Relative Importance of Diffusion and Chemical Reaction Rates in Determining Rate of Exchange of Gases in the Human Lung, With Special Reference to True Diffusing Capacity of Pulmonary Membrane and Volume of Blood in the Lung Capillaries

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ABSTRACT

ROUGHTON, F. J. W. AND R. E. FORSTER. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in lung capillaries. J. Appl. Physiol. 11(2): 290-302. 1957.—An equation, $1/DM + 1/V_e = 1/DL$, has been derived which relates the measured pulmonary diffusing capacity ($DL$), the true diffusing capacity of the pulmonary membrane ($DM$), the rate of uptake of CO by the red cells per mm Hg CO tension ($\theta$) and the blood volume of the pulmonary capillary bed ($V_e$). By making measurements of $DL$ at different alveolar O$_2$ tensions, thereby causing $\theta$ to vary, this equation can be solved graphically for $DM$ and $V_e$, which are assumed to be independent of $O_2$ tension. Calculations of $DM$ and $V_e$ were made utilizing $a$) values of $\theta$ previously obtained from the in vitro rates of CO uptake of suspensions of human red cells at 37°C and $b$) values of $DL$ in normal resting subjects at alveolar $O_2$ tensions from about 100 mm Hg to over 600 mm Hg measured by both steady state and breath holding CO techniques. $DM$ is about twice the value of $DL$ measured in subjects breathing air at sea level. $V_e$ is about 75 ml in approximate agreement with the previously reported estimate of Roughton. Similar results were obtained using values of $DL$ at different alveolar $O_2$ tensions reported in the literature. This means that, in determining the rate of CO absorption in the lungs, the resistance of the red cell to the uptake of CO is of the same order of importance as the resistance of the pulmonary membrane to the diffusion of gas across it. Arguments are advanced to show that red cell resistance is of at least equal importance in the case of $O_2$ uptake.

According to the ordinary physical laws of diffusion, the rate of passage of gas across the membrane separating the alveolar air from the blood in the lung capillaries is given by the equation

$$V = \frac{Ad(P_A - P_e)t}{x} \quad (1)$$

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2 Lowell M. Palmer Senior Fellow.
millimeters of mercury, DM, may thus be defined by the equation

\[ DM = \frac{Ad}{x} \]  

(2)

from which it follows, by combining equations 1 and 2 that

\[ DM = \frac{V}{(P_A - P_e) \times t} \]  

(3)

Among the first values of DM to be found in the literature are those of Loewy and Zuntz (1). These authors calculated DM for human lungs from existing estimates of A and x for human lungs, together with values of d, the diffusion coefficient of isolated animal tissues for gases. Their values for DM, on this basis, were about 200, i.e. of the order of 3 to 10 times greater than the values currently accepted for normal men (2-4). Bohr (5), however, rightly pointed out that the estimates of A and x were subject to great uncertainty and that DM ought to be determined by some much more direct method. It occurred to him that DM for carbon monoxide might readily be deduced from data on the rate of CO uptake in the lungs, when gas mixtures containing low percentages of CO were inhaled. Carbon monoxide was believed to be a uniquely suitable gas for this purpose, since it has such an enormous affinity for hemoglobin that any back pressure of CO in the blood plasma could be safely neglected. The gradient across the lung membrane at any instant should thus be equal to the partial pressure of CO in the alveolar air at that instant. The volume of CO absorbed in the lung during a given interval divided by the average alveolar CO tension during that interval would thus give DM for CO directly. The diffusing capacity for oxygen, DM, should then be calculable from DM by the equation

\[ DM = \frac{dO_2}{dCO} \]

where \(dO_2\) and \(dCO\) are the diffusion coefficients of isolated tissue to the respective gases. Actually \(dO_2/dCO = 1.21\).

In his first paper on the subject, Bohr contented himself with using figures for rate of CO uptake previously given by Haldane (6), but in a paper (7) shortly before his death he published some determinations of his own, which formed the basis for the much more accurate methods with which the Kroghs (8, 9) subsequently followed up the problem.

In the final method of M. Krogh (9), a maximal inspiration of a gas mixture containing CO was made from residual volume and followed immediately by an expiration of at least 1 liter of gas. The breath was held at the remaining volume for 6-10 seconds and then a maximal expiration was made. The terminal volumes of the two expirations were considered to be alveolar gas and were analyzed for CO concentration. The latter was assumed to decay exponentially according to the equation

\[ FA = F_{A0} \exp (-DMCOPB/VA) \]

(5)

where \(F_A\), \(F_{A0}\) are the respective alveolar CO concentrations at times t and zero, respectively, \(P_b\) is the total barometric pressure less 47 mm Hg water vapor pressure at 37°C, t is the time in minutes between the delivery of the two gas samples and VA the total alveolar volume (STPD), during the breath holding interval. The value of DM was calculated by substituting the observed values of \(F_A\), \(F_{A0}\), \(P_b\), t and VA in a rearranged form of equation 5.

In deriving equation 5 it was assumed that a) the CO concentration in the first sample was representative of all alveolar gas and b) the partial pressure of dissolved CO in the capillary plasma was always negligible as Bohr had also supposed. More recent work than that of Krogh (8, 9) has shown that assumption a is not valid. The consequences of the lack of homogeneity of the alveolar air, as regards determinations of diffusing capacity, are considered in detail by Forster, Fowler and Bates (10). These authors and van Lingen (11) have furthermore observed, in a series of breath holding experiments of varying duration, that

\[ \exp(-y) = e^{-y} \]

Although the subscript referring to CO has been included here, it is understood that if the subscript to DM or DL is omitted, the notation refers primarily to CO.

