

The Steady-State Transport of Oxygen through Hemoglobin Solutions

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ABSTRACT The steady-state transport of oxygen through hemoglobin solutions was studied to identify the mechanism of the diffusion augmentation observed at low oxygen tensions. A novel technique employing a platinum-silver oxygen electrode was developed to measure the effective diffusion coefficient of oxygen in steady-state transport. The measurements were made over a wider range of hemoglobin and oxygen concentrations than previously reported. Values of the Brownian motion diffusion coefficient of oxygen in hemoglobin solution were obtained as well as measurements of facilitated transport at low oxygen tensions. Transport rates up to ten times greater than ordinary diffusion rates were found. Predictions of oxygen flux were made assuming that the oxy-hemoglobin transport coefficient was equal to the Brownian motion diffusivity which was measured in a separate set of experiments. The close correlation between prediction and experiment indicates that the diffusion of oxyhemoglobin is the mechanism by which steady-state oxygen transport is facilitated.

INTRODUCTION

It has been observed by Scholander and Hemmingsen (9, 21) as well as others (14, 23) that, at relatively low oxygen concentrations, the steady-state rate of transport of oxygen through hemoglobin solutions significantly exceeds that predictable on the basis of Brownian motion diffusion of the oxygen molecules. These observations have led investigators to postulate that an oxygen transport-augmenting process exists in the system, effective at low oxygen concentrations.

This augmenting process has generated considerable interest because of its possible importance in respiratory diffusion. Transport mechanisms by which the augmentation might occur have been proposed and several analyses based on the various models have been published (3, 6, 22). The proposed mechanisms have certain features in common from which a qualitative description of the phenomenon can be evolved. As oxygen diffuses into a deoxygen-

ated, or partially oxygenated, solution of hemoglobin, some of it combines with the reduced hemoglobin. This results in an oxyhemoglobin concentration gradient and an oxyhemoglobin flux. The total flux of oxygen through the solution is the sum of its flux as molecular oxygen and its flux as oxyhemoglobin.

The greatest difficulty has arisen in reaching agreement on the molecular mechanism producing the oxyhemoglobin flux. The view is held by some (6, 22) that the flux is simply due to the Brownian motion diffusion of the hemoglobin molecule as a result of a concentration gradient driving force. However, others, including Scholander (21, 14) are of the opinion that the size of the hemoglobin molecule is sufficiently large to preclude any significant hemoglobin diffusion flux. They have suggested that, during collisions between adjacent hemoglobin molecules, oxygen is transferred from oxygenated to deoxygenated molecules. This results in an apparent flux of oxyhemoglobin without an accompanying translational motion of the large protein molecules.

Both the diffusional and collisional mechanisms depend upon a gradient in oxyhemoglobin to produce a flux and thus require that at least some of the hemoglobin be only partially oxygenated. This is consistent with the experimental observation that the augmentation effect only appears at low oxygen tension, the condition under which the hemoglobin is not fully saturated.¹

This paper reports the results of a study (10) of the augmentation mechanism. It should serve as an initial step in evaluating the importance of augmented transport in the red blood cell. Since the hemoglobin-oxygen system is, in certain respects, similar to other diffusing, reacting systems of physiological as well as nonphysiological interest, the results of this study should also provide a useful basis for interpreting and analyzing similar phenomena in a broad range of systems.

ANALYTICAL CONSIDERATIONS

From a physicochemical standpoint, the hemoglobin-oxygen system is one in which a species is diffusing through a solution containing a component with which it reacts. Since the hemoglobin-oxygen reaction rate is quite rapid and the reaction is reversible, the components of the system are always near, though not at, chemical equilibrium. Several analyses of the augmentation

¹ Hemmingsen has argued erroneously that this is not a requirement for the phenomenon (8). In a set of experiments conducted at oxygen tensions in excess of the saturation value, using $O^{16}O^{18}$ as a tracer, he found that the flux of $O^{18}O^{18}$ exhibited the same augmentation effect previously observed only at lower oxygen tensions. Since no gradient in HbO_2 existed during the experiments, he argued that the phenomenon did not depend on one. He neglected to take into account the fact that a gradient in $HbO^{16}O^{18}$ did exist during his experiments and thus a flux of $HbO^{16}O^{18}$ would occur, augmenting isotopic oxygen transport.

phenomenon, including those of Wang (22), Collins (3), and Fatt and La-Force (6) are based on the approximation that chemical equilibrium exists among the components at all points in the system. In this sense they are extensions of the work of Olander (15) who considered several cases for which the equilibrium assumption was valid.

