Measurement of \( \text{O}_2 \) Diffusing Capacity of the Lungs with a Stable \( \text{O}_2 \) Isotope*

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In 1946 Lilienthal and his colleagues (1) described an ingenious technique for determining the \( \text{O}_2 \) diffusing capacity of the lungs. Their method required the measurement of mixed venous and arterial oxygen pressure (\( \text{Po}_2 \)) while the subject was breathing air as well as breathing 12 to 14\% \( \text{O}_2 \) 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 \( \text{O}_2 \) gradient (2, 3). A method based on the uptake of isotopic \( \text{O}_2 \) provides the greatest possible alveolar-end capillary \( \text{O}_2 \) gradient for a given total alveolar \( \text{Po}_2 \) and diffusing capacity, since the mixed venous blood enters the capillaries in effect at a much lower \( \text{O}_2 \) tension than is possible for the total mixed venous \( \text{Po}_2 \) to attain in life.

The rate of disappearance of radioactive \( \text{O}_2 \) from the lungs has been measured previously (4), but to our knowledge this information has not been used to calculate the \( \text{O}_2 \) diffusing capacity of the lungs. This paper describes a technique for determining the \( \text{O}_2 \) diffusing capacity from the rate of disappearance of a stable \( \text{O}_2 \) isotope, \( ^{34}\text{O}_2 \), 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 \( \text{O}_2 \) diffusing capacity (\( \text{DLo}_2 \)) with increasing \( \text{O}_2 \) tension.

**Methods**

Determination of \( \text{DLo}_2 \) at low alveolar \( \text{Po}_2 \) (approximately 42 mm Hg). The stable \( \text{O}_2 \) 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 (\( \text{D}_2\text{O} \)) enriched eightfold over the natural abundance with \( ^{18}\text{O} \) oxygen.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\% \( \text{CO}_2 \) and 92\% nitrogen for four to six breaths so that the \( \text{Po}_2 \) and \( \text{PCo}_2 \), monitored by a rapidly responding mass spectrometer sampling at the mouthpiece, reached a plateau indicating that the \( \text{Po}_2 \) and \( \text{PCo}_2 \) 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\% \( ^{18}\text{O}_2 \), 0.2\% \( ^{18}\text{O}_2 \), 7\% \( \text{CO}_2 \), 1\% acetylene (\( \text{C}_2\text{H}_2 \)), 0.5\% neon (\( \text{Ne} \)), 0.4\% carbon monoxide (CO), and the balance nitrogen. The partial pressures of \( \text{O}_2 \) and \( \text{CO}_2 \) 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 \( \text{Po}_2 \) and \( \text{PCo}_2 \) constant at their respective mixed venous values during breath holding. As a result, capillary \( \text{O}_2 \) saturation is also constant along the capillary obviating the need for a Bohr integration procedure. Whereas the normal alveolar-capillary gradient for total \( \text{Po}_2 \) has been abolished, an alveolar-capillary gradient for \( ^{18}\text{O}_2 \) has been produced. The first liter of the expire was discarded to wash out the respiratory dead space, and the remaining gas was collected for analysis. The \( \text{P}^{18}\text{O}_2 \) and \( \text{P}^{18}\text{O}_2 \) of the expired sample were measured on a mass spectrometer, and the other gases were measured on a gas chromatograph. The mixed venous \( \text{P}^{18}\text{O}_2 \), \( \text{P}^{18}\text{O}_2 \), and \( \text{PCo}_2 \) were determined either from the continuous mass spectrometer record of expired breath

¹ Oxygen-enriched \( \text{D}_2\text{O} \) purchased from the Oak Ridge National Laboratory, Oak Ridge, Tenn.

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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 (VA) 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 \(^{18}\)O in excess of mixed venous \(^{18}\)O was plotted on semilogarithmic paper against time (Figure 2). From the \(\text{C}_2\text{H}_2\) disappearance, pulmonary capillary blood flow \((Q_c)\) and pulmonary parenchymal tissue volume \((V_t)\) were determined (7). Carbon monoxide diffusing capacity \((D_{LCO})\) was calculated from the CO disappearance (8).

### TABLE 1

**Natural occurrence of stable isotopes of oxygen**

<table>
<thead>
<tr>
<th>Mass</th>
<th>Molecular structure</th>
<th>Occurrence as per cent of total oxygen*</th>
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<tr>
<td>32</td>
<td>(^{16}\text{O}-^{16}\text{O})</td>
<td>99.518</td>
</tr>
<tr>
<td>33</td>
<td>(^{16}\text{O}-^{17}\text{O})</td>
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<td>36</td>
<td>(^{18}\text{O}-^{17}\text{O})</td>
<td>0.000042</td>
</tr>
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</table>

* Calculated by law of probabilities from data of Nier (5), who reported occurrence of \(^{18}\)Oxygen as 99.758% of total oxygen, \(^{16}\)Oxygen as 0.0373% of total oxygen, and \(^{18}\)Oxygen as 0.2039% of total \(\text{O}_2\). For these experiments \(\text{O}_2\) of mass 34 was used as the tracer gas.

### FIG. 2. GRAPH SHOWING THE DISAPPEARANCE OF \(\text{C}_2\text{H}_2\), Labeled \(\text{O}_2\) \((\text{Pa}_{\text{O}_2} \text{in excess of mixed venous } \text{Pa}_{\text{O}_2})\), AND CARBON MONOXIDE FROM THE ALVEOLAR GAS DURING BREATH HOLDING AT AN ALVEOLAR \(\text{P}_{\text{O}_2}\) 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. \(\text{DL}_{\text{CO}}\) was calculated from the \(\text{C}_2\text{H}_2\) and labeled \(\text{O}_2\) lines, \(\text{DL}_{\text{CO}}\) 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 \(\text{C}_2\text{H}_2\) by the pulmonary parenchyma (7). The depressed intercept for labeled \(\text{O}_2\) 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 \(\text{C}_2\text{H}_2\), diffusion limited in the case of CO, and a combination of both flow and diffusion limited in the case of labeled \(\text{O}_2\). \(\text{Pa}^{\text{O}_2}_{\text{O}_2}=\text{alveolar }^{18}\text{O}_2\) pressure.