The term 'capillary plasma' refers only to the plasma in that part of the pulmonary vascular bed which is exposed to alveolar gas. The CO tension referred to is not derived from the presence of COHb in the blood, as it is assumed either that mixed venous COHb is negligible or that it can be corrected for as described earlier (13).
the alveolar carbon monoxide concentration does not, in fact, decay exponentially with time, as equation 5 requires. Possible causes for this failure are discussed in their paper.

The present paper is concerned mainly with assumption b, the validity of which seems first to have been challenged by Roughton in his Ph.D. thesis (12). Assuming that the in vitro measurements of Hartridge and Roughton (13) on the rapid rate of entry of carbon monoxide and oxygen into sheep red cells could be applied to the human lung circulation in vivo, Roughton concluded that during CO uptake in the lung there must exist appreciable gradients of dissolved CO within the lung capillary blood. If so, the actual CO gradients across the lung membrane must have been less than those supposed by Bohr and Krogh, whose figures for the diffusing capacity of the lung membrane would also be correspondingly low.

Roughton's calculations were not published in full in the ordinary literature, since in several important respects they were believed to depend too much on guesswork. The two most serious gaps in the data at his disposal were 1) knowledge as to the rate at which human, as distinct from sheep, red cells take up carbon monoxide and oxygen at body temperature and 2) knowledge of the average time, tL, during which each red cell is in intimate contact with the alveolar air as it passes through the lung capillary.

In 1945 Roughton (14) put forward a theoretical method for computing tL, which was based upon 1) the observed difference in rate of carbon monoxide absorption in the human lung when low percentages of CO in air and in oxygen were breathed, respectively (15), together with 2) new data, on the rate at which carbon monoxide displaces oxygen from combination with hemoglobin in suspensions of oxygenated human red cells at 37°C (16). His values of tL ranged from 0.7 second for normal men at rest to 0.3 second for normal men at hard work, and were several times lower than the values assumed by him in his earlier thesis.

During recent years we have developed a simplified modification of Roughton's mathematical treatment leading finally to the equation

$$\frac{1}{D_L} = \frac{1}{D_m} + \frac{1}{\theta V_c} \quad (6)$$

where Dm is the true diffusing capacity of the membrane separating the alveolar air from the blood, as above defined, DL is the over-all diffusing capacity of the lung as measured by the Bohr-Krogh methods, Vc is the total volume in milliters of blood in the lung capillaries exposed to the alveolar air, θ is the number of milliters of gas taken up by the red cells in 1 ml of blood per minute per 1 mm Hg gradient of partial pressure of dissolved gas between the plasma and the interior of the red cell (10, 17). A similar equation has also been given by Kruhaffer (18).

In the present paper the theoretical deduction of equation 6 will first be given, and application of it will then be made to the data on the rate of replacement of oxygen by carbon monoxide in human red cell suspensions at 37°C in equilibrium with various oxygen pressures (19) and to the experimental values of DL at alveolar oxygen pressures varying from 100 to 670 mm Hg (20). These applications have led us not only to new and more reliable values of Vc which agree rather closely with Roughton's first estimates (14), but also suggest that in normal individuals Dm is of the order of twice DL. If this latter conclusion is correct, the rate of diffusion of carbon monoxide within the blood itself is a factor of comparable importance to its rate of diffusion through the lung membrane in limiting the rate of pulmonary gas exchange, and the justice of Roughton's original challenge would in consequence be sustained.

**THEORY**

A. Derivation of Equation 6 for Special Case of Inhalation of CO Mixtures With High O2 Content. Inhalation of a low percentage of carbon monoxide in oxygen (e.g. 0.3% CO + 98% O2) is an especially simple case to consider for the following reasons:

Roughton (14) has shown that when such a gas mixture is breathed, the blood entering the lung capillaries must become practically 100% saturated with oxygen by the time it has proceeded along the first one-hundredth of the length of the lung capillary. The amount of carbon monoxide entering the first hundredth of the lung capillary is, on the other hand, of the order of only 2% of that entering the remaining ninety-nine hundredths of the capillary. During the latter stage, the carbon
monoxide which has entered the blood plasma passes into the red cell, wherein it finds the hemoglobin at a practically constant percentage saturation with oxygen (i.e. 99 to 100% for the above gas mixture) and a practically constant partial pressure of dissolved oxygen \( (P_{CO_2}) \), i.e. about 84% atmospheres, or 638 mm Hg.

Let \( v \) = rate of formation of COHb in the lung capillary at any given point in milliliters per minute

\[
\frac{m_v}{60} \alpha_{CO} (O_2Hb)/\alpha_{O_2} \text{ml} = v \\
\]

where \( m_v \) (sec\(^{-1}\)) is the initial replacement constant of oxygen by carbon monoxide in human red cell suspensions at 37° C for low values of \( (CO)/(O_2) \) (see ref. 19 for its actual value); \( \alpha_{CO} \), \( \alpha_{O_2} \) are the respective solubility coefficients of carbon monoxide and oxygen. \( P_{CO_2} \), \( P_{O_2} \) are the respective partial pressures of carbon monoxide and oxygen in the capillary plasma at the point in question. It should be noted that \( m_v \) varies with \( O_2 \) tension (19).

Then \( v = \theta P_{CO} \) where \( \theta \) is another constant (ml/min. X mm Hg X ml), consisting of the product of all the terms other than \( P_{CO} \) on the right hand side of equation 7, each of these terms being itself constant at a given \( P_{O_2} \).

