg, $P_{A^{34}O_2}$ is the partial pressure of $^{34}O_2$ in the alveolar gas in millimeters Hg, $\dot{Q}_c$ is the pulmonary capillary blood flow in milliliters per minute, ab_{O_2} is the effective solubility coefficient for O_2 in milliliters of O_2 STPD in blood in chemical combination with hemoglobin per milliliter of blood per standard atmosphere of O_2 ,⁴ α_{O_2} is the solubility coefficient for O_2 in milliliters of O_2 STPD physically dissolved in blood per milliliter of blood per standard atmosphere of O_2 ,³ $P_{mv^{34}O_2}$ equals the mixed venous $P^{34}O_2$ in millimeters Hg, and $P_{c^{34}O_2}T_L$ is the average partial pressure of $^{34}O_2$ inside the red blood cells at the end of the pulmonary capillaries in millimeters Hg.⁵

In Equation 1, the end capillary partial pressure of $^{34}O_2$ ($P_{c^{34}O_2}T_L$) does not necessarily equal that in the alveolar gas, $P_{A^{34}O_2}$, and therefore the equation cannot be solved without a further relation between these two partial pressures. Such a relation can be obtained by describing the partial pressure of the gas within a volume of blood as it moves along the capillary as a function of alveolar $P^{34}O_2$ and time. Unfortunately, since alveolar and end capillary $P^{34}O_2$ are both functions of time, the solution of the simultaneous differential equations is complicated (see Appendix II), but if simplifying assumptions are made so that *a*) the red blood cells travel through the pulmonary capillaries so rapidly that the alveolar partial pressure of $^{34}O_2$ is constant during the time of transit, and *b*) storage of $^{34}O_2$ in the capillaries is negligible, the solution becomes much simpler. These assumptions result in an error of less than 6% in the estimate of DL_{O_2} if the capillary transit time is 1.5 seconds or less.

If we also assume that DL_{O_2} and the pulmonary capillary blood volume (V_c) are evenly distributed along the length of the capillaries, the O_2 diffusing capacity of an infinitesimal

⁴ ab_{O_2} varies with the partial pressure of oxygen present because of the alinearity of the O_2 dissociation curve. Therefore, in the experiments in this paper ab_{O_2} was always defined for a particular alveolar P_{O_2} and was calculated by the following formula:

$$ab_{O_2} = \frac{[O_2Hb] 760}{P_{A_{O_2}}} = \frac{[O_2 \text{ cap}][\%O_2Hb] 760}{100 P_{A_{O_2}}},$$

where $[O_2Hb]$ equals the concentration of O_2 in milliliters STPD chemically bound in each milliliter of blood at the specified P_{O_2} , $P_{A_{O_2}}$ is the alveolar P_{O_2} in millimeters Hg, $[\%O_2Hb]$ equals the O_2 saturation of the capillary blood in per cent, and $[O_2 \text{ cap}]$ equals the O_2 capacity of the subject's blood in milliliters per milliliter. See text for technique of measuring $[O_2 \text{ cap}]$ and $[\%O_2Hb]$.

⁵ Recent studies suggest that gas exchange in the lungs takes place in pulmonary arteries and veins as well as capillaries (9, 10), but the significance of extra capillary gas exchange has been disputed (11). In order to avoid this alteration we have arbitrarily defined the pulmonary capillaries as being those pulmonary blood vessels in which significant gas exchange takes place between the blood and the gas in the lungs.

mal segment will be DL_{O_2} times dx/L and the volume, V_c times dx/L , where dx is an infinitesimal distance along the pulmonary capillary and L is the total length of the capillary. Then at any instant the change in $^{34}O_2$ concentration in a segment equals the amount of $^{34}O_2$ diffusing into the segment, or:

$$\frac{dx}{L} DL_{O_2} (P_{A^{34}O_2} - P_{c^{34}O_2}) = \frac{dx}{L} \frac{(ab_{O_2} + \alpha_{O_2}) V_c}{760} \frac{dP_{c^{34}O_2}}{dT_L}, \quad [2]$$

where DL_{O_2} is the diffusing capacity of the capillaries in milliliters STPD or $^{34}O_2$ per minute per millimeter Hg, V_c is the volume of blood in the capillaries in milliliters, $P_{c^{34}O_2}$ is the average capillary $P^{34}O_2$ inside the red blood cells in millimeters Hg at any instant, and T_L is the pulmonary capillary transit time in minutes. Note that dx/L cancels out. If $P_{A^{34}O_2}$ is considered constant during one capillary transit, integrating over the interval of one capillary transit time (T_L) gives the following solution:

$$\frac{P_{A^{34}O_2} - P_{c^{34}O_2}T_L}{P_{A^{34}O_2} - P_{c^{34}O_2}T_0} = \exp \left[\frac{-760(DL_{O_2})(T_L)}{V_c(ab_{O_2} + \alpha_{O_2})} \right]. \quad [3]$$

$P_{c^{34}O_2}T_0$ is the partial pressure in the capillary blood at the beginning of the capillary and is, therefore, equal to $P^{34}O_2$ in the mixed venous blood ($P_{mv^{34}O_2}$), and $P_{c^{34}O_2}T_L$ is that at the end of the capillary. The exponent in Equation 3 is a constant, and therefore the ratio on the left side of the equation will also be a constant. For convenience this ratio can be called K , or:

$$\frac{P_{A^{34}O_2} - P_{c^{34}O_2}T_L}{P_{A^{34}O_2} - P_{c^{34}O_2}T_0} = K. \quad [4]$$

Note that T_L/V_c in Equation 3 equals the reciprocal of the capillary blood flow ($1/\dot{Q}_c$). The substitution of Equation 4 into 3 and $1/\dot{Q}_c$ for T_L/V_c gives:

$$DL_{O_2} = \frac{\dot{Q}_c(ab_{O_2} + \alpha_{O_2})}{760} \ln \frac{1}{K}. \quad [5]$$

Equation 4 can be solved for $P_{c^{34}O_2}T_L$, substituted into Equation 1, and integrated, giving:

$$\frac{P_{A^{34}O_2}t - P_{c^{34}O_2}T_0}{P_{A^{34}O_2}t_0 - P_{c^{34}O_2}T_0} = \exp \left[\frac{-\dot{Q}(1-K)(ab_{O_2} + \alpha_{O_2})t_{BH}}{760 \left(\frac{V_A}{P_B - P_{H_2O}} + \frac{Vt(\alpha_{O_2})}{760} \right)} \right], \quad [6]$$

where $P_{A^{34}O_2}t_0$ is the alveolar $P^{34}O_2$ at the start of breath holding, $P_{A^{34}O_2}t$ is the alveolar $P^{34}O_2$ at the end of breath holding, and t_{BH} equals the time of breath holding in minutes.

From Equation 6, K can be calculated because all the other terms can be measured (see below). Once K is known, DL_{O_2} can be calculated from Formula 5.

In order to calculate αbO_2 , the oxygen capacity and oxygen saturation of the capillary blood had to be known. The oxygen capacity (O_2 cap) in units of milliliters O_2 STPD per milliliter of blood was determined by measuring the hemoglobin concentration of the subject's venous blood in grams per milliliter (12) and multiplying by the conversion factor 1.34 (3a). The per cent O_2 saturation ($\%\text{O}_2\text{Hb}$) was determined from the alveolar PO_2 and PCO_2 at the end of the period of breath holding and a standard oxygen hemoglobin dissociation curve (13).

One limitation of the above method of measuring DL_{O_2} is that the derivation is dependent on making the mixed venous PO_2 equal to the alveolar PO_2 so that DL_{O_2} could only be determined at the oxygen tension of mixed venous blood or approximately 40 mm Hg in our normal subjects. Therefore, the above method had to be modified to enable the determination of DL_{O_2} at an alveolar PO_2 higher than mixed venous PO_2 .

Determination of DL_{O_2} at high PO_2 (approximately 250 mm Hg). The experimental observations are made in exactly the same manner as for the measurement of DL_{O_2} at low PO_2 , except that the inspired gas mixture contained a higher concentration of O_2 . It consisted of 7% CO_2 , 35% or more $^{32}\text{O}_2$, approximately 1% $^{34}\text{O}_2$, 2% neon, 1% acetylene, 0.4% CO , and the balance N_2 . However, when PA_{O_2} is greater than mixed venous PO_2 , in addition to the diffusion of $^{34}\text{O}_2$ from the alveolar gas into the blood in exchange for $^{32}\text{O}_2$, total $\%\text{O}_2\text{Hb}$ increases along the capillary. Theoretically, this means that the solubility of $^{34}\text{O}_2$ in capillary blood (αbO_2), which equals $[\text{O}_2\text{Hb}]/760/\text{PCO}_2$ varies along the capillary, and Equation 2 cannot be integrated. In addition, DL_{O_2} may vary along the capillary because the rate of the reaction of O_2 with intracellular reduced hemoglobin decreases as PO_2 rises (14). One solution of this dilemma would be to perform a numerical integration of each case, since we would know the alveolar and capillary PO_2 , but this is extremely tedious and complicated (Appendix III). A second and approximate solu-

FIG. 3. GRAPH SHOWING THE RATE OF DISAPPEARANCE OF C_2H_2 , LABELED O_2 ($\text{PA}_{^{34}\text{O}_2}$ IN EXCESS OF MIXED VENOUS P^{34}O_2), AND CARBON MONOXIDE FROM THE ALVEOLAR GAS DURING BREATH HOLDING AT AN ALVEOLAR PO_2 OF 203 MM HG IN SUBJECT GGP. Note that compared to Figure 2, at this high PO_2 the labeled O_2 has a much slower rate of disappearance. This decrease is principally due to the lower effective solubility for O_2 (αbO_2) at the higher PO_2 .