The decrease in \(\text{Pa}^{\text{O}_2}_{\text{O}_2}\) in the alveolar gas can be described by the following differential equation. The term on the left is the change in alveolar \(^{18}\text{O}_2\) and those on the right the net exchange in the blood.

\[
\left(\frac{V_A}{P_B-P_{\text{H}_2\text{O}}} + \frac{V_t(\text{at}_{\text{O}_2})}{760}\right) \cdot \frac{d\text{Pa}^{\text{O}_2}_{\text{O}_2}}{dt} = \frac{\dot{Q}_c(\text{ab}_{\text{O}_2}+\text{at}_{\text{O}_2})}{760} \cdot \text{P}_{\text{MV}^{\text{O}_2}_{\text{O}_2}} - \frac{\dot{Q}_c(\text{ab}_{\text{O}_2}+\text{at}_{\text{O}_2})}{760} \cdot \text{P}_{\text{MV}^{\text{O}_2}_{\text{O}_2}TL}, \tag{1}
\]

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, \(\text{at}_{\text{O}_2}\) equals the Bunsen solubility coefficient for \(\text{O}_2\) in \(V_t\)^2

\(\text{DL}_{\text{O}_2}\) equals the lung \(\text{O}_2\) diffusing capacity; \(\text{Pa}_2\) = oxygen pressure.

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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.

We assumed that the solubility of \(\text{O}_2\) in lung tissue \((\text{at}_{\text{O}_2})\) and the solubility of \(\text{O}_2\) physically dissolved in blood \((\text{ab}_{\text{O}_2})\) were the same, namely 0.0236 ml \(\text{O}_2\) STPD per standard atmosphere per ml.
in milliliters STPD per milliliter of tissue per standard atmosphere, P8 is the barometric pressure in millimeters Hg, P8H2O is the vapor pressure of water at body temperature in millimeters Hg, PA*O2 is the partial pressure of O2 in the alveolar gas in millimeters Hg, Pc is the pulmonary capillary blood flow in milliliters per minute, abO2 is the effective solubility coefficient for O2 in milliliters of O2 STPD in blood in chemical combination with hemoglobin per milliliter of blood per standard atmosphere of O2, 4 αO2 is the solubility coefficient for O2 in milliliters of O2 STPD physically dissolved in blood per milliliter of blood per standard atmosphere of O2, PmVO2 equals the mixed venous P4O2 in millimeters Hg, and PC4O2TL is the average partial pressure of P4O2 inside the red blood cells at the end of the pulmonary capillaries in millimeters Hg.

In Equation 1, the end capillary partial pressure of P4O2 (PcVO2TL) does not necessarily equal that in the alveolar gas, PmVO2, 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 P4O2 and time. Unfortunately, since alveolar and end capillary P4O2 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 P4O2 is constant during the time of transit, and b) storage of P4O2 in the capillaries is negligible, the solution becomes much simpler. These assumptions result in an error of less than 6% in the estimate of DL02 if the capillary transit time is 1.5 seconds or less.

If we also assume that DL02 and the pulmonary capillary blood volume (Vc) are evenly distributed along the length of the capillaries, the O2 diffusing capacity of an infinitesimal segment will be DL02 times dx/L and the volume, Vc 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 P4O2 concentration in a segment equals the amount of P4O2 diffusing into the segment, or:

$$\frac{dx}{L} DL_{02} (P_{A*O2} - P_{c*O2}) = \frac{dx (abO2 + \alphaO2)}{L} \frac{Vc dPc*O2}{760} \frac{dTL}{dL}$$  [2]

where DL02 is the diffusing capacity of the capillaries in milliliters STPD or P4O2 per minute per millimeter Hg, Vc is the volume of blood in the capillaries in milliliters Hg, and TL is the pulmonary capillary transit time in minutes. Note that dx/L cancels out. If PmVO2 is considered constant during one capillary transit, integrating over the interval of one capillary transit time (TL) gives the following solution:

$$\frac{P_{A*O2} - P_{c*O2} TL}{P_{A*O2} - P_{c*O2} T0} = \exp \left[ -\frac{760 (DL_{02}) (TL)}{Vc(abO2 + \alphaO2)} \right]$$  [3]

Pc*O2TL is the partial pressure in the capillary blood at the beginning of the capillary and is, therefore, equal to PmVO2 in the mixed venous blood (PmVO2) and PC4O2TL 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:

$$\frac{P_{A*O2} - P_{c*O2} TL}{P_{A*O2} - P_{c*O2} T0} = K.$$  [4]

Note that TL/Vc in Equation 3 equals the reciprocal of the capillary blood flow (1/Qc). The substitution of Equation 4 into 3 and 1/Qc for TL/Vc gives:

$$DL_{02} = \frac{\dot{Q}c(abO2 + \alphaO2)}{760} \ln \frac{1}{K}$$  [5]

Equation 4 can be solved for Pm*O2TL, substituted into Equation 1, and integrated, giving:

$$P_{A*O2} - P_{c*O2} T0 \frac{P_{A*O2} - P_{c*O2} T0}{P_{A*O2} - P_{c*O2} T0} = \exp \left[ -\frac{\dot{Q}c(abO2 + \alphaO2)TL}{760 \left( \frac{Vc}{P8 - P8H2O} + \frac{Vt(\alphaO2)}{760} \right)} \right]$$  [6]

where Pm*O2T0 is the alveolar PmVO2 at the start of breath holding, Pm*O2T0 is the alveolar PmVO2 at the end of breath holding, and tBH 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, DL02 can be calculated from Formula 5.
In order to calculate \( \text{abo}_{2} \), 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 (%\( O_{2} \) HB) was determined from the alveolar \( P_{O_{2}} \) and \( P_{CO_{2}} \) 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 \( P_{O_{2}} \) equal to the alveolar \( P_{O_{2}} \), 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 \( P_{O_{2}} \) higher than mixed venous \( P_{O_{2}} \).