The volume of carbon monoxide diffusing through the membrane surrounding the differential length, \( dl \), of a single capillary in time, \( dt \),

\[
\frac{Sd}{x} (P_{CO} - P_{CO}) \text{dl} dt \\
\]

where \( S \) is the surface area of the capillary per unit length, and \( P_{CO} \) is the alveolar partial pressure of carbon monoxide which can be taken as constant during the infinitesimal interval, \( dt \), and the remaining symbols as defined above. It is easy to show that, on the average, only about 1% at most, of the carbon monoxide entering the blood capillary accumulates in the plasma; the remaining 99% passes into the red cell, where its increase in time, \( dt \), amounts to \( \theta P_{CO} \text{ml} \text{dl} dt \), a being the cross-sectional area of the capillary. Neglecting the accumulation of carbon monoxide in the plasma it therefore follows that

\[
\frac{Sd}{x} (P_{CO} - P_{CO}) \text{dl} dt = \theta P_{CO} \text{ml} \text{dl} dt \\
\]

whence

\[
P_{CO} = \frac{P_{ACO}}{1 + \frac{\theta \text{a}x}{Sd}} \\
\]

The value of \( P_{CO} \) is thus a constant fraction of \( P_{ACO} \) along the whole length of the lung capillary except the first one-hundredth assuming \( a, x, s \) and \( d \) are constant (or are average values) for that capillary. The total volume of carbon monoxide passing into all the \( n \) capillaries of the lung, including the whole length, \( l \), of each capillary, in time \( dt \) is clearly equal to

\[
\left( \frac{P_{ACO} - P_{CO}}{x} \right) \int_0^1 \text{dl} dt \\
\]

assuming \( l, S, d, x \) and \( a \) are also average values for all the capillaries. Since \( nS = A \) and \( DM = Ad/x \).

\[
\frac{DMP_{ACO} \text{dt}}{\left( i + \frac{Sd}{\theta \text{a}x} \right)} = \frac{DMP_{ACO} \text{dt}}{\left( i + \frac{\theta \text{a}x}{Sd} \right)} \\
\]

where

\( V_e = \text{the total blood in all the lung capillaries} = anl. \)

But the total volume of carbon monoxide passing into the blood from the lung in time \( dt \) is also equal to

\[
DLP_{CO} \text{dt} \\
\]

where \( Dl \) is the over-all diffusing capacity. Comparison of equation 13 and 12 then leads to the relation

\[
DL = DM \left( i + \frac{\theta \text{a}x}{Sd} \right) \\
\]

whence, by taking reciprocals of each side

\[
\frac{1}{DL} = \frac{1}{DM} + \frac{1}{\theta \text{a}x} \\
\]

When the inspired \( O_2 \) concentration is lowered below 98%, \( \theta \) should still remain approximately constant over the length of the lung capillary until the alveolar \( O_2 \) tension reaches approximately 200 mm Hg (38% \( O_2 \)). However, the actual value of \( \theta \) will increase
(1/θ will decrease), as seen in figure 1 which is based on the kinetic data from the previous papers. If, furthermore, $D_M$ and $V_e$ remain constant over this range of inspired $O_2$ concentrations, as has been generally assumed by previous workers in this field (2) (for recent direct support of this assumption see ref. 21), then a plot of $1/D_1$, as measured experimentally, against $1/θ$, derived from figure 1, should give a straight line (fig. 2). The slope of this line is $1/V_e$ and the intercept on the vertical axis equals $1/D_M$.

It has been tacitly assumed to this point that there are no CO gradients within the plasma itself. Suppose the plasma is incompletely mixed. Let $P_{CO}^{'}$ = plasma CO pressure just inside the pulmonary membrane and $P_{CO}^{''}$ = average pressure of CO on the surface of the red cell. To a rough approximation the average CO gradient in the plasma should be proportional to the gradient across the pulmonary membrane, i.e.

$$P_{CO}^{''} - P_{CO}^{'} = f(P_{CO} - P_{CO}^{''})$$

(16)

Where $f$ is constant and independent of $P_{O_2}$. Under these circumstances it can be shown that *equation 6* becomes

$$\frac{1}{D_M} - \frac{1 + f}{D_1} = \frac{1}{V_e}$$

(17)

Therefore, if CO gradients do exist in the plasma itself our estimate of $V_e$ will be correct if $1/D_1$, as measured experimentally, against $1/θ$, derived from figure 1, should give a straight line (fig. 2). The slope of this line is $1/V_e$ and the intercept on the vertical axis equals $1/D_M$.

But we will underestimate $D_M$ by the factor $1 + f$. Probably the motion of the red cells through the pulmonary capillaries does impart some degree of mixing to the adjacent plasma, but at present we do not know how complete such mixing may be. In default of such knowledge it seems simplest to adopt the convention that the plasma is incompletely mixed, i.e. $f = 0$; the actual values of $D_M$ given in the remainder of this paper are all based on this convention and are thus minimal values. If by subsequent research it proves possible to obtain $f$, the values of $D_M$ given below can then be readily corrected by multiplying them all by the factor $1 + f$.

Of course, $\gamma = \varnothing$ corresponds to a red cell whose membrane offered no resistance to gas diffusion at all; in other words, a layer of hemoglobin solution of average thickness and concentration to the red cell. The curve for mixed hemoglobin solution is included for interest.