TABLE II
Physical characteristics of experimental subjects

Subject	Age	Height	Weight	Surface area
	years	inches	lb	m^2
RR	24	72	175	2.01
GGP	28	73	175	2.03
RWH	34	71	160	1.94
REF	44	74	170	2.02
JCC	46	64	132	1.65

tion is to assume that the high alveolar PO_2 (250 mm Hg) will lead to rapid equilibration of the capillary blood with alveolar gas before the blood has travelled a significant distance along the capillary and then to use Equation 3 with the effective solubility for $^{34}\text{O}_2$ at a PO_2 of 250 mm Hg.

To justify this assumption, we performed a numerical integration (Bohr) for total PO_2 using the method given by Staub, Bishop, and Forster (14) for the case when alveolar PO_2 is 250 mm Hg and assuming values for the pertinent constants that are approximately average for our subjects (see Appendix III). We found the simplifying assumption to produce an underestimation of DL_{O_2} in the neighborhood of 10%. (For details of the numerical integration see Appendix III.)

Once it is assumed that the high alveolar PO_2 will lead to rapid equilibration of the capillary blood with alveolar gas before the blood has travelled a significant distance along the capillary, then the P^{34}O_2 in the red blood cells of the capillary blood immediately after the hemoglobin has become almost completely saturated ($\text{PC}^{34}\text{O}_2\text{T}_0$) can be determined by the following formula:

$$\text{PC}^{34}\text{O}_2\text{T}_0 = \frac{\%\text{O}_2\text{Hb}}{100} \text{Pmv}^{34}\text{O}_2 + \left(1 - \frac{\%\text{O}_2\text{Hb}}{100}\right) \text{PA}^{34}\text{O}_2, \quad [7]$$

where $\%\text{O}_2\text{Hb}$ equals the per cent of the hemoglobin of the mixed venous blood present as oxyhemoglobin.

Substitution of Equation 7 into Equation 4 so as to eliminate $\text{PC}^{34}\text{O}_2\text{T}_0$ gives:

$$\frac{\text{PA}^{34}\text{O}_2 - \text{PC}^{34}\text{O}_2\text{TL}}{\frac{\%\text{O}_2\text{Hb}}{100} [\text{PA}^{34}\text{O}_2 - \text{Pmv}^{34}\text{O}_2]} = K. \quad [8]$$

Equation 8 can be substituted into Equation 1 so as to eliminate $\text{PC}^{34}\text{O}_2\text{TL}$. Integration of the resultant equation gives:

$$\frac{\text{PA}^{34}\text{O}_2\text{t} - \text{Pmv}^{34}\text{O}_2}{\text{PA}^{34}\text{O}_2\text{t}_0 - \text{Pmv}^{34}\text{O}_2} = \exp \left[\frac{-\dot{Q}c(\alpha\text{bO}_2 + \alpha\text{O}_2) \left(1 - K \frac{\%\text{O}_2\text{Hb}}{100}\right) (t_{\text{BH}})}{760 \left(\frac{V_A}{P_B - P_{\text{H}_2\text{O}}} + \frac{Vt(\alpha\text{tO}_2)}{760}\right)} \right]. \quad [9]$$

The analyses were performed in the same manner as for the case at lower alveolar PO_2 , and the data were substituted into Equations 9 and then 5 to solve for DL_{O_2} (see Figure 3).

TABLE III
 Measurements obtained in all subjects*

Subject	PAO ₂	PACO ₂	O ₂ sat	Hgb	αbo ₂	VA	Q̇c	Vt	DL _{CO}	K	DL _{O₂} appx	DL _{O₂} (Vc = 60)
	mm Hg	mm Hg	%	g/100 ml	ml/ml	ml	ml/min	ml	ml/min × mm Hg		ml/min × mm Hg	ml/min × mm Hg
A. Mixed venous Po ₂												
RR	36.0	50	62.1	14.1	2.47	4,780	8,190	540	43.8	0.182	45.4	45.6
GGP	41.5	48	71.5	15.4	2.69	5,530	7,980	320	50.7	0.302	34.1	34.3
RWH	42.0	50	71.4	15.0	2.60	5,860	7,070	570	43.7	0.240	34.8	35.0
REF	49.0	50	78.5	15.8	2.58	5,760	6,200	710	40.4	0.333	23.3	23.3
JCC	43.5	48	74.0	14.1	2.44	5,600	6,310	720	42.5	0.284	25.7	25.8
B. Hypoxia												
GGP	28.5	34	59.4	15.8	3.35	4,790	11,280	678	46.1	0.487	36.0	36.0
C. Hyperoxia												
GGP	203	47	99.8	14.5	0.724	5,400	7,730	648	28.6	0.316	8.8	
RWH	205	50	99.8	14.1	0.701	5,340	8,020	485	27.3	0.436	6.1	
REF	250	50	99.8	15.7	0.639	6,020	6,870	440	26.1	0.663	2.5	

* Abbreviations: PAO₂ = alveolar oxygen pressure (Po₂); O₂ sat = O₂ saturation of pulmonary capillary blood; Hgb = hemoglobin concentration of venous blood; αbo₂ = effective solubility of O₂ in capillary blood; VA = alveolar volume; Q̇c = pulmonary capillary blood flow; Vt = pulmonary tissue volume; DL_{CO} = single breath CO diffusing capacity; K = (alveolar P⁵⁰_{O₂} - end capillary P⁵⁰_{O₂}) ÷ (alveolar P⁵⁰_{O₂} - precapillary P⁵⁰_{O₂}); DL_{O₂} appx = single breath O₂ diffusing capacity calculated by approximation method described in text; and DL_{O₂} (Vc = 60) = single breath O₂ diffusing capacity calculated by the method described in Appendix II assuming a capillary blood volume of 60 ml.

Subjects. The subjects in this study were healthy male laboratory personnel familiar with the performance of respiratory maneuvers. Their physical characteristics are given in Table II. All measurements were made in the sitting position after the subject had rested for 5 minutes. The inspired volume during the three to four breath-holding periods performed for each measurement of DL_{O₂} differed by no more than 300 ml.

Results

Measurement of lung diffusing capacity at mixed venous O₂ pressures. In all five subjects DL_{O₂} was initially measured at an alveolar O₂ tension at or near their mixed venous Po₂, which was approximately 42 mm Hg (see Table III, A). The average DL_{O₂} was 33 (SD ± 8) ml per

(minute × mm Hg), and the alveolar to end capillary ³⁴O₂ gradient as a percentage of the gradient present at the start of the capillary was 27% (SD = 5%). Figure 4 shows that DL_{O₂} falls with increasing alveolar Po₂.

The average value for the simultaneously measured carbon monoxide diffusing capacity (DL_{CO}) was 44.2 (SD ± 3.7) ml per (minute × mm Hg). In all but one subject DL_{CO} was greater than DL_{O₂}. In three of the subjects (RWH, REF, and GGP), DL_{CO} was also measured by the standard breath-holding technique (8) at an alveolar Po₂ of about 110 mm Hg. The DL_{CO} for the three subjects at the higher Po₂ were 36.8, 28.4, and 41.6 ml per (minute × mm Hg), respectively, which are 21% (SD = 6%) lower than the measurements made at mixed venous Po₂. This difference is presumably secondary to the faster carbon monoxide-hemoglobin reaction rate at mixed venous Po₂ (15) or the higher alveolar PCO₂ present during the measurements at the lower Po₂ (16).

Measurement of O₂ diffusing capacity of the lungs at a hypoxic level. In one subject an additional determination of DL_{O₂} was made after breathing 9% O₂, 91% N₂ for 10 minutes before the breath-holding maneuver (see Table III, B). The hypoxic breathing lowered the mixed venous Po₂ from 41.5 mm Hg to 28.5 mm Hg and the mixed venous PCO₂ from 48 mm Hg to 34 mm Hg. Pulmonary capillary blood flow increased from 8.0 to 11.3 L minute. There was a slight rise in

FIG. 4. FALL IN DL_{O₂} WITH INCREASING ALVEOLAR PO₂. The lines connect measurements of DL_{O₂} performed in the same subjects but at different PAO₂.

DL_{O_2} from 34.1 to 36.0 ml per (minute $\times$ mm Hg) and a slight fall in DL_{CO} from 50.7 to 46.1 ml per (minute $\times$ mm Hg). This experiment indicates that a fall in alveolar PO_2 from 41.5 to 28 mm Hg is not accompanied by much change in DL_{O_2} or DL_{CO} , which is not surprising because over this range of alveolar PO_2 there is little change in the reaction rate of O_2 and CO with hemoglobin (14, 15).

Measurements of O_2 diffusing capacity of the lungs at elevated alveolar O_2 tensions. In three of the subjects additional measurements of DL_{O_2} and DL_{CO} were made at an alveolar PO_2 of approximately 220 mm Hg (see Table III, C). This high alveolar PO_2 during breath holding was produced by increasing the concentration of O_2 in the inspired test gas from approximately 7% to about 50%. Compared with measurements made at mixed venous PO_2 no marked changes in pulmonary capillary blood flow were found, but DL_{O_2} decreased from an average value of 30.7 (SD $\pm$ 5.3) ml per (minute $\times$ mm Hg) to 5.8 (SD $\pm$ 2.6), and DL_{CO} fell from 44.9 (SD $\pm$ 4.3) to 27.3 (SD $\pm$ 3.2) ml per (minute $\times$ mm Hg). The fall in DL_{CO} with this change in PO_2 is of the same order of magnitude as observed

before (15), but the low value of DL_{O_2} at increased alveolar PO_2 has not been noted previously to our knowledge and would be expected to be present on a theoretical basis (14).