Determination of \( DL_{O_{2}} \) at high \( P_{O_{2}} \) (approximately 250 mm Hg). The experimental observations are made in exactly the same manner as for the measurement of \( DL_{O_{2}} \) at low \( P_{O_{2}} \), except that the inspired gas mixture contained a higher concentration of \( O_{2} \). It consisted of 7% \( CO_{2} \), 35% or more \( ^{18}O_{2} \), approximately 1% \( ^{18}O_{2} \), 2% neon, 1% acetylene, 0.4% \( CO_{2} \), and the balance \( N_{2} \). However, when \( P_{O_{2}} \) is greater than mixed venous \( P_{O_{2}} \), in addition to the diffusion of \( ^{18}O_{2} \) from the alveolar gas into the blood in exchange for \( ^{18}O_{2} \), total \( %O_{2} \) HB increases along the capillary. Theoretically, this means that the solubility of \( ^{18}O_{2} \) in capillary blood (\( \text{abo}_{2} \)), which equals \( [O_{2} \text{HB}] / (P_{O_{2}} - P_{CO_{2}}) \) 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 \( P_{O_{2}} \) 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 \( P_{O_{2}} \), but this is extremely tedious and complicated (Appendix III). A second and approximate solution is to assume that the high alveolar \( P_{O_{2}} \) (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 \( ^{18}O_{2} \) at a \( P_{O_{2}} \) of 250 mm Hg.

To justify this assumption, we performed a numerical integration (Bohr) for total \( P_{O_{2}} \) using the method given by Staub, Bishop, and Forster (14) for the case when alveolar \( P_{O_{2}} \) 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 understimation 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 \( P_{O_{2}} \) 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 \( ^{18}O_{2} \) in the red blood cells of the capillary blood immediately after the hemoglobin has become almost completely saturated (\( P_{mv}O_{2}T_{s} \)) can be determined by the following formula:

\[
P_{mv}O_{2}T_{s} = \frac{\%O_{2} \text{ HB}}{100} \left[ P_{mv}O_{2} - \frac{\%O_{2} \text{ HB}}{100} \right]
\]

where \( \%O_{2} \) 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 \( P_{mv}O_{2}T_{s} \) gives:

\[
\frac{P_{mv}O_{2} - P_{mv}O_{2}T_{s}}{P_{mv}O_{2}} = K
\]

Equation 8 can be substituted into Equation 1 so as to eliminate \( P_{mv}O_{2}T_{s} \). Integration of the resultant equation gives:

\[
\int \frac{P_{mv}O_{2} - \%O_{2} \text{ HB}}{100} \text{ d}(\%O_{2} \text{ HB}) = \frac{V_{A} (P_{O_{2}} - P_{MV})}{760 (V_{T} (\%O_{2} \text{ HB}) + \frac{V_{A}}{P_{B} - P_{MV}})}
\]

The analyses were performed in the same manner as for the case at lower alveolar \( P_{O_{2}} \), and the data were substituted into Equations 9 and then 5 to solve for \( DL_{O_{2}} \) (see Figure 3).

**Figure 3.** Graph showing the rate of disappearance of \( C_{2}H_{2} \), labeled \( O_{2} \) (\( P_{mv}O_{2} \) in excess of mixed venous \( P_{mv}O_{2} \) and carbon monoxide from the alveolar gas during breath holding at an alveolar \( P_{O_{2}} \) of 203 mm Hg in subject GGP. Note that compared to Figure 2, at this high \( P_{O_{2}} \) the labeled \( O_{2} \) has a much slower rate of disappearance. This decrease is principally due to the lower effective solubility for \( O_{2} \) (\( \text{abo}_{2} \)) at the higher \( P_{O_{2}} \).
**TABLE III**

Measures obtained in all subjects

<table>
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<tr>
<th>Subject</th>
<th>$P_{A02}$</th>
<th>$P_{ACO2}$</th>
<th>$O_2$ sat</th>
<th>$Hgb$</th>
<th>$abO_2$</th>
<th>$V_A$</th>
<th>$Q_c$</th>
<th>$V_t$</th>
<th>$DLCO$</th>
<th>K</th>
<th>$DL_{O2}$ appx</th>
<th>$DL_{O2}$ (VC=60)</th>
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<tbody>
<tr>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>%</td>
<td>ml/ml</td>
<td>ml/ml</td>
<td>ml/min</td>
<td>ml/min</td>
<td>ml/min</td>
<td>ml/mm Hg</td>
<td>mm Hg</td>
<td>ml/min</td>
<td>ml/mm Hg</td>
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<td>A. Mixed venous PO$_2$</td>
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<td>0.663</td>
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* Abbreviations: $P_{A02} = $ alveolar oxygen pressure (PO$_2$); $O_2$ sat = $O_2$ saturation of pulmonary capillary blood; $Hgb$ = hemoglobin concentration of venous blood; $abO_2$ = effective solubility of $O_2$ in capillary blood; $V_A$ = alveolar volume; $Q_c$ = pulmonary capillary blood flow; $V_t$ = pulmonary tissue volume; $DLCO$ = single breath CO diffusing capacity; $K = $ (alveolar PO$_2$ - capillary PO$_2$) + (alveolar PO$_2$ - precapillary PO$_2$); $DL_{O2}$ appx = single breath $O_2$ diffusing capacity calculated by the method described in text; and $DL_{O2}$ (VC=60) = single breath $O_2$ 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 $O_2$ pressures. In all five subjects $DL_{O2}$ was initially measured at an alveolar $O_2$ tension at or near their mixed venous PO$_2$, 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 $^{15}O_2$ 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 PO$_2$.

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 PO$_2$ of about 110 mm Hg. The $DL_{CO}$ for the three subjects at the higher PO$_2$ 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 PO$_2$. This difference is presumably secondary to the faster carbon monoxide-hemoglobin reaction rate at mixed venous PO$_2$ (15) or the higher alveolar PCO$_2$ present during the measurements at the lower PO$_2$ (16).

Measurement of $O_2$ diffusing capacity of the lungs at a hypoxic level. In one subject an additional determination of $DL_{O2}$ was made after breathing 9% $O_2$, 91% N$_2$ for 10 minutes before the breath-holding maneuver (see Table III, B). The hypoxic breathing lowered the mixed venous PO$_2$ from 41.5 mm Hg to 28.5 mm Hg and the mixed venous PCO$_2$ 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 PO$_2$.** The lines connect measurements of $DL_{O2}$ performed in the same subjects but at different $PA_{O2}$.  