**B. Justification of Equation 6 When Alveolar $O_2$ Tension is Less Than 200 mm Hg and More Than 85 mm Hg.** As the alveolar $O_2$ tension falls below about 200 mm Hg (which corresponds to an inspired $O_2$ tension of 28% atmosphere), *equation 6* becomes progressively more inexact because the $O_2$ tension changes markedly along the capillary: $θ$ is therefore no longer a constant and in consequence the integral in *equation 11* cannot be evaluated exactly. It was, however, of special importance.
corresponding to the venous and arterial \( O_2 \) tensions are 56 and 65 ml/min/mm Hg and of \( V_c \) 110 and 103 ml, respectively. The mean values of \( D_m \) and \( V_c \) have therefore an uncertainty, at most, of \( \pm 7\% \) and \( \pm 3\% \), respectively. Actually the mean \( O_2 \) tension in the lung capillary is much nearer the arterial \( O_2 \) tension than the mixed venous tension. Usually the mean capillary \( O_2 \) tension (at rest) is taken to be about 10 mm Hg below the alveolar \( O_2 \) tension (2) but in view of our finding (vide infra) that \( D_m \) is of the order of twice \( D_l \) it seems fair to assume that the mean \( O_2 \) tension in the lung capillary plasma is only about 5 mm Hg below the alveolar \( O_2 \) tension, and it is on this latter basis that our values of \( 1/\theta \) have been chosen in the subsequent figures of the present paper.

C. Calculation of \( D_m \) and \( V_c \). Figure 1 shows the relation between \( 1/\theta \) and \( O_2 \) tension surrounding the red cell for various values of \( \lambda \), the ratio of the permeability of the red cell membrane to the permeability of the red cell interior (the curve relating \( \theta \) and \( O_2 \) tension for homogeneous hemoglobin solutions is also included). \( 1/\theta \) has been used on the ordinate instead of \( \theta \) because it gives an approximately linear relationship and is more convenient. Actually \( \lambda \) equals \( D_2/b_2 \div D_1/b_1 \), where \( D_1, D_2 \) are the respective diffusion coefficients of carbon monoxide in the red cell interior and in the red cell membrane, and \( b_1, b_2 \) are the respective thicknesses of the red cell interior and the red cell membrane. The four \( \lambda \) curves plotted in the figure are theoretical ones calculated from equation 7 and figure 1 of the second paper of the present series (19), the actual values of \( \theta \) being obtained by multiplying (the initial cell rate)/(the initial solution rate) by \( 174/(O_2 \text{ tension surrounding the red cells in mm Hg}) \). The figure 174 equals \( 18.8 \times 60 \times 0.77 \times 0.20 \); 18.8 is the average \( m_\theta^2 \) of the photocolorimetric data from the previous paper (19), 60 the seconds in a minute, 0.77 the ratio of CO to \( O_2 \) solubility in plasma and 0.20 the CO (or \( O_2 \)) capacity of blood in milliliters of gas per milliliters of blood. It should be stressed that the values of \( \theta \) in figure 1 apply to low (CO)/(\( O_2 \)) values, such as are met with in the diffusing capacity determinations, having been corrected from the observed results on cell suspensions.
exposed to higher (CO)/(O₂) values in the manner described in the second paper of this series (19). Actually the observed data, as corrected, correspond quite well to the curve for λ = 1.5 in the case of zero O₂ tension and at tensions above 300 mm Hg; in the vicinity of 100 mm Hg O₂ tension a value of λ = 2.5 was found to give a better fit, but the uncertainty in λ, especially at slow rates of reaction, is too great to be sure that there is a real difference at varying O₂ tensions. Reference to equations 7 and 8 shows that θ is proportional to the amount of O₂Hb present at a given O₂ tension; in other words, the hemoglobin CO or O₂ capacity. In this paper an average value for O₂ capacity of 0.2 ml gas per milliliter of blood was used. The actual values for the O₂ capacity of venous blood samples for the different subjects varied only ±5% from this figure, which produces an equal uncertainty in Vc, but does not affect DM. In addition, the pulmonary capillary hematocrit may not be equal to that in the venous blood sample. Rapaport et al. (22) calculated that the average hematocrit in the pulmonary vasculature was only 84 to 95% of that in the large vessels in lightly anesthetized dogs. However, these measurements apply to more than just the pulmonary capillary bed. Gibson et al. (23), using labeled plasma and red cells, measured the hematocrit of blood in the finer vessels of the lung parenchyma in healthy dogs killed with Nembutal and found it to be about 23% less than that in the large vessels. In other words, we may be overestimating the hematocrit in the pulmonary capillary bed by this amount, thereby overestimating θ and underestimating Vc. When human data on the capillary hematocrit, as opposed to the hematocrit of the whole pulmonary circuit, become available, the present values of Vc can easily be corrected in proportion.

In previous work on sheep red cell suspensions (24), much higher values of λ (up to 10 or more) have sometimes been observed and it is possible that the in vitro conditions under which the reactions were observed in human red cell suspensions may have produced an artificial lowering of λ. Figure 1 accordingly includes a calculated curve for λ equal to infinity, implying an infinite permeability of the red cell membrane such as has been approached in certain sheep red cell suspensions (24). The curve for λ = 1.5 is given because this value of λ is obtained in one of the preceding papers (19).

Figure 3 is a plot of 1/DL versus 1/θ for the same experiments on R.E.F., as in figure 2, but with the values of θ corresponding to λ = 1.5, λ = 2.5 and λ = infinity, the last case giving a lower limit to the value of DM and an upper limit to Vc. The second and third columns of table 1 give the values of DM and Vc as calculated by equation 6 for the three values of λ used in figure 3; the fourth and fifth columns of table 1 give values of DM and Vc similarly calculated from diffusion capacity measurements on the same subject by the steady state method, at alveolar oxygen tensions ranging from 118 to 677 mm Hg. The values of Vc by the two diffusing capacity methods agree closely and are furthermore

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**Fig. 3.** Graphs of 1/DL (breath holding) against 1/θ for the three cases, λ = 1.5, λ = 2.5 and λ = ∞ applied to the data of R.E.F.