The attractiveness of this approach is that the nonlinear reaction rate terms can be eliminated from the equations of conservation of species, simplifying their solution. However, the method does not permit a check on the validity of the equilibrium assumption. It was shown by Friedlander and Keller (7) that when there is a flux of one of the reactants across a boundary of a reacting system, the deviation from chemical equilibrium is greatest at the boundary. The deviation decreases with distance from the interface and becomes negligible at points further away than a relaxation distance, λ , which depends on the ratio of the forward reaction rate coefficient to the diffusion coefficients. Friedlander and Keller showed that when the ratio of the thickness of the system to λ exceeds 100, the equilibrium approximation is valid. For the hemoglobin-oxygen system, λ is given by (10):

$$\lambda = \left[k' n_{O_2} \left(\frac{n_t(1-y)}{D_{O_2} n_{O_2}} + \frac{1}{D_{Hb}} + \frac{(1-y)}{y D_{HbO_2}} \right) \right]^{-1} \quad (1)$$

where n_t is the total concentration of oxygenated and deoxygenated hemoglobin expressed as g-equivalents/cm³ (4 g-equivalents/g-mole), and y is the fraction of hemoglobin present in the oxygenated form. k' is the forward reaction rate of the hemoglobin-oxygen reaction when it is approximated as:



The maximum value of λ under the experimental conditions of this study was calculated to be 1.2×10^{-4} cm (10). In the experiments described below, the thickness of the diffusion layer was 0.13 cm. Thus the use of an equilibrium approach in analyzing the data is justified.

By modifying the existing equilibrium analyses a quantity related to the total oxygen flux can be defined which is easily measurable. In a one-dimensional, steady-state hemoglobin-oxygen system, the total flux of oxygen at any point is given by

$$J_{O_2} = -D_{O_2} \frac{dn_{O_2}}{dx} - D_{HbO_2} \frac{dn_{HbO_2}}{dx} \quad (3)$$

By definition,

$$n_{HbO_2} = n_t y \quad (4)$$

If $D_{\text{Hb}} = D_{\text{HbO}_2}$ or if the variation in y across the system is small, n_t is constant. Under these conditions, Equation (3) can be rewritten in terms of an effective diffusion coefficient as follows:—

$$J_{\text{O}_2} = -D_{\text{eff}} \frac{dn_{\text{O}_2}}{dx} \quad (5)$$

where

$$D_{\text{eff}} = D_{\text{O}_2} + D_{\text{HbO}_2} n_t \frac{dy}{dn_{\text{O}_2}} \quad (6)$$

The amount of oxygen transport augmentation will depend on the magnitude of the last term in Equation (6). At chemical equilibrium, dy/dn_{O_2} is the slope of the hemoglobin oxygenation curve so that for a given hemoglobin concentration, augmentation will be greatest when the slope of that curve is greatest. When the hemoglobin is fully saturated, dy/dn_{O_2} approaches zero and the problem reduces to one of simple one-dimensional diffusion of oxygen. The quantity D_{eff} is thus a useful measure of the augmentation effect. However, measurements must be made across a finite oxygen difference and it is necessary to introduce an integral diffusion coefficient, $\overline{D}_{\text{eff}}$. For a layer of hemoglobin solution of thickness a , Equation (3) has the following boundary conditions:

$$\text{At } x = 0, \quad n_{\text{O}_2} = (n_{\text{O}_2})_0, \quad n_{\text{HbO}_2} = (n_{\text{HbO}_2})_0 \quad (7a)$$

$$x = a, \quad n_{\text{O}_2} = (n_{\text{O}_2})_a, \quad n_{\text{HbO}_2} = (n_{\text{HbO}_2})_a \quad (7b)$$