1182 HYDE, FORSTER, POWER, NAIRN, AND RYNES


DL\textsubscript{O}\textsubscript{2} from 34.1 to 36.0 ml per (minute × mm Hg) and a slight fall in DL\textsubscript{CO} from 50.7 to 46.1 ml per (minute × mm Hg). This experiment indicates that a fall in alveolar \textsubscript{PO}\textsubscript{2} from 41.5 to 28 mm Hg is not accompanied by much change in DL\textsubscript{O}\textsubscript{2} or DL\textsubscript{CO}, which is not surprising because over this range of alveolar \textsubscript{PO}\textsubscript{2} there is little change in the reaction rate of \textsubscript{O}\textsubscript{2} and CO with hemoglobin (14, 15).

**Measurements of \textsubscript{O}2 diffusing capacity of the lungs at elevated alveolar \textsubscript{PO}2 tensions.** In three of the subjects additional measurements of DL\textsubscript{O}\textsubscript{2} and DL\textsubscript{CO} were made at an alveolar \textsubscript{PO}\textsubscript{2} of approximately 220 mm Hg (see Table III, C). This high alveolar \textsubscript{PO}\textsubscript{2} during breath holding was produced by increasing the concentration of \textsubscript{O}2 in the inspired test gas from approximately 7% to about 50%. Compared with measurements made at mixed venous \textsubscript{PO}\textsubscript{2} no marked changes in pulmonary capillary blood flow were found, but DL\textsubscript{O}\textsubscript{2} decreased from an average value of 30.7 (SD ± 5.3) ml per (minute × mm Hg) to 5.8 (SD ± 2.6), and DL\textsubscript{CO} fell from 44.9 (SD ± 4.3) to 27.3 (SD ± 3.2) ml per (minute × mm Hg). The fall in DL\textsubscript{CO} with this change in \textsubscript{PO}2 is of the same order of magnitude as observed before (15), but the low value of DL\textsubscript{O}\textsubscript{2} at increased alveolar \textsubscript{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 \textsubscript{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 \textsuperscript{40}O\textsubscript{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:

\[ \text{isotope intercept at time zero in per cent} \]
\[
\frac{100}{V_A + (a_{O_2})(V_t) \left[ \frac{(P_B - P_{H_2}O)}{760} \right] + (a_{O_2} + a_{O_2})(V_c) \left[ \frac{(P_B - P_{H_2}O)}{760} \right]} = \frac{V_A}{\left( \frac{100}{\text{isotope intercept at time zero in per cent}} - 1 \right) \left( \frac{760}{P_B - P_{H_2}O} \right) - (a_{O_2})(V_t)}
\]

where the terms in the denominator on the right side of the equation represent the effective volume into which the \textsuperscript{40}O\textsubscript{2} may go. Solving for \( V_c \) gives

\[ V_c = \frac{V_A \left( \frac{100}{\text{isotope intercept at time zero in per cent}} - 1 \right) \left( \frac{760}{P_B - P_{H_2}O} \right) - (a_{O_2})(V_t)}{a_{O_2} + a_{O_2}} \]

Values for \( V_c \) in our subjects are shown in Table IV. Like the results for \( V_t \) by the \textsubscript{C}H\textsubscript{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 DL\textsubscript{CO}SB at different alveolar \textsubscript{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 \textsuperscript{40}O\textsubscript{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.
cause the rate at which O₂ is taken up by the red blood cells (θ) is a function of the O₂ saturation and tends to fall progressively as O₂ saturation rises over 70% (14). In the measurement of DLo₂SB, O₂ 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 O₂ onto the hemoglobin molecule within the red cell are simplified because oxyhemoglobin and reduced hemoglobin remain constant (20, 21).

On the other hand, DLo₂SB 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 O₂ isotopes, which is costly and time consuming.

Despite the markedly different techniques required for the measurement of DLo₂SB and DLo₂SS, the average DLo₂SB of 33 ml per (minute × mm Hg) obtained at mixed venous Po₂ in this study is only moderately larger than the values reported by several investigators for DLo₂SS in normal resting subjects. For example, values for DLo₂SS of 21 ml per (minute × mm Hg) (1) and 26 ml per (minute × mm Hg) (22) have been reported. The discrepancy between the two methods is similar to that seen when comparing the single breath DLco (DLcoSB) with the steady state DLco methods (DLcoSS) (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 DLo₂SS and DLo₂SB because the latter method uses O₂ of a heavier mass, which would be expected to diffuse more slowly (36). This difference in mass, which would make DLo₂SS greater than DLo₂SB 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 DLO$_2$SS to be 60 ml per (minute X 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 DLO$_2$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 DLO$_2$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 DLO$_2$SS in resting man.

Several authors have described a method for indirectly calculating DLO$_2$SB from measurements of DLCO$_2$SB (14, 28). In resting man at an alveolar Po$_2$ of 42 mm Hg this calculation shows that DLO$_2$SB should be considerably higher than DLCO$_2$SB. In our subjects DLO$_2$SB measured directly with $^4$O$_2$ was on the average lower than DLCO$_2$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 summed error in DLO$_2$SB produced by errors in the data, in part because of the complicated nature of Equations 5 and 6. Experimentally determined variation in DLO$_2$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 DLO$_2$SB from the variability of the component measurements.

Reproducibility of measurements with the gas chromatograph and mass spectrometer used for determining DLO$_2$SB was within 1%. Overall 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$_2$H$_2$ and $^4$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 $^4$O$_2$ to total O$_2$ on the mass spectrometer in quintuplicate. Random variation was further reduced by calculating DLO$_2$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 DLO$_2$SB is in essence calculating the effective solubility of $^4$O$_2$ by comparing its disappearance rate with that of C$_2$H$_2$, whose solubility is known, errors in alveolar volume and time of breath holding will affect the slopes of C$_2$H$_2$ and $^4$O$_2$ in excess of mixed venous $^4$O$_2$ to the same degree and thereby tend to cancel each other out and not be critical in the determination of DLO$_2$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 DLO$_2$SB. These are $aO_2$ and $(V_t)aO_2/760$. The following items are possible sources of large errors in the calculation:

- $aO_2$ is calculated from the relation $[O_2 \text{ cap}]$ [\%$O_2\text{Hb}$] 760/100 PAo$_2$. The [\%$O_2\text{Hb}$]/PAo$_2$ is obtained from a dissociation curve (13). [O$_2$ 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 the aorta (30). Since it is the dynamic hematocrit that we need in calculating $aO_2$, the observed low values of pulmonary hematocrit do not affect our calculations. We estimate the accuracy of the measurement of $aO_2$ to be in the order of ± 2%.