Calculation of pulmonary capillary blood volume from the initial rate of O_2 isotope disappearance. It has been previously shown that a soluble inert gas equilibrates extremely rapidly (in less than a second) with the finer parenchymal tissues of the lung (7), and that the initial disappearance of such a gas can be used to calculate the pulmonary parenchymal tissue volume. Experimentally the logarithm of alveolar inert gas concentration is plotted against time of breath holding and the curve extrapolated to time zero. Any rapid initial solution of inert gas in the lung tissue will be seen as a depression of the intercept 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 $^{34}\text{O}_2$, although a small amount will dissolve in the tissue, a larger amount will combine with the hemoglobin in the capillaries. If we assume that the latter process is complete in 3 seconds (our shortest period of breath holding), then:

$$\frac{\text{isotope intercept at time zero in per cent}}{100}$$

$$= \frac{V_A}{V_A + (\alpha_{\text{O}_2})(V_t) \left[\frac{(\text{P}_B - \text{P}_{\text{H}_2\text{O}})}{760} \right] + (\alpha_{\text{bO}_2} + \alpha_{\text{O}_2})(V_c) \left[\frac{(\text{P}_B - \text{P}_{\text{H}_2\text{O}})}{760} \right]}, \quad [10]$$

where the terms in the denominator on the right side of the equation represent the effective volume into which the $^{34}\text{O}_2$ may go. Solving for V_c gives

$$V_c = \frac{V_A \left(\frac{100}{\text{isotope intercept at time zero in per cent}} - 1 \right) \left(\frac{760}{\text{P}_B - \text{P}_{\text{H}_2\text{O}}} \right) - (\alpha_{\text{O}_2})(V_t)}{\alpha_{\text{bO}_2} + \alpha_{\text{O}_2}}. \quad [11]$$

Values for V_c in our subjects are shown in Table IV. Like the results for V_t by the C_2H_2 method, the measurement of V_c by this method is markedly affected by slight variations in the intercept. Therefore, the calculated value for any single determination of V_c has a large standard deviation (128 ml). In comparison, measurements of V_c made by determining $\text{DL}_{\text{CO}}\text{SB}$ at different alveolar PO_2 in our hands has a standard deviation of approximately 20 ml and in addition

is technically a much less difficult measurement to perform. We, therefore, do not believe V_c calculated from the disappearance of $^{34}\text{O}_2$ will be of much practical value. But the average V_c of 127 ml for all nine determinations is in reasonably good agreement with the results reported by others using different methods (17, 18) and lends strength to the belief that in normal resting subjects V_c is approximately 100 ml.

TABLE IV

Calculation of pulmonary capillary blood volume (V_c) from the initial rapid disappearance of alveolar $^{34}\text{O}_2$

Subject	Alveolar Po_2	Isotope intercept at time zero	V_c^*
	<i>mm Hg</i>	<i>%</i>	<i>ml</i>
RR	36.0	99.2	+13
GGP	28.5	87.2	+217
	41.5	95.2	+107
	203.0	99.8	-5
RWH	42.0	96.0	+63
	205.0	94.9	+407
REF	49.0	99.0	+18
	250.0	99.2	+63
GC	43.5	91.0	+233

* Average: 127 (SD = 128).

Discussion

Previously reported methods of measuring O_2 diffusing capacity ($\text{DL}_{\text{O}_2}\text{SS}$) have required the estimation of O_2 uptake, alveolar Po_2 , and end capillary Po_2 during steady state (SS) breathing of the test gas mixture. The measurement of O_2 uptake has been no problem, but considerable difficulty has been encountered in the measurement of PA_{O_2} and especially end capillary Po_2 . Although ingenious methods have been devised to measure these quantities, they have limitations that have been reviewed in detail elsewhere (19). The measurement of DL_{O_2} during breath holding with $^{34}\text{O}_2$ ($\text{DL}_{\text{O}_2}\text{SB}$) avoids some of the problems encountered with the older steady state methods. For instance, $\text{DL}_{\text{O}_2}\text{SB}$ does not require the direct measurement of the alveolar-arterial O_2 gradient and the separation of this gradient into its three sources, namely venous-arterial shunting, ventilation-perfusion imbalances, and diffusion. Since $\text{DL}_{\text{O}_2}\text{SB}$ is measured over a short time interval, errors resulting from the failure to achieve a steady state are minimized.

Another advantage of the single breath method results from the fact that during the measurement pulmonary capillary Po_2 , PCO_2 , and hemoglobin saturation are kept constant, whereas in the determination of $\text{DL}_{\text{O}_2}\text{SS}$, O_2 saturation is increasing as blood traverses the capillary. Because of the changing O_2 saturation, $\text{DL}_{\text{O}_2}\text{SS}$ progressively decreases along the capillary (14). This decrease takes place be-

cause the rate at which O_2 is taken up by the red blood cells (θ) is a function of the O_2 saturation and tends to fall progressively as O_2 saturation rises over 70% (14). In the measurement of $\text{DL}_{\text{O}_2}\text{SB}$, O_2 saturation and θ are constant, and thereby diffusing capacity remains constant along the capillary. Therefore, the complexities resulting from θ and diffusing capacity being variable functions are avoided. In addition the analyses of the complex physical-chemical processes taking place during the movement of O_2 onto the hemoglobin molecule within the red cell are simplified because oxyhemoglobin and reduced hemoglobin remain constant (20, 21).

On the other hand, $\text{DL}_{\text{O}_2}\text{SB}$ has some problems, namely that it requires breath holding, which is unphysiological and may itself alter the pulmonary capillary blood flow and diffusing capacity. In addition, the method requires analyses of O_2 isotopes, which is costly and time consuming.

Despite the markedly different techniques required for the measurement of $\text{DL}_{\text{O}_2}\text{SB}$ and $\text{DL}_{\text{O}_2}\text{SS}$, the average $\text{DL}_{\text{O}_2}\text{SB}$ of 33 ml per (minute $\times$ mm Hg) obtained at mixed venous Po_2 in this study is only moderately larger than the values reported by several investigators for $\text{DL}_{\text{O}_2}\text{SS}$ in normal resting subjects. For example, values for $\text{DL}_{\text{O}_2}\text{SS}$ of 21 ml per (minute $\times$ mm Hg) (1) and 26 ml per (minute $\times$ mm Hg) (22) have been reported. The discrepancy between the two methods is similar to that seen when comparing the single breath DL_{CO} ($\text{DL}_{\text{CO}}\text{SB}$) with the steady state DL_{CO} methods ($\text{DL}_{\text{CO}}\text{SS}$) (23). Factors that are believed to affect the various carbon monoxide diffusion methods in different ways, such as lung volume or uneven distribution of ventilation and alveolar volume to diffusing capacity (24) could also explain the difference between the two oxygen diffusion methods. On a theoretical basis, one would expect a slight difference between $\text{DL}_{\text{O}_2}\text{SS}$ and $\text{DL}_{\text{O}_2}\text{SB}$ because the latter method uses O_2 of a heavier mass, which would be expected to diffuse more slowly (3b). This difference in mass, which would make $\text{DL}_{\text{O}_2}\text{SS}$ greater than $\text{DL}_{\text{O}_2}\text{SB}$ probably produces a difference in the order of 2% (25) and is thereby insignificant compared with other variables associated with both methods.

In contrast to the above measurements Lam-

bertsen and co-workers found $\text{DL}_{\text{O}_2}\text{SS}$ to be 60 ml per (minute $\times$ mm Hg) (26). More recently Cosby and his colleagues, while using a continuously recording intra-arterial oxygen polarograph, found even smaller alveolar-arterial O_2 gradients at hypoxic levels (27). This finding suggests that $\text{DL}_{\text{O}_2}\text{SS}$ may be even higher than reported by Lambertsen's group. In addition, their data showed that during the breathing of 12 to 16% O_2 , a steady state for alveolar and arterial PO_2 was not obtainable within 10 minutes. Because the determination of $\text{DL}_{\text{O}_2}\text{SS}$ with reasonable precision requires a steady state and an alveolar-arterial gradient of sufficient size to permit accurate measurement, the above information casts doubt on the validity of measurements of $\text{DL}_{\text{O}_2}\text{SS}$ in resting man.

Several authors have described a method for indirectly calculating $\text{DL}_{\text{O}_2}\text{SB}$ from measurements of $\text{DL}_{\text{CO}}\text{SB}$ (14, 28). In resting man at an alveolar PO_2 of 42 mm Hg this calculation shows that $\text{DL}_{\text{O}_2}\text{SB}$ should be considerably higher than $\text{DL}_{\text{CO}}\text{SB}$. In our subjects $\text{DL}_{\text{O}_2}\text{SB}$ measured directly with $^{34}\text{O}_2$ was on the average lower than $\text{DL}_{\text{CO}}\text{SB}$.

There are several possible explanations for the above discrepancies, one of which is an absolute error produced by error in the data used for the calculation. It is difficult to estimate the summated error in $\text{DL}_{\text{O}_2}\text{SB}$ produced by errors in the data, in part because of the complicated nature of Equations 5 and 6. Experimentally determined variation in $\text{DL}_{\text{O}_2}\text{SB}$ is not available because of the difficulty in making the measurements. Therefore, we have attempted to make a reasonable estimate of the reliability of $\text{DL}_{\text{O}_2}\text{SB}$ from the variability of the component measurements.