**Fig. 4.** Graphs of 1/DL, as measured by Kruhøffer (18), against 1/θ for λ = 1.5 and λ = ∞.
September 1957

DIFFUSING CAPACITY OF PULMONARY MEMBRANE

Table I. DM and \( V_c \) calculated for three values of \( \lambda \) using breath holding and steady state estimates of DL in subject R.E.F.

<table>
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<th>( \lambda )</th>
<th>Breath Holding</th>
<th>Steady State</th>
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<tbody>
<tr>
<td></td>
<td>DM (ml)</td>
<td>( V_c ) (ml)</td>
</tr>
<tr>
<td>(1) 1.5</td>
<td>86</td>
<td>97</td>
</tr>
<tr>
<td>(2) 2.5</td>
<td>65</td>
<td>103</td>
</tr>
<tr>
<td>(3) Infinity</td>
<td>50</td>
<td>106</td>
</tr>
<tr>
<td>Average of (1) and (3)</td>
<td>68</td>
<td>102</td>
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very insensitive to changes in the selected value of \( \lambda \). We therefore have considerable confidence in the determination of \( V_c \), not only in the present case in which the experimental conditions were especially favorable but also in many other cases which we have examined. The values of DM by the two methods agree closely though there is a clear trend towards lower values by the steady state method as is the case, of course, with the DL values. DM, it will be noted, is much more sensitive to the particular value of \( \lambda \) used in the computation, its value varying from 30 to 40% in the examples given in table 1. In view of our uncertainty about the true value of \( \lambda \) in vivo we have as a compromise taken the average of the two extreme values of DM, corresponding to \( \lambda = 1.5 \) and \( \lambda = \infty \), which in the case of table 1 happen to approximate the value of DM corresponding to \( \lambda = 2.5 \). We believe that the average values of DM in table 1, i.e. 68 by the breath holding method and 50 by the steady state method, are correct to 20% or less. They are about 1.8 times greater than the measured value of DL when breathing 21% \( O_2 \) thus showing that in the case of subject R.E.F. the speed of the processes within the blood itself is about four-fifths as important as the speed of the diffusion through the pulmonary membrane in the over-all uptake of CO in the lung when breathing 21% \( O_2 \) at sea level.

RESULTS AND DISCUSSION

A. Effect of Processes Within the Blood on Rate of CO Uptake in the Lung. The average values of DL (breathing 21% \( O_2 \) at sea level), DM and \( V_c \) were calculated from the results of the preceding paper (20), in the manner described above for seven normal subjects using the steady state and 10-second breath holding methods of estimating DL. The results are given in the first eight columns in table 2. DL (10-sec. breath holding) at an alveolar \( O_2 \) tension of 100 mm Hg is on the average 39% larger than DL (steady state). Actually DL (breath holding) = \(-2.6 + 1.53 \) \( D_2 \) (steady state) with a correlation coefficient of 0.81 (probability less than 0.05). Likewise, \( V_c \) for the breath holding data is, on the average, 39% greater than \( V_c \) for the steady state data, but the correlation is not significant (correlation coefficient equals 0.49). DM is highly variable, with occasional values which are negative or infinite. The basis of this variation can be seen in any of the graphs of \( 1/DL \) against \( 1/\theta \) (figs. 2, 3 or 4). If the intercept is close to the origin, a small error in the estimated DL can lower the intercept to the origin or below, producing estimates of DM which are infinite or negative. This is much more likely to happen when DL is larger, and it is therefore reasonable that it should occur for the breath holding values and not for the steady state values. It is also more likely for \( \lambda = 1.5 \) than for \( \lambda = \infty \), since the intercept is less in the former case. In those instances in table 2 where the value of DM for \( \lambda = 1.5 \) was infinite or negative, the value of \( V_c \) for \( \lambda = \infty \) was given as a lower limit of the average value. \( V_c \) is a more reliable datum because it is not subject to this difficulty.

It should be emphasized that our theoretical calculations of DM and \( V_c \) implicitly assume uniform distribution of capillary blood volume and diffusing capacity to alveolar ventilation in the case of the steady state method of DL estimation, and to alveolar volume in the case of the breath holding method. These assumptions are not precisely true, but are probably reasonable in normal subjects, and, moreover, we have no alternative at present. The fact that the three estimates of DM and \( V_c \) in table 2 agree as well as they do indicates that the assumptions are not greatly in error.

In general, the values of \( V_c \), DL, and DM appear to be larger for the 10-second breath holding data than for the data obtained by other methods, and it is thus clear that the
### Table 2. $D_{M}$, $V_{c}$ and $D_{L}$ (100 mm Hg $P_{O2}$) measured by steady state and breath holding method in seven subjects

<table>
<thead>
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<th>Subj.</th>
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<th>30-sec Breath Holding</th>
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</tbody>
</table>

$D_{L}$ is the apparent pulmonary diffusing capacity obtained at an alveolar $O_{2}$ tension approximating 100 mm Hg. $D_{M}$ and $V_{c}$ are the pulmonary membrane diffusing capacity and pulmonary capillary blood volume, respectively, calculated as the average of the values obtained using $\lambda = 1.5$ and $\lambda = \infty$ in the manner described in the text. (Resistance cell)/(resistance membrane) equals $\frac{1}{\theta V_{c}} / \frac{1}{D_{M}}$. Substituting the value of $\frac{D_{M}}{\theta V_{c}}$ from eq. 6, one obtains $\frac{D_{M}}{D_{L}} = 1$.

* Indicates that the value of $D_{M}$ for $\lambda = 1.5$ was negative or infinite so that the average of value for $\lambda = 1.5$ and $\lambda = \infty$ is meaningless. In this circumstance the value of $D_{M}$ and (resistance cell)/(resistance membrane) calculated for $\lambda = \infty$ are given as lower limits. Corresponding average values do not include these extreme results.