Integrating Equation (3) one obtains

$$aJ_{\text{O}_2} = -D_{\text{O}_2}[(n_{\text{O}_2})_a - (n_{\text{O}_2})_0] - D_{\text{HbO}_2}[(n_{\text{HbO}_2})_a - (n_{\text{HbO}_2})_0] \quad (8)$$

The integral diffusion coefficient is defined as

$$\overline{D}_{\text{eff}} = D_{\text{O}_2} + D_{\text{HbO}_2} n_t \frac{y_a - y_0}{(n_{\text{O}_2})_a - (n_{\text{O}_2})_0} \quad (9)$$

so that

$$aJ_{\text{O}_2} = -\overline{D}_{\text{eff}}[(n_{\text{O}_2})_a - (n_{\text{O}_2})_0] \quad (10)$$

The experimental system was designed to measure the integral effective diffusion coefficient at varying conditions of oxygen tension and hemoglobin concentration.

EXPERIMENTAL APPARATUS AND PROCEDURES

Diffusion Cell

Measurements of $\overline{D}_{\text{eff}}$ were made in the oxygen diffusion cell shown in Fig. 1. This cell was constructed of Type 304 stainless steel and was divided into two sections; the

upper section was a gas reservoir and the lower section was a holder for a Clark-type polarographic oxygen electrode.

Sample solutions were placed in a small well, 0.12 cm deep and 0.36 cm in diameter, located at the bottom of the gas reservoir. The well bottom was the membrane

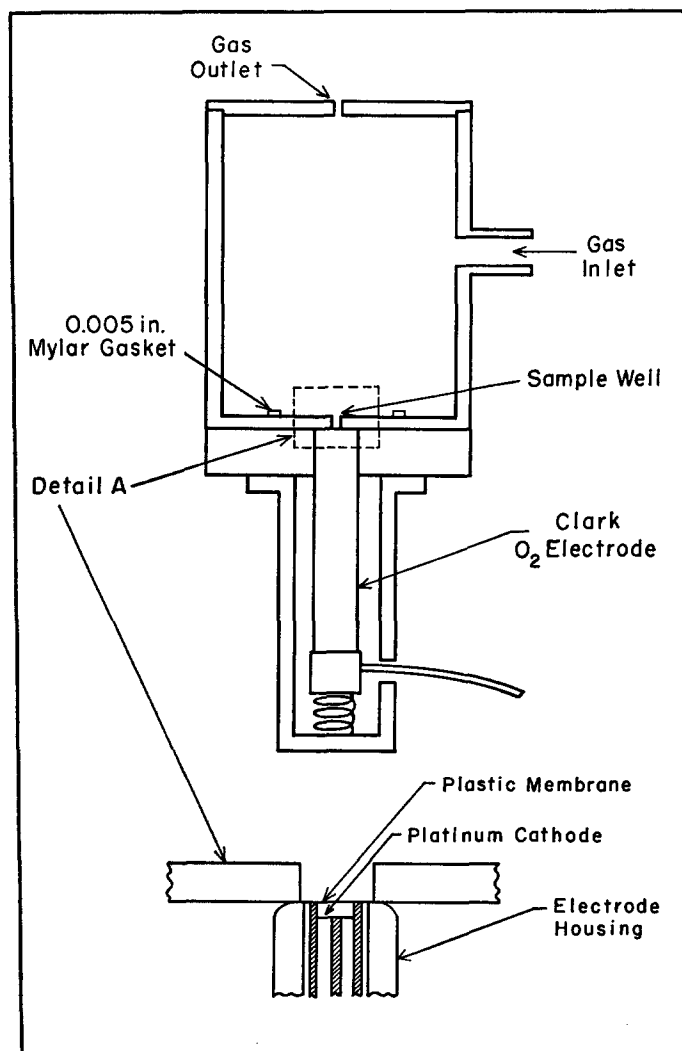


FIGURE 1. Schematic diagram of oxygen diffusion cell used in measurements of $\overline{D_{eff}}$.