Reproducibility of measurements with the gas chromatograph and mass spectrometer used for determining $\text{DL}_{\text{O}_2}\text{SB}$ was within 1%. Over-all accuracy was checked by diluting the inspired gas mixture with an equal volume of air or 7% oxygen in nitrogen and then calculating from the neon dilution the total amount of C_2H_2 and $^{34}\text{O}_2$ in excess of natural abundance recovered. Recovery was between 101% and 99%. Random errors in the analysis were minimized by doing the gas chromatographic measurements in duplicate and the measurement of the ratio of $^{34}\text{O}_2$ to

total O_2 on the mass spectrometer in quintuplicate. Random variation was further reduced by calculating $\text{DL}_{\text{O}_2}\text{SB}$ from the slope of 3 to 4 breath-holding points (see Figures 2 and 3) instead of depending on one breath-holding sample.

Since the measurement of $\text{DL}_{\text{O}_2}\text{SB}$ is in essence calculating the effective solubility of $^{34}\text{O}_2$ by comparing its disappearance rate with that of C_2H_2 , whose solubility is known, errors in alveolar volume and time of breath holding will affect the slopes of C_2H_2 and $^{34}\text{O}_2$ in excess of mixed venous $^{34}\text{O}_2$ to the same degree and thereby tend to cancel each other out and not be critical in the determination of $\text{DL}_{\text{O}_2}\text{SB}$.

Two items in Equations 5 and 6 are so small that they can almost be neglected. Certainly, variations in them will have little effect on $\text{DL}_{\text{O}_2}\text{SB}$. These are αO_2 and $(\text{Vt})\alpha\text{O}_2/760$. The following items are possible sources of large errors in the calculation:

a) αbo_2 is calculated from the relation $[\text{O}_2 \text{ cap}] [\% \text{O}_2 \text{Hb}] 760/100 \text{ PAO}_2$. The $[\% \text{O}_2 \text{Hb}]/\text{PAO}_2$ is obtained from a dissociation curve (13). $[\text{O}_2 \text{ cap}]$ is calculated from the subject's venous oxyhemoglobin concentration. The pulmonary hematocrit may be less than in the peripheral blood (29). However, this represents a pooling of plasma in the pulmonary vasculature, and the dynamic hematocrit, that is the ratio of cell volume to plasma volume flowing past a point in the pulmonary circulation, is the same as that in the mixed venous blood and in the aorta (30). Since it is the dynamic hematocrit that we need in calculating αbo_2 , the observed low values of pulmonary hematocrit do not affect our calculations. We estimate the accuracy of the measurement of αbo_2 to be in the order of $\pm 2\%$.

b) $\dot{Q}_c$ depends upon the ratio of expired alveolar $[\text{C}_2\text{H}_2]$ to inspired $[\text{C}_2\text{H}_2]$ and the solubility coefficient for C_2H_2 in blood ($\alpha\text{bc}_2\text{H}_2$). Errors in the analysis of C_2H_2 are critical, and we estimate our experimental error in the ratio to about $\pm 2\%$. An error in $\alpha\text{bc}_2\text{H}_2$ would produce an error in $\text{DL}_{\text{O}_2}\text{SB}$ of the same magnitude as an error in αbo_2 (see Tables V and VI), and our estimate of the error in $\alpha\text{bc}_2\text{H}_2$ is the same.

c) $\text{PA}^{34}\text{O}_{2t_0}$ and $\text{PA}^{34}\text{O}_{2t}$, the alveolar P^{34}O_2 at the start of breath holding and the alveolar P^{34}O_2 at the end of breath holding, respectively, were

TABLE V

Effect of variation in component measurements on calculated single breath lung O_2 diffusing capacity at mixed venous oxygen pressures*

Measurement	Variation	Recalculated $DL_{O_2}SB$	Per cent change
	%	ml/(min $\times$ mm Hg)	
1. αBO_2	+2	33.5	-2
	-2	34.9	+2
2. Inspired $[C_2H_2]$	+2†	31.0	-9
Expired $[C_2H_2]$	-2†	36.2	+6
3. a. $[^{34}O_2]$	+2‡	37.2	+9
Total $[O_2]$	-2‡	31.4	-8
b. Total inspired $[O_2]$	+1†	36.5	+7
Total alveolar $[O_2]$	-1†	31.4	-8
c. Inspired [neon]	+1†	34.5	+1
Alveolar [neon]	-1†	33.9	-1
4. Mixed venous $P^{34}O_2$	+10	36.5	+7
	-10	32.3	-6

* Data used were for subject GGP, whose $DL_{O_2}SB$ was 34.14 ml per (minute $\times$ mm Hg) at an alveolar P_{O_2} of 41.5 mm Hg. SB = single breath.

† The change in the longest breath-holding point (13.2 seconds) produced by this variation was calculated, and the line of least mean squares at this point was then moved up or down by the same amount. The point on the regression line at time zero was kept constant to assure a change in its slope. $DL_{O_2}SB$ was then recalculated with data computed from the adjusted line.

‡ Two per cent change was produced by increasing the inspired sample by 1% and decreasing the alveolar sample by 1% for a plus error and the converse for a minus error.

§ An increase of 1% in $[^{34}O_2]/total [O_2]$ in the alveolar sample and a decrease of 1% in $[^{34}O_2]/total [O_2]$ in the inspired sample was produced (see Equation 16) for a plus error and the converse for a minus error.

|| One per cent change was produced by increasing the inspired sample by $\frac{1}{2}$ % and decreasing the expired sample by $\frac{1}{2}$ % and the converse for a minus error.

TABLE VI

Effect of variation in component measurements on calculated single breath lung O_2 diffusing capacity at an alveolar O_2 pressure of 200 mm Hg*

Measurement	Variation	Recalculated $DL_{O_2}SB$	Per cent change
	%	ml/(min $\times$ mm Hg)	
1. αBO_2	+2	8.4	-4
	-2	9.2	+4
2. Inspired $[C_2H_2]$	+2†	7.2	-17
Expired $[C_2H_2]$	-2†	10.1	+15
3. a. $[^{34}O_2]$	+1‡	10.6	+21
Total $[O_2]$	-1‡	7.6	-14
b. Total inspired $[O_2]$	+1†	10.9	+24
Total alveolar $[O_2]$	-1†	7.2	-18
c. Inspired [neon]	+1†	8.0	-9
Alveolar [neon]	-1†	9.6	+10
4. Mixed venous $P^{34}O_2$	+10	9.6	+9
	-10	8.3	-6

* Data used were for subject GGP, whose $DL_{O_2}SB$ was 8.8 ml per (minute $\times$ mm Hg) at an alveolar P_{O_2} of 203 mm Hg. Variation of the ratio $[^{34}O_2]/total [O_2]$ was considered to be only 1% at the higher alveolar P_{O_2} compared with 2% at mixed venous P_{O_2} , because the $^{34}O_2$ concentration was five times higher and could therefore be measured with greater precision.

† For explanation see Table V.

‡ An increase of $\frac{1}{2}$ % in $[^{34}O_2]/total [O_2]$ in the alveolar sample and a decrease of $\frac{1}{2}$ % in $[^{34}O_2]/total [O_2]$ in the inspired sample was produced for a plus error and the converse for a minus error.

calculated from the neon dilution, the total P_{O_2} in the inspired and expired samples, and the ratio of $^{34}O_2$ to total O_2 in each sample (see Appendix I). The ratio $[^{34}O_2]/total [^{34}O_2]$ is measured on the mass spectrometer with an estimated accuracy of $\pm 2\%$ at mixed venous P_{O_2} and $\pm 1\%$ at about 200 mm Hg alveolar P_{O_2} . Total inspired and total alveolar P_{O_2} are measured at different times on the mass spectrometer with an estimated accuracy of $\pm 1\%$. The neon dilution ratio was measured on the gas chromatograph with an estimated accuracy of $\pm 1\%$.

d) $P_{CO_2}T_0$ (mixed venous P_{O_2}) in these experiments was too great to be neglected. Compared with the measurements of mixed venous P_{CO_2} more difficulty was encountered in reaching a mixed venous plateau with O_2 . This difficulty would be expected on a theoretical basis because the alveolar P_{CO_2} equilibrates with the mixed venous blood faster than the alveolar P_{O_2} equilibrates with the mixed venous blood (31). We believe our estimation of mixed venous P_{O_2} may be in error by as much as 10%, but fortunately $DL_{O_2}SB$ is not extremely sensitive to errors in this measurement.

The effects of errors in the different data upon calculated $DL_{O_2}SB$ are given in Tables V and VI. If all of the individual errors at mixed venous P_{O_2} tended to increase $DL_{O_2}SB$, we would overestimate it by 32%, and if all the individual errors decreased $DL_{O_2}SB$, we would underestimate it by 34%. Comparative errors in $DL_{O_2}SB$ at about 200 mm Hg P_{O_2} would be 83 and 68%. It seems unlikely that all the component errors would cause the same directional change in $DL_{O_2}SB$, and we estimate the error to be as high as $\pm 15\%$ at mixed venous P_{O_2} and $\pm 40\%$ at the higher P_{O_2} .