Absolute values of $V_{c}$ and $D_{M}$ both depend on the method used to measure them. However, the relative importance of the CO gradients within the whole blood compared with the gradient across the pulmonary membrane can, with a reasonable assumption, be shown to be independent of the particular method used to measure $D_{L}$. If any given method of estimation of $D_{M}$ is in error by a factor $F$, then from equation 6,

$$\frac{1}{FD_{L}} = \frac{1}{FD_{M}} + \frac{1}{F\theta V_{c}}$$  \hspace{1cm} (18)

provided we assume that $D_{L}$ is in error by the same factor over the range of alveolar $O_{2}$ tensions studied, which is reasonable as a first approximation. The measured resistance of the pulmonary membrane equals the CO pressure gradient across it divided by the CO uptake, i.e. $1/FD_{M}$. In a similar manner, the resistance of the red cell to CO uptake is $1/F\theta V_{c}$. Therefore, the cell resistance/membrane resistance $= D_{M}/\theta V_{c}$ and is independent of the factor $F$. The values of this ratio have been calculated and are also given in table 2. While the ratio varies markedly it is always greater than 41%.

In a further effort to demonstrate that this last conclusion is independent of the particular method used to measure $D_{L}$ we calculated $D_{L}$ (breath holding) at 30 seconds instead of the usual 10 seconds. These data are in the last three columns of table 2. $D_{L}$ is less than when it is measured by the 10-second method as expected (11), but the resistance ratio is greater than 36% in all cases. In addition we have calculated $D_{M}$, $V_{c}$ and the ratio (cell resistance)/(membrane resistance) in resting normal subjects for the published data of a) Roughton (14), b) Kruh&ffer (15) and c) data which Dr. D. V. Bates was kind enough to send us, and all these values, as well as our average results, are presented in table 3. The values of $D_{L}$ from Roughton’s data were obtained by dividing the rate of increase of total blood COHb by alveolar $PcO$ calculated from an estimated respiratory dead space and knowledge of inspired and expired CO concentrations and tidal volume. Kruh&ffer’s estimate of $D_{L}$ was obtained by...
measuring the rate of change of CO concentration in a closed rebreathing circuit, using the equation of Krogh and Krogh (8). Radioactively labeled CO was used so blood COHb was effectively zero. Since the data they presented on two subjects at many different alveolar O₂ tensions were not significantly different, they have been combined and are plotted as 1/DL against 1/θ in figure 4. The values of DL supplied by Bates et al. (25) were obtained under steady state conditions by an end-tidal sampling method, similar to that reported in the previous paper in this series (20). The average Vₑ ranges from 58 to 110 ml and average DM from 26 to 89 ml/min/mm Hg, but the average (cell resistance)/(membrane resistance) is greater than 47% in each case. Since our data, particularly the later experiments on R.E.F. and L.C. were obtained under steady state conditions by an end-tidal sampling method, similar to that reported in the previous paper in this series (20), the average Vₑ ranges from 58 to 110 ml and average DM from 26 to 89 ml/min/mm Hg, but the average (cell resistance)/(membrane resistance) is greater than 47% in each case.

The values of DL obtained at varying alveolar O₂ tensions are compared with those of Roughton (89-127) and Kruhöfffer (56-85) in table 3. From this table, it is evident that DL is of the order of twice DM, and that full account was taken of the variation in alveolar CO tension and that the alveolar CO tension varies even though the same CO percentage is present in the inspired gas. Reference to Roughton's paper (14) shows, however, that full account was taken of the variation in alveolar CO tension in his earlier work and have shown that this inverse relationship is not correct (see fig. 1); applying the average of our recent results on 8 to Kruhöfffer's data (18) leads to a ratio of DM to DL of 1.48 to 1, as table 3 indicates. Kruhöfffer furthermore objects to Roughton's (14) calculation of the average time spent by the blood in the alveolar capillaries from observations of the rate of CO uptake with increasing alveolar O₂ tensions on the ground that the alveolar CO tension varies even though the same CO percentage is present in the inspired gas. Reference to Roughton's paper (14) shows, however, that full account was taken of the variation in alveolar CO tension and that therefore this criticism of Kruhöfffer's is invalid. As a matter of fact, calculations of DL at varying alveolar O₂ tensions are implicit in Roughton's earlier estimations (14) of tₑ, which—though admittedly somewhat involved—are theoretically on about as sound a basis as the present estimations based directly on values of DL at different O₂ tensions.

### Table 3. Comparison of values of DL (100 mm Hg Pₐo₂), DM and Vₑ from different sources

<table>
<thead>
<tr>
<th></th>
<th>DL (100 mm Hg)</th>
<th>DM (ml/mm Hg)</th>
<th>Vₑ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ml/min/mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roughton (14), 2 subjects</td>
<td>90 (51-127)</td>
<td>70 (56-85)</td>
<td>200 (76-35)</td>
</tr>
<tr>
<td>Kruhöfffer (18), 2 subjects</td>
<td>97</td>
<td>110</td>
<td>48</td>
</tr>
<tr>
<td>Bates (personal communication), 6 subjects</td>
<td>15 (10-20)</td>
<td>26 (10-48)</td>
<td>73 (42-112)</td>
</tr>
<tr>
<td>Forster and Roughton (steady state), 6 subjects</td>
<td>21 (15-28)</td>
<td>40 (20-70)</td>
<td>59 (28-98)</td>
</tr>
<tr>
<td>Forster and Roughton (10-sec. breath holding), 7 subjects</td>
<td>30 (18-39)</td>
<td>57 (35-76)</td>
<td>79 (30-168)</td>
</tr>
</tbody>
</table>

Figures in parentheses are ranges.