- $Qc$ depends upon the ratio of expired alveolar [C$_2$H$_2$] to inspired [C$_2$H$_2$] and the solubility coefficient for C$_2$H$_2$ in blood ($aO_2\text{Hb}$). Errors in the analysis of C$_2$H$_2$ are critical, and we estimate our experimental error in the ratio to about ± 2%. An error in $aO_2\text{Hb}$ would produce an error in DLO$_2$SB of the same magnitude as an error in $aO_2$ (see Tables V and VI), and our estimate of the error in $aO_2\text{Hb}$ is the same.

- PA$^{H_2}$t$_0$ and PA$^{H_2}$t, the alveolar P$^{H_2}$ at the start of breath holding and the alveolar P$^{H_2}$ at the end of breath holding, respectively, were
calculated from the neon dilution, the total \( P_{O_2} \) in the inspired and expired samples, and the ratio of \(^{18}O_2\) to total \( O_2 \) in each sample (see Appendix I). The ratio \( [^{18}O_2]/[^{18}O_2] \) is measured on the mass spectrometer with an estimated accuracy of ±2% at mixed venous \( P_{O_2} \) and ±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 ±1%. The neon dilution ratio was measured on the gas chromatograph with an estimated accuracy of ±1%.

d) \( P_{CO_2}T_o \) (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 \( DL_{O2}SB \) is not extremely sensitive to errors in this measurement.

The effects of errors in the different data upon calculated \( DL_{O2}SB \) are given in Tables V and VI. If all of the individual errors at mixed venous \( P_{O_2} \) tended to increase \( DL_{O2}SB \), we would overestimate it by 32\%, and if all the individual errors decreased \( DL_{O2}SB \), we would underestimate it by 34\%. Comparative errors in \( DL_{O2}SB \) 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 \( DL_{O2}SB \), and we estimate the error to be as high as ±15\% at mixed venous \( P_{O_2} \) and ±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, \( a_{O_2} \), would be reduced by an average of 1.1\%, which would cause an underestimation of 1.3% in \( DL_{O2}SB \). (Allowance was made for the shift in the hemoglobin dissociation curve

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Variation</th>
<th>Recalculated ( DL_{O2}SB )</th>
<th>Per cent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_{O_2} )</td>
<td>+2</td>
<td>33.5</td>
<td>−2</td>
</tr>
<tr>
<td></td>
<td>−2</td>
<td>34.9</td>
<td>+2</td>
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<tr>
<td>2. Inspired ([C_2H_2])</td>
<td>+2%</td>
<td>31.0</td>
<td>−9</td>
</tr>
<tr>
<td>Expired ([C_2H_2])</td>
<td>−2%</td>
<td>36.2</td>
<td>+6</td>
</tr>
<tr>
<td>3. a. ([NO_2])</td>
<td>+1%</td>
<td>37.2</td>
<td>+9</td>
</tr>
<tr>
<td>Total ([O_2])</td>
<td>−1%</td>
<td>31.4</td>
<td>−8</td>
</tr>
<tr>
<td>b. Total inspired ([O_2])</td>
<td>+1%</td>
<td>36.5</td>
<td>+7</td>
</tr>
<tr>
<td>Total alveolar ([O_2])</td>
<td>−1%</td>
<td>31.4</td>
<td>−8</td>
</tr>
<tr>
<td>c. Inspired ([neon])</td>
<td>+1%</td>
<td>34.5</td>
<td>+1</td>
</tr>
<tr>
<td>Alveolar ([neon])</td>
<td>−1%</td>
<td>33.9</td>
<td>−1</td>
</tr>
<tr>
<td>4. Mixed venous (P_{NO_2})</td>
<td>+10</td>
<td>36.5</td>
<td>+7</td>
</tr>
<tr>
<td></td>
<td>−10</td>
<td>32.3</td>
<td>−6</td>
</tr>
</tbody>
</table>

* Data used were for subject GGp, whose \( DL_{O2}SB \) was 34.14 ml per (minute \( \times \) mm Hg) at an alveolar \( P_{O_2} \) of 41.5 mm Hg. \( SB \) = single breath.
† The increase 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. \( DL_{O2}SB \) 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 \([NO_2]/[O_2]\) in the alveolar sample and a decrease of 1% in \([NO_2]/[O_2]\) 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 1% and decreasing the expired sample by 1% and the converse for a minus error.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Variation</th>
<th>Recalculated ( DL_{O2}SB )</th>
<th>Per cent change</th>
</tr>
</thead>
<tbody>
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<td>( a_{O_2} )</td>
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<td></td>
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<td>+21</td>
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<tr>
<td>Total ([O_2])</td>
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<td>−14</td>
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<tr>
<td>b. Total inspired ([O_2])</td>
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<td>+24</td>
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<tr>
<td>Total alveolar ([O_2])</td>
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<td>7.2</td>
<td>−18</td>
</tr>
<tr>
<td>c. Inspired ([neon])</td>
<td>+1%</td>
<td>8.0</td>
<td>−9</td>
</tr>
<tr>
<td>Alveolar ([neon])</td>
<td>−1%</td>
<td>9.6</td>
<td>+10</td>
</tr>
<tr>
<td>4. Mixed venous (P_{NO_2})</td>
<td>+10</td>
<td>9.6</td>
<td>+9</td>
</tr>
<tr>
<td></td>
<td>−10</td>
<td>8.3</td>
<td>−6</td>
</tr>
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</table>

* Data used were for subject GGp, whose \( DL_{O2}SB \) was 8.8 ml per (minute \( \times \) mm Hg) at an alveolar \( P_{O_2} \) of 203 mm Hg. Variation of the ratio \([NO_2]/[O_2]\) was considered to be only 1% at the higher alveolar \( P_{O_2} \) compared with 2% at mixed venous \( P_{O_2} \), because the \( NO_2 \) concentration was five times higher and could therefore be measured with greater precision.
† For explanation see Table V.
‡ An increase of 1% in \([NO_2]/[O_2]\) in the alveolar sample and a decrease of 1% in \([NO_2]/[O_2]\) in the inspired sample was produced for a plus error and the converse for a minus error.
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, DLo2SB was underestimated by 4.5%. However, we have concluded that these corrections are small enough to be neglected.