Because of the addition of CO to the inspired mixture, its movement into the pulmonary capillary blood during breath holding would slightly reduce the O_2 -carrying capacity of the blood. For example, in subject GGP during breath holding for 14 seconds at mixed venous P_{O_2} of 42 mm Hg, the potential oxygen-carrying capacity of the blood, αBO_2 , would be reduced by an average of 1.1%, which would cause an underestimation of 1.3% in $DL_{O_2}SB$. (Allowance was made for the shift in the hemoglobin dissociation curve

that occurs in the presence of carboxyhemoglobin.) This carbon monoxide effect is somewhat greater at higher oxygen tensions. For example, when subject GGP was restudied with a mean intracapillary oxygen tension of 203 mm Hg, $\text{DL}_{\text{O}_2}\text{SB}$ was underestimated by 4.5%. However, we have concluded that these corrections are small enough to be neglected.

Calculation of alveolar-end capillary O_2 difference. Because it seemed likely that the discrepancy among reported values of $\text{DL}_{\text{O}_2}\text{SS}$ in resting man might be due to a small alveolar-end capillary (A-a) O_2 difference that precluded accurate measurement of $\text{DL}_{\text{O}_2}\text{SS}$, we calculated this difference from the average values of $\text{DL}_{\text{O}_2}\text{SB}$ obtained in our subjects. The actual values used were $\text{DL}_{\text{O}_2}\text{SB}$ of 33 ml per (minute $\times$ mm Hg) at PA_{O_2} of 42 mm Hg and 6 ml per (minute $\times$ mm Hg) at PA_{O_2} of 220 mm Hg. $\text{DL}_{\text{O}_2}\text{SB}$ for any PA_{O_2} between the range of 42 and 220 mm Hg can then be interpolated by plotting $1/\text{DL}_{\text{O}_2}\text{SB}$ against PA_{O_2} (14). The A-a O_2 gradient was then calculated with the charts of Riley, Courmand, and Donald (32). In resting man, $\text{DL}_{\text{O}_2}\text{SS}$ is measured while breathing 10 to 14% O_2 , and we chose the conditions reported by Staub and his colleagues, namely PA_{O_2} of 47 mm Hg, O_2 consumption of 250 ml per minute, mixed venous PO_2 of 27.5 mm Hg, and an arterial-venous O_2 difference of 19% (14). The interpolated $\text{DL}_{\text{O}_2}\text{SB}$ at PA_{O_2} of 47 mm Hg would be 28 ml per (minute $\times$ mm Hg) and the calculated A-a gradient would be only 2 to 3 mm Hg. If our measurements and calculations are correct, it would be extremely difficult to measure so small an A-a O_2 difference, let alone take into account the effects of uneven ventilation to blood flow and the shunting of blood around the lungs. Therefore, the accurate measurement of $\text{DL}_{\text{O}_2}\text{SS}$ in resting normal subjects appears to be close to impossible.

At higher alveolar PA_{O_2} the A-a O_2 difference calculated by the above method becomes even smaller. For instance, at PA_{O_2} of 100 mm Hg, $\text{DL}_{\text{O}_2}\text{SB}$ would be 12 ml per (minute $\times$ mm Hg), but the A-a O_2 difference would be less than 0.5 mm Hg.

Cause of decrease in single breath lung O_2 diffusing capacity measured at high O_2 pressure. The fall in $\text{DL}_{\text{O}_2}\text{SB}$ from an average value of 33 ml

per (minute $\times$ mm Hg) at a PA_{O_2} of 42 mm Hg to 6 ml per (minute $\times$ mm Hg) at a PA_{O_2} of approximately 220 mm Hg would be expected on a theoretical basis because of the reported decrease in the diffusing capacity of the red blood cell for O_2 (θ_{O_2}) with increasing PO_2 (14). The very low value of $\text{DL}_{\text{O}_2}\text{SB}$ obtained at the higher PO_2 indicates that at this PO_2 the major site of resistance to diffusing is in the red blood cell rather than the alveolar-capillary membrane. For instance, if the membrane diffusing capacity for O_2 , $\text{DM}_{\text{O}_2}\text{SB}$, were 75 ml per (minute $\times$ mm Hg) ($\text{DM}_{\text{O}_2}\text{SB}$ being the diffusing capacity for O_2 of the alveolar capillary membrane and distinguished from $\text{DL}_{\text{O}_2}\text{SB}$, which also includes the diffusion from the membrane into the interior of the red blood cells), only 8% of the total resistance to O_2 diffusion would be interposed by the alveolar-capillary membrane (14). In contrast, the total resistance to CO diffusion produced by the membrane at this PO_2 in our subjects would be approximately 39%. At the present time we have no clear explanation for this discrepancy, except that there may be considerable error in $\text{DL}_{\text{O}_2}\text{SB}$ measured at high PO_2 due either to the technique used (*vide supra*) or to uneven distribution of diffusing capacity with respect to $\dot{Q}_c$ (*vide infra*). An additional explanation might be that at a PO_2 in the range of 250 mm Hg, θ_{O_2} , the diffusing capacity of the red blood cells for O_2 may be considerably smaller than θ for carbon monoxide. Unfortunately, θ_{O_2} has not been measured at PO_2 higher than 88 mm Hg, so that this explanation cannot be excluded. In addition, $\text{DM}_{\text{O}_2}\text{SB}$ and V_c cannot be calculated from the determinations of $\text{DL}_{\text{O}_2}\text{SB}$ at different alveolar PO_2 without knowing θ_{O_2} .

The very low figure for $\text{DL}_{\text{O}_2}\text{SB}$ at PO_2 of 250 mm Hg does not imply that the transport of O_2 in the lungs is diffusion limited at high PO_2 . For example, when mixed venous blood first enters the pulmonary capillary bed, $\text{DL}_{\text{O}_2}\text{SB}$ would be about 30 ml per (minute $\times$ mm Hg), but as soon as the blood becomes almost completely saturated $\text{DL}_{\text{O}_2}\text{SB}$ would fall to a much lower value. Further uptake of O_2 by the blood would be almost entirely as physically dissolved O_2 , and it is not limited by the rate of its combination with the red cells. An analogous marked drop in $\text{DL}_{\text{CO}}\text{SB}$ has recently been produced by making

measurements in a hyperbaric chamber at an alveolar PO_2 of 3,000 mm Hg (33).

Effect of nonuniform distribution of alveolar volume and pulmonary capillary blood flow. All measurements of $DL_{O_2}SB$ were made while the subjects were seated. On the basis of data reported by Dollery, Dyson, and Sinclair (34) and West (35), this posture produces considerable uneven distribution of VA with respect to diffusing capacity (uneven VA/DL) and uneven distribution of VA with respect to $\dot{Q}_c$ (uneven VA/ $\dot{Q}_c$). They observed that the ratios VA/DL and VA/ $\dot{Q}_c$ gradually decrease as one moves from the apices to the base of the lungs. Using representative data reported by the above investigators, we calculated that this type of uneven distribution compared with a pattern of perfectly even distribution produces approximately a 10% decrease in $DL_{O_2}SB$. We therefore do not think that $DL_{O_2}SB$ will be markedly altered by assuming different body positions.

A number of anatomical studies suggest that an entirely different form of uneven distribution may be present in the lung, namely uneven distribution of diffusing capacity with respect to $\dot{Q}_c$ (uneven DL/ $\dot{Q}_c$) within each gas exchange unit (18, 36, 37). For instance, if each of the units in the lungs contained two capillary pathways, one 100 and the other 300 μ long, and each received equal amounts of $\dot{Q}_c$, the lungs would contain two DL/ $\dot{Q}_c$ compartments, one three times as large as the other. This form of uneven DL/ $\dot{Q}_c$ would not necessarily be related to the posture of the subject. The measured value of $DL_{O_2}SB$ can be significantly reduced by this form of uneven distribution, whereas $DL_{CO}SB$ is barely affected. For example, in our subjects the above pattern of uneven DL/ $\dot{Q}_c$ we calculate would cause approximately a 25% decrease in $DL_{O_2}SB$, whereas $DL_{CO}SB$ would be unchanged. At the present time we believe that uneven DL/ $\dot{Q}_c$ of this variety accounts for the unexpectedly low values of $DL_{O_2}SB$ observed in our subjects.

Summary

The rate of disappearance of a stable O_2 isotope, $^{34}O_2$, from the alveoli during breath holding was used to measure the single breath oxygen diffusing capacity of the lungs ($DL_{O_2}SB$). In five resting subjects at an average oxygen pres-

sure (PO_2) of 42 mm Hg, $DL_{O_2}SB$ was 33 ml per (minute $\times$ mm Hg), and the simultaneously measured carbon monoxide diffusing capacity was 44 ml per (minute $\times$ mm Hg).

In three of the subjects $DL_{O_2}SB$ was measured at a PO_2 of approximately 220 mm Hg and found to be only 6 ml per (minute $\times$ mm Hg). This finding is attributed to the slower velocity of the reaction of O_2 and hemoglobin at the higher O_2 tensions.

The alveolar-end capillary O_2 gradient (A-a gradient) calculated from the average values of $DL_{O_2}SB$ found in our subjects by the Bohr integration procedure was 2 to 3 mm Hg at an alveolar PO_2 of 47 mm Hg and less than 0.5 mm Hg at an alveolar PO_2 of 100 mm Hg.

$DL_{O_2}SB$ calculated from the carbon monoxide diffusing capacity was almost twice as great as the value obtained by directly measuring $DL_{O_2}SB$ with $^{34}O_2$. This discrepancy could not be explained by uneven distribution of alveolar volume and diffusing capacity throughout the lungs but could be explained by uneven distribution of diffusing capacity and blood flow (uneven DL/ $\dot{Q}_c$).