*In some instances these values have been obtained at approximately 100 mm Hg alveolar O₂ tension from the least squares regression of 1/DL on 1/θ.

Kruhöfffer (18) arrived at the conclusion that DL was only 10 to 15% less than DM; in other words, the (cell resistance)/(membrane resistance) equals 11 to 18%, compared with our estimate of 50% or more. The reason for this discrepancy lies in Kruhöfffer's assumption, based on Roughton's earlier work (16) that θ for red cell suspensions is inversely proportional to O₂ tension at O₂ tensions greater than 200 mm Hg. Our recent studies (19) have, however, brought to light errors in Roughton's earlier work and have shown that this inverse relationship is not correct (see fig. 1); applying the average of our recent results on θ to Kruhöfffer's data (18) leads to a ratio of DM to DL of 1.48 to 1, as table 3 indicates. Kruhöfffer furthermore objects to Roughton's (14) calculation of the average time spent by the blood in the alveolar capillaries from observations of the rate of CO uptake with increasing alveolar O₂ tensions on the ground that the alveolar CO tension varies even though the same CO percentage is present in the inspired gas. Reference to Roughton's paper (14) shows, however, that full account was taken of the variation in alveolar CO tension and that therefore this criticism of Kruhöfffer's is invalid. As a matter of fact, calculations of DL at varying alveolar O₂ tensions are implicit in Roughton's earlier estimations (14) of tₑ, which—though admittedly somewhat involved—are theoretically on about as sound a basis as the present estimations based directly on values of DL at different O₂ tensions.

**B. Effect of Processes Within the Blood on Rate of O₂ Uptake in the Lung.** So far, in this paper, attention has been concentrated on the rate of distribution of carbon monoxide within the blood as regards its effect on the carbon monoxide diffusing capacity of the lung, DₑCO. During the past 50 years there have been repeated discussions of many of the factors limiting the rate of oxygen uptake in the lungs of man, especially during exercise at high altitudes, but so far no significant attention has been paid to the influence exerted by the rate of distribution...
of oxygen within the blood, which has been tacitly assumed to be instantaneous. It is now desirable, therefore, to consider, as far as our present theoretical and experimental data permit, this analogous problem in regard to oxygen.

*Equation 6*, relating $D_{LO_2}$ to $D_m$, $\theta$ and $V_c$, should apply to oxygen as well as to carbon monoxide, but of these various parameters only $V_c$ should be exactly the same under equivalent conditions of inspired $O_2$ tension, breathing and physiological activity. $D_m$ for oxygen, i.e. $D_{MO_2}$ should be equal to 1.21 times $D_m$ for CO (see eq. 4). As regards $\theta$ for oxygen, there are not only serious gaps on the experimental side but also theoretical difficulties to each of which we shall now refer.

The data in the first paper of the present series (26) yield an average value of $\theta = 1.5$ for the initial rate of combination of oxygen with fully reduced human red cell suspensions at 37°C. The only data so far available for partially saturated red cells are those given in a) a single experiment plotted in figure 6 of the paper by Gibson et al. (17) for the rate of uptake of oxygen by the red cells of subject H. G. and b) a single experiment on the blood of F. K. quoted in the first paper of the present series (26). In both of these experiments the red cells initially contained about 35% $O_2$Hb. The corresponding values of $\theta$ in the two cases were 1.3 and 1.28, respectively (mean 1.14), as compared with the normal value of $\theta = 1.5$ for the cells of the same subjects when completely reduced. During rest and/or light activity the mixed venous blood commonly contains 50-70% $O_2$Hb, at which percentages the initial value of $\theta$ may possibly be lower than in the range 0-35% $O_2$Hb, but unfortunately there has been, as yet, no opportunity to carry out direct determinations in this range, which is experimentally much more difficult to handle accurately with our present techniques. Adaptations of the technique to initial values of 50% $O_2$Hb and higher are obviously needed.

On the theoretical side there is the difficulty that the blood, in its passage through the lung capillary, approaches so close to equilibrium with the $O_2$ tension in the alveolar air that it is far from sound to assume that the average value of $\theta$ in the lung capillary, $\theta_{02}$, is equal to the initial value of $\theta$ at the percentage of $O_2$Hb in the mixed venous blood entering the lung capillary. Unfortunately, it is only this latter value of $\theta$ that our present techniques are readily suited to measure: $\theta_{02}$ is almost certainly appreciably less, but so far we have found no satisfactory way of estimating its actual value in any given set of conditions either by theoretical calculations or by attempts to match by experiments with *in vitro* red cell suspensions the conditions existing *in vivo* in the lung capillary.

It is, however, possible—and has proved useful—to calculate by means of *equation 6* the values of $D_{LO_2}$ corresponding to the range within which $\theta_{02}$ might reasonably be expected to lie, assuming fixed values of $D_{MO_2}$ and $V_c$. Table 4 shows the results of such calculations of $D_{LO_2}$ together with the value of (red cell resistance)/(pulmonary membrane resistance) for $O_2$ for the case of subject R. E. F., at rest, using the average values from table 1 of $V_c$ and $D_m$, the latter being multiplied by 1.21 to give $D_{MO_2}$. Even at the maximal value of $\theta_{02}$, i.e. 1.5, corresponding to the initial value of $\theta$ for completely reduced red cells, the (resistance of the cells)/(resistance of the pulmonary membrane) is 49%—a quite significant figure—and rises to 63% for $\theta_{02} = 1.15$, the average initial value of $\theta_{02}$ for cells containing 35% $O_2$Hb. Actually $\theta_{02}$ in the resting subject may well be less than 1.0—perhaps considerably less—in which case the (cell resistance)/(membrane resistance) would be of the order of unity or greater. A plot of the plasma $O_2$ tension along the capillary derived by apply-