Calculation of alveolar-end capillary O₂ difference. Because it seemed likely that the discrepancy among reported values of DLo2SS in resting man might be due to a small alveolar-end capillary (A-a) O₂ difference that precluded accurate measurement of DLo2SS, we calculated this difference from the average values of DLo2SB obtained in our subjects. The actual values used were DLo2SB of 33 ml per (minute X mm Hg) at PAo₂ of 42 mm Hg and 6 ml per (minute X mm Hg) at PAo₂ of 220 mm Hg. DLo2SB for any PAo₂ between the range of 42 and 220 mm Hg can then be interpolated by plotting 1/DLo2SB against PAo₂ (14). The A-a O₂ gradient was then calculated with the charts of Riley, Cournand, and Donald (32). In resting man, DLo2SS is measured while breathing 10 to 14% O₂, and we chose the conditions reported by Staub and his colleagues, namely PAo₂ of 47 mm Hg, O₂ consumption of 250 ml per minute, mixed venous Po₂ of 27.5 mm Hg, and an arterial-venous O₂ difference of 19% (14). The interpolated DLo2SB at PAo₂ of 47 mm Hg would be 28 ml per (minute X 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 O₂ 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 DLo2SS in resting normal subjects appears to be close to impossible.

At higher alveolar PAo₂ the A-a O₂ difference calculated by the above method becomes even smaller. For instance, at PAo₂ of 100 mm Hg, DLo2SB would be 12 ml per (minute X mm Hg), but the A-a O₂ difference would be less than 0.5 mm Hg.

Cause of decrease in single breath lung O₂ diffusing capacity measured at high O₂ pressure. The fall in DLo2SB from an average value of 33 ml per (minute X mm Hg) at a PAo₂ of 42 mm Hg to 6 ml per (minute X mm Hg) at a PAo₂ 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 O₂ (θO₂) with increasing Po₂ (14). The very low value of DLo2SB obtained at the higher Po₂ indicates that at this Po₂ the major site of resistance to diffusion is in the red blood cell rather than the alveolar-capillary membrane. For instance, if the membrane diffusing capacity for O₂, DMO2SB, were 75 ml per (minute X mm Hg) (DMO2SB being the diffusing capacity for O₂ of the alveolar capillary membrane and distinguished from DLo2SB, which also includes the diffusion from the membrane into the interior of the red blood cells), only 8% of the total resistance to O₂ diffusion would be interposed by the alveolar-capillary membrane (14). In contrast, the total resistance to CO diffusion produced by the membrane at this Po₂ 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 DLo2SB measured at high Po₂ due either to the technique used (vide infra) or to uneven distribution of diffusing capacity with respect to Óc (vide infra). An additional explanation might be that at a Po₂ in the range of 250 mm Hg, θO₂, the diffusing capacity of the red blood cells for O₂ may be considerably smaller than θ for carbon monoxide. Unfortunately, θO₂ has not been measured at Po₂ higher than 88 mm Hg, so that this explanation cannot be excluded. In addition, DMO2SB and Vc cannot be calculated from the determinations of DLo2SB at different alveolar Po₂ without knowing θO₂.

The very low figure for DLo2SB at Po₂ of 250 mm Hg does not imply that the transport of O₂ in the lungs is diffusion limited at high Po₂. For example, when mixed venous blood first enters the pulmonary capillary bed, DLo2SB would be about 30 ml per (minute X mm Hg), but as soon as the blood becomes almost completely saturated DLo2SB would fall to a much lower value. Further uptake of O₂ by the blood would be almost entirely as physically dissolved O₂, and it is not limited by the rate of its combination with the red cells. An analogous marked drop in DLo2SB has recently been produced by making
measurements in a hyperbaric chamber at an alveolar Po₄ of 3,000 mm Hg (33).

Effect of nonuniform distribution of alveolar volume and pulmonary capillary blood flow. All measurements of DLo₂SB 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 Qc (uneven VA/Qc). They observed that the ratios VA/DL and VA/Qc 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 DLo₂SB. We therefore do not think that DLo₂SB 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 Qc (uneven DL/Qc) 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 Qc, the lungs would contain two DL/Qc compartments, one three times as large as the other. This form of uneven DL/Qc would not necessarily be related to the posture of the subject. The measured value of DLo₂SB can be significantly reduced by this form of uneven distribution, whereas DLo₂SB is barely affected. For example, in our subjects the above pattern of uneven DL/Qc we calculate would cause approximately a 25% decrease in DLo₂SB, whereas DLo₂SB would be unchanged. At the present time we believe that uneven DL/Qc of this variety accounts for the unexpectedly low values of DLo₂SB observed in our subjects.

Summary

The rate of disappearance of a stable O₂ isotope, ³⁴O₂, from the alveoli during breath holding was used to measure the single breath oxygen diffusing capacity of the lungs (DLo₂SB). In five resting subjects at an average oxygen pressure (Po₂) of 42 mm Hg, DLo₂SB was 33 ml per (minute × mm Hg), and the simultaneously measured carbon monoxide diffusing capacity was 44 ml per (minute × mm Hg).

In three of the subjects DLo₂SB was measured at a Po₂ of approximately 220 mm Hg and found to be only 6 ml per (minute × mm Hg). This finding is attributed to the slower velocity of the reaction of O₂ and hemoglobin at the higher O₂ tensions.

The alveolar-end capillary O₂ gradient (A-a gradient) calculated from the average values of DLo₂SB found in our subjects by the Bohr integration procedure was 2 to 3 mm Hg at an alveolar Po₂ of 47 mm Hg and less than 0.5 mm Hg at an alveolar Po₂ of 100 mm Hg.

DLo₂SB calculated from the carbon monoxide diffusing capacity was almost twice as great as the value obtained by directly measuring DLo₂SB with ³⁴O₂. 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/Qc).