Acknowledgments

We thank Dr. George Karreman for essential aid in the derivation of the expressions in Appendix II. We also thank Mrs. Mary Friedmann for expert technical assistance.

Appendix I

Method of calculating the relative disappearance of $^{34}O_2$ from the alveoli. To solve Equation 6, we needed to calculate the relative change with time in the difference between the alveolar $P^{34}O_2$ and the $P^{34}O_2$ at the start of the capillary, the left-hand term in the equation, which in symbols is:

$$\frac{PA^{34}O_2t - PC^{34}O_2T_0}{PA^{34}O_2t_0 - PC^{34}O_2T_0}, \quad [12]$$

and which we will call hereafter the fraction remaining. The alveolar partial pressure after the mixing of inspired gas with the gas in the residual volume but before any $^{34}O_2$ has exchanged with the blood ($PA^{34}O_2t_0$), and the partial pressure in the blood at the start of the capillary ($PC^{34}O_2T_0$) cannot be measured directly but have to be calculated from other experimental measurements.

Because the inert and relatively insoluble gas neon was added to the inspired gas mixture, $PA^{34}O_2t_0$ can be calculated by the following expression:

$$PA^{34}O_2t_0 = \frac{PE_{neon}}{PI_{neon}} PI^{34}O_2 + PA^{34}O_2VR \left(1 - \frac{PE_{neon}}{PI_{neon}}\right), \quad [13]$$

where P^{34}O_2 equals the P^{34}O_2 in the test gas mixture that is inspired, P_{neon} is the PNEON in the test gas mixture, P_{Eneon} is the PNEON in the expired sample, and $\text{P}^{34}\text{O}_2\text{VR}$ is the P^{34}O_2 in the gas in the residual lung volume just before inspiration of the test gas mixture. The first term on the right-hand side represents the contribution of the inspired gas to the particular gas sample in question, and the second term is the contribution to the same sample from the residual gas in the lungs. Since in the experiments performed at the PO_2 of mixed venous blood, the rebreathing maneuver made $\text{P}^{34}\text{O}_2\text{T}_0$ equal to $\text{P}^{34}\text{O}_2\text{VR}$, the above equation can be written in the following form:

$$\text{P}^{34}\text{O}_2\text{t}_0 - \text{P}^{34}\text{O}_2\text{T}_0 = \frac{\text{P}_{\text{Eneon}}}{\text{P}_{\text{neon}}} (\text{P}^{34}\text{O}_2 - \text{P}^{34}\text{O}_2\text{T}_0). \quad [14]$$

Substitution of this expression into Equation 12 gives:

$$\text{fraction remaining} = \frac{\text{P}_{\text{neon}}}{\text{P}_{\text{Eneon}}} \left[\frac{\text{P}^{34}\text{O}_2\text{t} - \text{P}^{34}\text{O}_2\text{T}_0}{\text{P}^{34}\text{O}_2 - \text{P}^{34}\text{O}_2\text{T}_0} \right]. \quad [15]$$

Although we sought the partial pressure of $^{34}\text{O}_2$ in millimeters Hg, in practice we found it to be more accurate to measure the ratio of P^{34}O_2 to total PO_2 of the inspired and expired samples in the mass spectrometer and multiply these by the total PO_2 of each sample measured independently. Note that because the experiment was designed to make the inspired, expired, and alveolar total O_2 tensions equal: (P_{IO_2}) (natural abundance) = $\text{P}^{34}\text{O}_2\text{T}_0$ and (P_{AO_2}) (natural abundance) = $\text{P}^{34}\text{O}_2\text{T}_0$, where natural abundance is the fraction of alveolar ambient O_2 that is in the isotopic form of $^{34}\text{O}_2$ measured for each subject before the start of the day's experiment, P_{IO_2} equals the total inspired O_2 tension, and P_{AO_2} equals the total alveolar O_2 tension. Then, Equation 15 can be expressed in the following form:

$$\text{fraction remaining} = \frac{(\text{P}_{\text{neon}})(\text{P}_{\text{AO}_2})}{(\text{P}_{\text{Eneon}})(\text{P}_{\text{IO}_2})} \times \left[\frac{\frac{\text{P}^{34}\text{O}_2\text{t}}{\text{P}_{\text{AO}_2}} - \text{natural abundance}}{\frac{\text{P}^{34}\text{O}_2}{\text{P}_{\text{IO}_2}} - \text{natural abundance}} \right], \quad [16]$$

where $\text{P}^{34}\text{O}_2\text{t}/\text{P}_{\text{AO}_2}$ and $\text{P}^{34}\text{O}_2/\text{P}_{\text{IO}_2}$ are the experimentally measured ratios of abundance of $^{34}\text{O}_2$ to total O_2 in the expired (alveolar) sample and inspired samples, respectively.

If the fraction remaining is calculated in this manner, contamination of the sample with air, which inadvertently occurred owing to the dead space of the apparatus, does not change the value obtained. The change in the dilution ratio of the inert insoluble gas neon compensates for the change in alveolar PO_2 and in the ratio $\text{P}^{34}\text{O}_2\text{t}/\text{P}_{\text{AO}_2}$.

At the higher alveolar PO_2 , where the O_2 tension in the mixed venous blood does not equal that in the alveolar gas, Equation 16 cannot be used, and each term in Equation 9 must be calculated individually, $\text{P}^{34}\text{O}_2\text{t}_0$ from Equation 14 and $\text{P}^{34}\text{O}_2\text{t}_0$ by multiplying the total mixed venous PO_2 determined from the rebreathing maneuver by the natural abundance. Unfortunately, in this case, the fraction re-

maining is not independent of contamination with air, which does change the abundance of $^{34}\text{O}_2$ significantly, and we found it necessary to flush the apparatus with helium before each collection to prevent contamination with air. Total P^{34}O_2 was measured by the rapidly responding mass spectrometer sampling the expired alveolar gas at the mouthpiece.

Appendix II

Calculation of alveolar oxygen pressure as a function of time and distance along the capillary without neglecting the transit time of the blood or the storage of $^{34}\text{O}_2$ in it. During the diffusion of gas between alveolar air and capillary blood during breath holding, alveolar partial pressure changes with time and, in addition, capillary blood partial pressure of the gas changes as it moves along the capillary. The solution for the diffusion-limited case in which the capillary partial pressure of the gas is either neglected (38) or considered finite but constant (39) along the capillary is widely known. The solution for the other extreme, the blood flow-limited case, in which diffusion is considered complete by the end of the capillary, so that the end capillary partial pressure of the gas equals the alveolar partial pressure again avoids the problem of considering capillary partial pressure as a function of time independent of alveolar partial pressure (7). We are not aware of a published solution of the general case in which capillary partial pressure and alveolar pressure are considered independent functions of time, which is as follows: $\text{P}^{34}\text{O}_2 = \text{P}^{34}\text{O}_2\text{t}_0 e^{-Zt}$, where Z is a solution of the equation

$$\left[\frac{\text{VA}}{\text{P}_\text{B} - \text{P}_{\text{H}_2\text{O}}} + \frac{\text{Vt}(\alpha\text{O}_2)}{760} \right] \frac{Z}{\text{DL}_{\text{O}_2}} = 1 - \frac{\dot{\text{Q}}\text{cDL}_{\text{O}_2} 760}{\text{Vc}^2(\alpha\text{bO}_2 + \alpha\text{O}_2)} \left[\frac{1}{\frac{\text{DL}_{\text{O}_2} 760}{\text{Vc}(\alpha\text{bO}_2 + \alpha\text{O}_2)} - Z} \right] \times \left[\frac{\exp\left\{ - \left[\frac{\text{DL}_{\text{O}_2} 760}{\dot{\text{Q}}\text{c}(\alpha\text{bO}_2 + \alpha\text{O}_2)} + Z \right] \right\} - 1}{\frac{\text{DL}_{\text{O}_2} 760}{\text{Vc}(\alpha\text{bO}_2 + \alpha\text{O}_2)} - Z} \right]. \quad [17]$$

No explicit analytical solution for DL_{O_2} or for Z can be obtained from Equation 17. Numerical or graphical methods must be used.

The approximate equation used in the text, in which the transit time of the blood is considered negligible in comparison with the rate of change of alveolar P^{34}O_2 , can be put into a form analogous to that of Equation 17 by eliminating K between Equations 3 and 6. The solution is of the same form as Equation 17, but the exponential constant corresponding to Z equals:

$$\frac{\dot{\text{Q}}\text{c}(\alpha\text{bO}_2 + \alpha\text{O}_2)}{760 \left[\frac{\text{VA}}{\text{P}_\text{B} - \text{P}_{\text{H}_2\text{O}}} + \frac{\text{Vt}(\alpha\text{O}_2)}{760} \right]} \times \left\{ 1 - \exp \left[\frac{-\text{DL} 760}{\dot{\text{Q}}\text{c}(\alpha\text{bO}_2 + \alpha\text{O}_2)} \right] \right\}. \quad [18]$$

FIG. 5. THE RELATIONSHIP OF DL_{O_2} FOR SUBJECT RR CALCULATED BY THE APPROXIMATION METHOD DESCRIBED IN THE TEXT IN WHICH ALVEOLAR PO_2 IS CONSIDERED CONSTANT DURING A SINGLE TRANSIT TIME AND THE STORAGE OF O_2 IN THE CAPILLARY BLOOD VOLUME IS NEGLECTED, COMPARED TO THE MORE PRECISE AND COMPLEX METHOD DESCRIBED IN APPENDIX II. In the precise calculation a value for V_c must be assumed, and the resulting value of DL_{O_2} obtained is to some extent a function of it. This relation is shown by the solid line, and it can be seen that larger assumed values of V_c result in slightly increased values of DL_{O_2} . The open circle on the line at $DL_{O_2} = 45.4$ ml per (minute $\times$ mm Hg) represents the single value obtained by the approximation method. Note that if in the precise calculation V_c is assumed to be 35 ml, the two methods give the same numerical value, but if V_c is in actuality 100 ml, which seems more likely, the precise method gives a DL_{O_2} of 46.3 ml per (minute $\times$ mm Hg) as opposed to 45.4 ml by the approximation method. Such a difference is small compared to other sources of variation (see Table V). V_c = capillary blood volume.