<table>
<thead>
<tr>
<th>Value of $\theta$</th>
<th>1.5</th>
<th>1.3</th>
<th>1.2</th>
<th>0.8</th>
<th>0.6</th>
<th>0.4</th>
<th>0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{LO_2}$ ml/m</td>
<td>10</td>
<td>6.7</td>
<td>4.6</td>
<td>2.3</td>
<td>1.8</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Resistance of cells</td>
<td>40</td>
<td>36</td>
<td>63</td>
<td>73</td>
<td>92</td>
<td>122</td>
<td>183</td>
</tr>
<tr>
<td>Resistance of membrane in %</td>
<td>283</td>
<td>243</td>
<td>31.9</td>
<td>25.8</td>
<td>21.3</td>
<td>18.3</td>
<td>15.1</td>
</tr>
</tbody>
</table>

Table 4. Relation between $D_{LO_2}$ and $\theta_{02}$ for the case $D_{MO_2} = 1.21$, $D_{MO_2} = 73$ and $V_c = 100$, as calculated from *equation 6*.
FIG. 5. A plot of $O_2$ tension in the plasma as the blood moves through the capillary, for four different values of $\theta$ for $O_2$. Curve for $\theta = \infty$ corresponds to a situation where plasma $O_2$ tension is always in equilibrium with the intracellular hemoglobin, i.e. the Bohr integration. The example is taken from Riley and Cournand (27).

ing the ratios, (cell resistance)/(membrane resistance), in table 4 to an example of a Bohr integration published by Riley and Cournand (27) is given in figure 5. As the blood enters the pulmonary capillary $O_2$ diffuses across the pulmonary membrane into the plasma, increasing the tension there until the rate at which $O_2$ is entering the red cells equals the rate at which $O_2$ diffuses into the plasma outside the alveoli. If $\theta$ were infinite, the plasma tension would always be in equilibrium with intracellular hemoglobin. Since this is the condition assumed in the Bohr integration, the curve for $\theta = \infty$ corresponds to that determined by the Bohr method. If $\theta$ is not infinite, which appears to be the case, a fraction equal to (cell resistance)/(membrane resistance) divided by (one plus this ratio) of the total $O_2$ gradient from the alveolar gas to the intracellular hemoglobin will exist between the plasma and the intracellular hemoglobin. This is indicated by the curves for different values of $\theta$. Of course the instant the blood leaves the capillary, the relatively small amount of $O_2$ dissolved in the plasma continues to react with intracellular hemoglobin and the plasma and cells come rapidly into $O_2$ equilibrium.

Further reference to table 4 shows that a decrease of $\theta_{O_2}$ from 1.0 to 0.3 reduces the calculated value of $D_{LO_2}$ about twofold. This point may have a bearing on the relative value of $D_{LO_2}$ at rest and in exercise as measured by Lilienthal et al. (2). Although the value of $D_{LO_2}$ at rest is admittedly somewhat uncertain, it seems fairly clear that the increase in the measured value of $D_{LO_2}$ on exercise tends to be more marked than the increase in the value of $D_{LCO}$, whether determined by steady state or by breath holding methods. On exercise the percentage of $O_2 Hb$ in the mixed venous blood entering the lung capillaries may be appreciably less than at rest: in line with this the initial value of $\theta_{O_2}$ would be expected to be greater, and so also would the value of $\theta_{O_2}$, not only on account of the higher initial $\theta_{O_2}$ but also because the blood tends not to approach so closely to equilibrium with the $O_2$ tension in the alveolar air as during rest.

The true chemical rate of combination of $O_2$ with reduced or partially oxygenated human hemoglobin at 37°C is of the order of 20-150 times greater than the true chemical rate of replacement of $O_2$ from combination with hemoglobin by CO, according as the initial $O_2$ tension in equilibrium ranges from 100 to 700 mm Hg. The overall rate of $O_2$ uptake by reduced or partially saturated red cell suspensions should therefore be more sensitive to changes in $\lambda$, the ratio of the permeability of the red cell membrane to the red cell interior, than is the case in the overall rate of $O_2$ replacement by CO. So far the in vitro values of $\lambda$ for red cell suspensions, made from blood drawn from normal men at rest or in light activity at sea level, have shown relatively small variations. Changes in $\lambda$ of the order of threefold have, however, been seen in the blood drawn from the same sheep in summer and in winter (24). It would obviously be of interest to compare the value of $\lambda$ for the blood of normal men in rest, after prolonged exercise and if possible in the polycythemia which occurs during acclimatization to low $O_2$ tensions at high altitudes. A significant rise in $\lambda$, with a concomitant increase in $\theta_{O_2}$, would decrease the difference between $D_{LO_2}$ and $D_{LCO}$ and thus provide a more efficient $O_2$ uptake in the lung without any necessary increase in $D_{LCO}$, i.e. any increase in the area (or decrease in thickness) of the pulmonary membrane. Similar studies of $\theta_{O_2}$, $\lambda$, and $D_{LO_2}$ should of course be extended to as wide a
range as possible of abnormal conditions in man. In conclusion, we would stress that although we have been unable to estimate precisely the significance of the rate of oxygen distribution within the blood, there is nevertheless good reason to suppose that the O₂ gradients within the blood are not only of the same order of importance as the CO gradients but also may exert a more widely varying influence on the over-all rate of O₂ uptake in the lung than is the case in the analogous problem of CO uptake during diffusing capacity determinations.

REFERENCES