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Appendix I

Method of calculating the relative disappearance of ³⁴O₂ from the alveoli. To solve Equation 6, we needed to calculate the relative change with time in the difference between the alveolar Po₄ and the Po₄ at the start of the capillary, the left-hand term in the equation, which in symbols is:

\[
\frac{P_{\text{AO}2t} - P_{\text{CO}2T_{\text{ao}}}}{P_{\text{AO}2t_0} - P_{\text{CO}2T_{\text{ao}}}}
\]

[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 ³⁴O₂ has exchanged with the blood (P\text{AO}2t₂), and the partial pressure in the blood at the start of the capillary (P\text{CO}2T₂) 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, P\text{AO}2t₂ can be calculated by the following expression:

\[
P_{\text{AO}2t_2} = \frac{P_{\text{AO}2T_{\text{ao}}}}{P_{\text{AO}2T_{\text{ao}}}} P_{\text{AO}2T_2} + P_{\text{AO}2T_{\text{ao}}} V_R \left(1 - \frac{P_{\text{AO}2T_{\text{ao}}}}{P_{\text{AO}2T_{\text{ao}}}}\right).
\]

[13]
where $P_{i02}$ equals the $P_{n02}$ in the test gas mixture that is inspired, $P_{i02}$ is the $P_{n02}$ in the test gas mixture, $P_{a02}$ is the $P_{n02}$ in the expired sample, and $P_{a02}$ is the $P_{a02}$ 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 $P_{02}$ of mixed venous blood, the rebreathing maneuver made $P_{c02}T_e$ equal to $P_{i02}V_R$, the above equation can be written in the following form:

$$P_{a02} = P_{i02} - P_{i02}T_e = P_{i02}T_e (P_{i02} - P_{i02}T_e). \quad [14]$$

Substitution of this expression into Equation 12 gives:

$$\text{fraction remaining} = \frac{P_{i02}T_e}{P_{i02}} \left( \frac{P_{a02}}{P_{a02}T_e} \right). \quad [15]$$

Although we sought the partial pressure of $O_2$ in millimeters Hg, in practice we found it to be more accurate to measure the ratio of $P_{i02}$ to total $P_{02}$ of the inspired and expired samples in the mass spectrometer and multiply these by the total $P_{02}$ of each sample measured independently. Note that because the experiment was designed to make the inspired, expired, and alveolar total $O_2$ tensions equal: $(P_{i02})$ (natural abundance) = $P_{c02}T_e$ and $(P_{a02})$ (natural abundance) = $P_{c02}T_e$, where natural abundance is the fraction of alveolar ambient $O_2$ that is in the isotopic form of $O_2$ measured for each subject before the start of the day’s experiment, $P_{i02}$ equals the total inspired $O_2$ tension, and $P_{a02}$ equals the total alveolar $O_2$ tension. Then, Equation 15 can be expressed in the following form:

$$\text{fraction remaining} = \left( \frac{P_{i02}}{P_{i02}T_e} \right) \left( \frac{P_{a02}T_e}{P_{a02}} \right) \left( \frac{P_{c02}T_e}{P_{a02}} \right), \quad [16]$$

where $P_{a02}/P_{a02}$ and $P_{c02}/P_{i02}$ are the experimentally measured ratios of abundance of $O_2$ to total $O_2$ in the inspired (alveolar) sample and inspired samples, respectively.

If the ratio 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 $P_{02}$ and in the ratio $P_{a02}/P_{a02}$. At the higher alveolar $P_{02}$, 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, $P_{a02}T_e$ from Equation 14 and $P_{mv}T_e$ by multiplying the total mixed venous $P_{02}$ determined from the rebreathing maneuver by the natural abundance. Unfortunately, in this case, the fraction remaining is not independent of contamination with air, which does change the abundance of $O_2$ significantly, and we found it necessary to flush the apparatus with helium before each collection to prevent contamination with air. Total $P_{i02}$ 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 $O_2$ 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: $P_{a02} = P_{a02}e^{-z}$, where $Z$ is a solution of the equation

$$\frac{V_A}{P_B - PHOH + VT(a02)} + \frac{760}{DL_02} \left[ 1 - \frac{DLO_2 760}{Qc(ab02 + a02)} \left( \frac{1}{760} \right) \right]$$

$$= 1 - \frac{DLO_2 760}{Qc(ab02 + a02)} \left( \frac{1 + Z}{760} \right), \quad [17]$$

No explicit analytical solution for $DL_02$ 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 $P_{i02}$, 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:

$$\frac{Qc(ab02 + a02)}{760} - \frac{V_A}{P_B - PHOH + VT(a02)} \left[ 1 - \exp \left( - \frac{760}{Qc(ab02 + a02)} \right) \right], \quad [18]$$

with $Qc(ab02 + a02)$ and $760$ being replaced by $Qc(ab02 + a02) - Qc(ab02 + a02)$ and $760$ in the modified equation.
Such variation is in if two the obtained of assumed value for $V_c$.

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 $D_L O_2$ 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 $D_L O_2$. The open circle on the line at $D_L O_2 = 45.4$ 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 $D_L O_2$ 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 $D_L 760/[V_c(aHbO_2 + aO_2)]$ becomes much greater than $Z$, the exponent in Equation 17 becomes equal to Equation 18, as is approximately the case here.