If $DL_{760}/[V_c(\alpha b^{H_2O_2} + \alpha O_2)]$ becomes much greater than Z , the exponent in Equation 17 becomes equal to Equation 18, as is approximately the case here.

Calculations of DL from experimental data by the more correct but more tedious Equation 17 differed from the more convenient and simpler equations in the text by less than 6% at V_c of 200 ml or less, so the latter was used throughout (see Figure 5).

Appendix III

Comparison of a numerical solution of red cell O_2 tension and $^{34}O_2$ tension along the capillary with an approximation that assumes instantaneous equilibration of total O_2 tension at the start of the capillary at an alveolar O_2 tension of 250 mm Hg. Alveolar PO_2 was assumed constant at 250 mm Hg. A normal hemoglobin oxygen dissociation curve at pH 7.4 was assumed (3) but had to be extrapolated, assuming the Hill equation to be correct (40). The actual relationship used was $\log_{10}(\%O_2Hb)/(100 - \%O_2Hb) = 1.03 + 3 \log_{10} PO_2$. O_2Hb capacity was chosen as 0.2 ml per ml. In column 1 of Table VII is listed the O_2 partial pressure inside the red cells in arbitrary steps. Column 2 merely gives the difference between alveolar and RBC PO_2 . Column 3 contains the total O_2 content of the blood in milliliters per milliliter, composed of the bound O_2 obtained from the approximation equation above and the dissolved O_2 obtained using a solubility factor of 0.000316 ml per (ml $\times$ mm Hg). Column 4 is the change in total O_2 content for each PO_2 step. The time required for the given increment of O_2 to diffuse into the blood and react with the intracellular hemoglobin is calculated from the equation

$$\Delta t = \frac{V_c}{DL} \frac{\Delta [O_2]}{\Delta PRBC_{O_2}} \ln \frac{PA_{O_2} - PRBC_{O_2}^i}{PA_{O_2} - PRBC_{O_2}^j} \quad [19]$$

TABLE VII
*Numerical solution of partial pressure of O_2 and $^{34}O_2$ in the red cell along the pulmonary capillary at alveolar O_2 pressure of 250 mm Hg**

$PRBC_{O_2}$	$PA_{O_2} - PRBC_{O_2}$	$[O_2]$	$\Delta [O_2]$	$\frac{V_c}{DL}$	Δt	$\Sigma \Delta t$	$PRBC_{^{34}O_2}$	$PA_{^{34}O_2} - PRBC_{^{34}O_2}$	$\Delta [^{34}O_2]$ in RBC	$[^{34}O_2]$ in RBC
mm Hg	mm Hg	ml/ml	ml/ml	mm Hg/sec	sec	sec	mm Hg	mm Hg	ml/ml	ml/ml
40	210	0.1512		225			0.16	4.34		0.00060
50	200	0.1685	0.0173	227	0.019	0.019	0.29	4.21	0.000366	0.00097
60	190	0.1798	0.0113	229	0.013	0.032	0.40	4.10	0.00024	0.00121
70	180	0.1901	0.0103	231	0.0127	0.0447	0.53	3.97	0.00023	0.00145
80	170	0.1940	0.0039	233	0.0052	0.0499	0.64	3.85	0.00009	0.00154
90	160	0.1969	0.0029	235	0.0039	0.0538	0.73	3.77	0.000064	0.00160
100	150	0.1986	0.0017	238	0.0026	0.0574	0.83	3.67	0.000042	0.00164
150	100	0.2032	0.0046	249	0.0088	0.0652	1.31	3.19	0.00014	0.00178
200	50	0.2055	0.0023	260	0.0079	0.0731	1.82	2.68	0.00010	0.00188
230	20	0.2065	0.0010	267	0.0107	0.0838	2.20	2.30	0.00011	0.00199
			0.0006		0.018				0.00022	
249	1	0.2071		272		0.1018	2.65	1.85		0.00221
250	0	0.2071		272		0.2000	3.28			
250	0	0.2071		272		0.3000	3.72			
250	0	0.2071		272		0.4500	4.09			

* RBC = red blood cell. For a further explanation of abbreviations, see Appendix III.

This relation assumes that blood Po_2 changes exponentially within each Po_2 step, which is reasonable for all blood Po_2 and becomes precisely true as the hemoglobin approaches saturation. As equilibrium between blood and alveolar Po_2 is approached, the diffusion gradient decreases so much over a single Po_2 step that the use of an average gradient leads to gross aberrances. It is for this reason that the more complicated equation above was introduced. Vc/DL is calculated from the relation $1/\theta_{\text{O}_2} + \text{Vc/DL}$ (14).

θ_{O_2} was calculated from the relationship: $1/\theta_{\text{O}_2} = 0.45 + 0.00029 \text{ Po}_2$. This equation was derived from the data of Staub and co-workers by assuming that θ_{O_2} is proportional to θ_{CO} (14). θ_{O_2} is an extrapolated value at Po_2 much over 65 mm Hg. Vc was chosen as 60 ml and DM_{O_2} as 19 ml per (minute $\times$ mm Hg). These values are probably both lower than the correct values. Vc for CO , which should be the same for O_2 , is 60 ml or higher (17), and DM_{O_2} should be 1.19 times DM_{CO} (3b), which is several times greater than 19 ml per (mm Hg $\times$ minute). There is some question as to the size of DL for O_2 , because it is so much less than the simultaneously measured value of DL for CO . Most important, these calculations were carried out to investigate the error resulting from the assumption that the mixed venous blood equilibrated immediately with the alveolar Po_2 , thus permitting the use of a continuous exponential equation, rather than requiring a laborious numerical integration. The difference between the two cases, that is between the solid and interrupted dashed lines in Figure 6, will be greater the slower the diffusion process. Therefore, assuming an extremely low value for DL will exaggerate this error. The value of DL chosen does give approximately the alveolar-end capillary P^{34}O_2 gradient found experimentally. Pulmonary capillary blood flow was assumed to be 8 L per minute, so that the transit time (TL) equaled 0.45 second.

In Table VII, column 7 gives the cumulative time of transit. Column 8 gives the partial pressure of $^{34}\text{O}_2$ corresponding to the total concentration of $^{34}\text{O}_2$ in the blood in column 11, both applying to the total Po_2 in the red cell on the same line. The partial pressure of $^{34}\text{O}_2$ is equal to $\text{Po}_2 \times [\text{O}_2]/[\text{O}_2 \text{ total}]$. The increment in $[\text{O}_2]$, listed in column 10, equals $(\Delta t \text{DL}/\text{Vc})[\text{PA}^{34}\text{O}_2 - \text{PRBC}^{34}\text{O}_2]$ at the start of the interval of PRBC_{O_2} . The concentration of $^{34}\text{O}_2$ in alveolar air was 1.8%, so that $\text{PA}^{34}\text{O}_2 = 0.018 \times 250 = 4.5$ mm Hg. The natural abundance of $^{34}\text{O}_2$ is 0.4%, so that $\text{PRBC}^{34}\text{O}_2$ at the start of the capillary is $40 \times 0.004 = 0.16$ mm Hg.

Whereas the numerical integration could be continued in the same manner until the end of the capillary is reached, that is until t equals 0.45 second, once the total PRBC_{O_2} equals 250 mm Hg, which is true by about 0.107 second, the $\text{PRBC}^{34}\text{O}_2$ can be described by an exponential equation (see Equation 3), obviating the more laborious numerical calculation. This equation is

$$\frac{\text{PRBC}^{34}\text{O}_2 - \text{PA}^{34}\text{O}_2}{\text{PRBC}^{34}\text{O}_2 - \text{PA}^{34}\text{O}_2} = \exp\left[\frac{-\text{DL}(t - t_0) \text{PRBC}_{\text{O}_2}}{\text{Vc}[\text{O}_2]}\right], \quad [20]$$

FIG. 6. GRAPH OF THE TOTAL PARTIAL PRESSURE OF THE MAJOR ISOTOPES OF O_2 AND OF THE PARTIAL PRESSURE OF $^{34}\text{O}_2$ IN THE RED BLOOD CELLS AS THEY MOVE ALONG THE PULMONARY CAPILLARY AT A TOTAL ALVEOLAR Po_2 OF 250 mm Hg. Total Po_2 is shown as a dotted line. P^{34}O_2 obtained by numerical solution is given as an interrupted dashed line. All numerical constants and assumed data are given in Appendix III. The approximate solution for P^{34}O_2 is derived assuming that the total Po_2 in the red cells equilibrates instantaneously with alveolar total Po_2 when it enters the pulmonary capillaries and that the only process occurring along the capillary is the exchange of $^{32}\text{O}_2$ for $^{34}\text{O}_2$. This curve is indicated as a solid line. It is this calculation of capillary red cell P^{34}O_2 that is used in the text because of its greater convenience.

where $\text{PA}^{34}\text{O}_2 = 4.5$ mm Hg; $\text{PRBC}^{34}\text{O}_{20} = 2.65$; $\text{DL}/\text{Vc} = 1/272$ mm Hg $^{-1}$ second $^{-1}$; $\text{PRBC}_{\text{O}_2} = 250$ mm Hg; $[\text{O}_2] = 0.2071$ ml per ml, and $t_0 = 0.107$ second. This relationship was used to calculate PA^{34}O_2 from 0.107 to 0.45 second in Figure 1.