Calculations of $D_L$ 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 $^4O_2$ 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 $P_O_2$ 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 $\log_{10}([%O_2 Hb]/[100 - [%O_2 Hb] = 1.03 + 3 \log_{10} P_O_2$. $O_2 Hb$ capacity was chosen as 0.2 ml per ml. In column I 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 $P_O_2$. 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 0.0000316 ml per (ml $\times$ mm Hg). Column 4 is the change in total $O_2$ content for each $P_O_2$ 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

$$\Delta t = \frac{V_c \Delta [O_2]}{D_L \Delta P_{RBCO_2}} \ln \left( \frac{P_{A O_2} - P_{RBCO_2}}{P_{A O_2} - P_{RBCO_2}} \right)$$

**TABLE VII**

Numerical solution of partial pressure of $O_2$ and $^4O_2$ in the red cell along the pulmonary capillary at alveolar $O_2$ pressure of 250 mm Hg

| $P_{RBCO_2}$ | $P_{A O_2} - P_{RBCO_2}$ | $[O_2]$ | $\Delta [O_2]$ | $V_c$ | $D_L$ | $\Delta t$ | $\Sigma \Delta t$ | $P_{RBCO_2}$ | $P_{RBCO_2}$ | $\Delta [O_2]$ | $[O_2]$ | $V_c$ | $D_L$ | $\Delta t$ | $\Sigma \Delta t$ |
|------------|---------------------|-------|---------------|------|--------|----------|--------------|----------|----------|---------------|-------|------|--------|----------|--------------|--------------|
| mm Hg      | mm Hg               | ml/ml | ml/ml         | mm Hg/sec | sec | mm Hg | mm Hg | ml/ml | ml/ml | mm Hg/sec | sec | mm Hg | mm Hg | ml/ml |
| 40         | 210                 | 0.1512 | 0.0173        | 225   | 0.019 | 0.16   | 4.34 | 0.00366 | 0.00060 |
| 50         | 200                 | 0.1685 | 0.0113        | 227   | 0.013 | 0.019 | 4.21 | 0.00024 | 0.00097 |
| 60         | 190                 | 0.1798 | 0.0103        | 229   | 0.0127 | 0.032 | 4.10 | 0.00023 | 0.00121 |
| 70         | 180                 | 0.1901 | 0.0039        | 231   | 0.0052 | 0.0447 | 3.97 | 0.00009 | 0.00015 |
| 80         | 170                 | 0.1940 | 0.0029        | 233   | 0.0039 | 0.0499 | 3.85 | 0.000064 | 0.00016 |
| 90         | 160                 | 0.1969 | 0.0017        | 235   | 0.0026 | 0.0538 | 3.77 | 0.0000942 | 0.000164 |
| 100        | 150                 | 0.1986 | 0.0046        | 238   | 0.0088 | 0.0574 | 3.67 | 0.00014 | 0.000178 |
| 150        | 100                 | 0.2032 | 0.0023        | 249   | 0.0079 | 0.0652 | 3.19 | 0.00010 | 0.000188 |
| 200        | 50                  | 0.2055 | 0.0010        | 260   | 0.0107 | 0.0731 | 2.68 | 0.00011 | 0.000199 |
| 230        | 20                  | 0.2065 | 0.0006        | 267   | 0.018 | 0.0838 | 2.30 | 0.00022 | 0.000221 |

* RBC = red blood cell. For a further explanation of abbreviations, see Appendix III.
This relation assumes that blood P02 changes exponentially within each P02 step, which is reasonable for all blood P02 and becomes precisely true as the hemoglobin approaches saturation. As equilibrium between blood and alveolar P02 is approached, the diffusion gradient decreases so much over a single P02 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/DM is calculated from the relation 1/θ02 = 0.45 + 0.00002 P02. This equation was derived from the data of Staub and co-workers by assuming that θ02 is proportional to θCO (14). θ02 is an extrapolated value at P02 much over 65 mm Hg. VC was chosen as 60 ml and DMO2 as 19 ml per (minute × mm Hg). These values are probably both lower than the correct values. VC for CO, which should be the same for O2, is 60 ml or higher (17), and DMO2 should be 1.19 times DMO2 (35), which is several times greater than 19 ml per (mm Hg × minute). There is some question as to the size of DL for O2, 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 P02, 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 P02 gradient found experimentally. Pulmonary capillary blood flow was assumed to be 8 l per minute, so that the transit time (TL) equals 0.45 second.

In Table VII, column 7 gives the cumulative time of transit. Column 8 gives the partial pressure of 18O2 corresponding to the total concentration of 18O2 in the blood in column 11, both applying to the total P02 in the red cell on the same line. The partial pressure of 18O2 is equal to P02 X [18O2]/[O2] total. The increment in [18O2], listed in column 10, equals (AtDL/VC)[PA02 - PRBC18O2] at the start of the interval of PRBC02. The concentration of 18O2 in alveolar air was 1.8%, so that PA18O2 = 0.018 X 250 = 4.5 mm Hg. The natural abundance of 18O is 0.4%, so that PRBC18O2 at the start of the capillary is 40 X 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 PRBCO2 equals 250 mm Hg, which is true by about 0.107 second, the PRBC18O2 can be described by an exponential equation (see Equation 3), obviating the more laborious numerical calculation. This equation is

\[
\text{PRBC}^{18}\text{O}_2 - \text{PA}^{18}\text{O}_2 = \exp \left[ - \frac{\text{DL}(t - t_d) \text{PRBCO}_2}{\text{VC}[\text{O}_2]} \right].
\]  

\[\text{[20]}\]

\[
\text{PRBC}^{18}\text{O}_2 - \text{PA}^{18}\text{O}_2 = \exp \left[ - \frac{\text{DL}(t - t_d) \text{PRBCO}_2}{\text{VC}[\text{O}_2]} \right].
\]

\[\text{[20]}\]

where PA18O2 = 4.5 mm Hg; PRBC18O2 = 2.65; DL/VC = 1/272 mm Hg^{-1} second^1; PRBCO2 = 250 mm Hg; [O2] = 0.2071 ml per ml, and t0 = 0.107 second. This relationship was used to calculate PA18O2 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 P02, the effective solubility of 18O2 in the blood becomes constant, and Equation 20 can be used to calculate the end capillary partial pressure of 18O2. In this case PA18O2, DL/VC, PRBCO2, and [O2] are the same as above. PRBC18O2, the partial pressure at the start of the capillary, consists of that of the mixed venous blood equilibrated with alveolar gas. [PO2] at the start of the capillary therefore consists of 0.151 X 0.004 = 0.0006 ml per ml of naturally occurring isotope contributed by the mixed venous blood, plus the remainder of the total O2 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 18O2 in the blood at the start of the capillary. PRBCO2 is then 0.0016/0.207 X 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 P18O2 in the blood that differ by less than 0.05 mm Hg, producing an underestimation of DLO2 of 10% or less, which was not considered prohibitive under the circum-
stances. We, therefore, calculated DlO₂ by this more convenient approximation at high alveolar P0₂, which is implicit in Equations 5 and 6.

References