If we assume that the mixed venous blood entering the capillary equilibrates instantaneously with alveolar Po_2 , the effective solubility of $^{34}\text{O}_2$ in the blood becomes constant, and Equation 20 can be used to calculate the end capillary partial pressure of $^{34}\text{O}_2$. In this case PA^{34}O_2 , DL/Vc , PRBC_{O_2} , and $[\text{O}_2]$ are the same as above. $\text{PRBC}^{34}\text{O}_2$, the partial pressure at the start of the capillary, consists of that of the mixed venous blood equilibrated with alveolar gas. $[\text{O}_2]$ at the start of the capillary therefore consists of $0.151 \times 0.004 = 0.0006$ ml per ml of naturally occurring isotope contributed by the mixed venous blood, plus the remainder of the total O_2 capacity of 0.207 ml per ml or 0.0559 ml per ml saturated with the enriched alveolar concentration of 1.8%, giving 0.001 ml per ml. This makes a total of 0.0016 ml per ml of $^{34}\text{O}_2$ in the blood at the start of the capillary. $\text{PRBC}^{34}\text{O}_{20}$ is then $0.0016/0.207 \times 250 = 1.94$ mm Hg. The result is plotted in Figure 6 (interrupted solid line).

At the end of the capillary the approximation method and the exact numerical integration method give values of P^{34}O_2 in the blood that differ by less than 0.05 mm Hg, producing an underestimation of DL_{O_2} of 10% or less, which was not considered prohibitive under the circum-

stances. We, therefore, calculated DL_{O_2} by this more convenient approximation at high alveolar PO_2 , which is implicit in Equations 5 and 6.

References

1. Lilienthal, J. L., Jr., R. L. Riley, D. D. Proemmel, and R. E. Franke. An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude. *Amer. J. Physiol.* 1946, **147**, 199.
2. Kety, S. S. Gas-blood diffusion *in* *Methods in Medical Research*, J. H. Comroe, Jr., Ed. Chicago, Year Book, 1950, vol. 2, p. 234.
3. Comroe, J. H., Jr., R. E. Forster, A. B. DuBois, W. A. Briscoe, and E. Carlsen. *The Lung*, 2nd ed. Chicago, Year Book, 1962, a) p. 124, b) p. 350.
4. Dyson, N. A., J. D. Sinclair, and J. B. West. A comparison of the uptakes of oxygen-15 and oxygen-16 in the lung. *J. Physiol. (Lond.)* 1960, **152**, 325.
5. Nier, A. O. A redetermination of the relative abundances of isotopes of carbon, nitrogen, oxygen, argon, and potassium. *Physical Rev.* 1950, **77**, 789.
6. Bates, D. V., and R. V. Christie. Intrapulmonary mixing of helium in health and emphysema. *Clin. Sci.* 1950, **9**, 17.
7. Cander, L., and R. E. Forster. Determination of pulmonary parenchymal tissue volume and pulmonary capillary blood flow in man. *J. appl. Physiol.* 1959, **14**, 541.
8. Ogilvie, C. M., R. E. Forster, W. S. Blakemore, and J. W. Morton. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. clin. Invest.* 1957, **36**, 1.
9. Staub, N. C. Microcirculation of the lung utilizing very rapid freezing. *Angiology* 1961, **12**, 469.
10. Sobol, B. J., G. Bottex, C. Emirgil, and H. Gissen. Gaseous diffusion from alveoli to pulmonary vessels of considerable size. *Circulat. Res.* 1963, **13**, 71.
11. Sackner, M. A., K. A. Feisal, and D. N. Karsch. Size of gas exchange vessels in the lung. *J. clin. Invest.* 1964, **43**, 1847.
12. Drabkin, D. L., and J. H. Austin. Spectrophotometric studies. V. A technique for the analysis of undiluted blood and concentrated hemoglobin solutions. *J. biol. Chem.* 1935, **112**, 105.
13. Bronk, D. W. *Handbook of Respiratory Data in Aviation*. Washington, D. C., National Research Council, 1944, section B, chart B-1, a and b.
14. Staub, N. C., J. M. Bishop, and R. E. Forster. Importance of diffusion and chemical reaction rates in O_2 uptake in the lung. *J. appl. Physiol.* 1962, **17**, 21.
15. Forster, R. E., F. J. W. Roughton, L. Cander, W. A. Briscoe, and F. Kreuzer. Apparent pulmonary diffusing capacity for CO at varying alveolar O_2 tensions. *J. appl. Physiol.* 1957, **11**, 277.
16. Rankin, J., R. S. McNeill, and R. E. Forster. Influence of increased alveolar CO_2 tension on pulmonary diffusing capacity for CO in man. *J. appl. Physiol.* 1960, **15**, 543.
17. Johnson, R. L., Jr., W. S. Spicer, J. M. Bishop, and R. E. Forster. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J. appl. Physiol.* 1960, **16**, 893.
18. Weibel, E. R. Morphometrics of the lung *in* *Handbook of Physiology*, section 3: Respiration, W. O. Fenn and H. Rahn, section Eds. Washington, D. C., American Physiological Society, 1964, vol. 1, p. 285.
19. Forster, R. E. Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol. Rev.* 1957, **37**, 391.
20. Roughton, F. J. W. Transport of oxygen and carbon dioxide *in* *Handbook of Physiology*, section 3: Respiration, W. O. Fenn and H. Rahn, section Eds. Washington, D. C., American Physiological Society, 1964, vol. 1, p. 767.
21. Forster, R. E. Rate of gas uptake by red cells *in* *Handbook of Physiology*, section 3: Respiration, W. O. Fenn and H. Rahn, section Eds. Washington, D. C., American Physiological Society, 1964, vol. 1, p. 827.
22. Turino, G. M., E. H. Bergofsky, R. M. Goldring, and A. P. Fishman. Effect of exercise on pulmonary diffusing capacity. *J. appl. Physiol.* 1963, **18**, 447.
23. Bates, D. V., and R. V. Christie. *Respiratory Function in Disease*. Philadelphia and London, W. B. Saunders, 1964, p. 92.
24. Forster, R. E., W. S. Fowler, and D. V. Bates. Considerations on the uptake of carbon monoxide by the lungs. *J. clin. Invest.* 1954, **33**, 1128.
25. Lane, G. A., and M. Dole. Fractionation of oxygen isotopes during respiration. *Science* 1956, **123**, 574.
26. Lambertsen, C. J., J. K. Clark, D. M. Aviado, Jr., R. G. Pontius, E. S. Barker, J. Moyer, and C. F. Schmidt. The pulmonary oxygen diffusion coefficient (abstract). *Amer. J. med. Sci.* 1949, **218**, 715.
27. Cosby, R. S., E. C. Stowell, Jr., D. M. Morrison, M. Mayo, F. B. Ruymann, and B. Bernard. Continuous measurement of alveolo-arterial gradients at ambient and anoxic levels. *J. appl. Physiol.* 1962, **17**, 1.
28. Johnson, R. L., Jr., H. F. Taylor, and A. C. DeGraff, Jr. Functional significance of a low pulmonary diffusing capacity for carbon monoxide. *J. clin. Invest.* 1965, **44**, 789.
29. Staub, N. C. The interdependence of pulmonary structure and function. *Anesthesiology* 1963, **24**, 831.
30. Eisenberg, S. An appraisal of the venous hematocrit. *J. clin. Invest.* 1961, **40**, 1036.
31. DuBois, A. B. Alveolar CO_2 and O_2 during breath holding, expiration, and inspiration. *J. appl. Physiol.* 1952, **5**, 1.
32. Riley, R. L., A. Cournand, and K. W. Donald. Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: methods. *J. appl. Physiol.* 1951, **4**, 102.

33. Nairn, J. R., G. G. Power, R. W. Hyde, R. E. Forster, C. J. Lambertsen, and J. Dickson. Diffusing capacity and pulmonary capillary blood flow at hyperbaric pressures. *J. clin. Invest.* 1965, **44**, 1591.
34. Dollery, C. T., N. A. Dyson, and J. D. Sinclair. Regional variations in uptake of radioactive CO in the normal lung. *J. appl. Physiol.* 1960, **15**, 411.
35. West, J. B. Regional differences in gas exchange in the lung of erect man. *J. appl. Physiol.* 1962, **17**, 893.
36. Von Hayek, H. The human lung, V. E. Krahel, Trans. New York, Hafner, 1960, p. 253.
37. Reeves, J. T., J. E. Leathers, and M. B. Quigley. Microradiography of pulmonary arterioles, capillaries, and venules of the rabbit. *Anat. Rec.* 1965, **151**, 531.
38. Krogh, A. The diffusion of gases through the lungs of man. *J. Physiol. (Lond.)* 1914, **49**, 271.
39. Forster, R. E., F. J. W. Roughton, L. Cander, W. A. Briscoe, and F. Kreuzer. Apparent pulmonary diffusing capacity for CO at varying alveolar O_2 tensions. *J. appl. Physiol.* 1957, **11**, 277.
40. Hill, A. V. The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J. Physiol. (Lond.)* 1910, **40**, iv.