of the oxygen electrode. Thus a thin film of solution separated the gas reservoir above from the oxygen electrode below. A 1.9 cm i.d., 0.013 cm thick Mylar gasket was cemented to the flat surface above the well to form a retaining lip. 0.05 ml of test solution was pipetted into the well at the beginning of a run. This carefully controlled volume and the large radius of the overflow section allowed very close control of the depth of the sample solution (± 0.0008 cm). Moreover, any change in the sample

depth was easily observable as a change in the opacity of the solution in the overflow section, and when such changes occurred, the run was begun again.

Gas of known oxygen/nitrogen composition was allowed to flow continuously from a supply system through the chamber above the test solution. The gas was presaturated with water vapor to minimize sample evaporation and the flow rate was adjusted to insure that the oxygen concentration at the gas-liquid interface was essentially the same as the mean oxygen concentration. The entire system was immersed in a $25 \pm 0.02^\circ\text{C}$ thermostat for temperature control.

Since the oxygen electrode consumes oxygen, oxygen diffused continuously from the gas phase to the electrode during experimental runs, giving rise to an oxygen concentration gradient in the test solution. Both flux and gradient could be measured and related to $\overline{D_{\text{eff}}}$. A Clark Oxygen Electrode (Yellow Springs Instrument Co., Antioch, Ohio) was used in the cell. The anode is a silver-silver chloride half-cell and the cathode is platinum. The cell was filled with a 0.1 M KCl, phosphate-buffered electrolyte. To facilitate using the electrode in an inverted position, the electrolyte was supported in an agar gel made of 1.5 g of agar per 100 ml of solution.

Both 1 mil polyethylene and 1 mil Teflon membranes were used during the experiments to provide a variation in the over-all electrode permeability. A Sargent Model III Manual Polarograph was used to operate the electrode. The galvanometer on this instrument had an accuracy of ± 0.003 microampere. Polarograms were made to establish the diffusion current range and, based on these, an electrode operating voltage of -0.9 v was selected.

In the one-dimensional steady state, the flux of oxygen across the membrane surface is equal to the rate of reduction of oxygen at the cathode surface which, in turn, is simply related to the measured current as follows:—

$$(\pi r_m^2) J_{\text{O}_2} = I/\nu F \quad (11)$$

where I is the current in amperes, F is Faraday's constant, r_m is the radius of the membrane surface, and ν is the number of electrons involved in the reduction of an oxygen molecule.

In the diffusion current range the concentration of oxygen approaches zero at the cathode surface, so that the flux of oxygen may also be expressed as

$$J_{\text{O}_2} = k_c(n_{\text{O}_2})_0 \quad (12)$$

where $(n_{\text{O}_2})_0$ is the concentration of oxygen at the bottom of the well (the surface of the membrane). k_c is an over-all electrode permeability which is a function of membrane and electrolyte characteristics.

By combining Equations (10), (11), and (12) a simple expression for $\overline{D_{\text{eff}}}$ in a one-dimensional system can be obtained in terms of the measured quantities:

$$\overline{D_{\text{eff}}} = \frac{(a/\pi r_m^2) \left(\frac{1}{\nu F}\right)}{(n_{\text{O}_2})_a/I - \left(1/\pi r_m^2 k_c\right) \left(\frac{1}{\nu F}\right)} = \frac{\Phi'_1}{\frac{(n_{\text{O}_2})_a}{I} - \Phi_2} \quad (13)$$

In the experimental apparatus, deviations from one-dimensional geometry oc-

curred at the top of the well because of the overflow lip and at the bottom of the well because of a nonuniform boundary condition. As shown in Fig. 1, the diameter of the well was larger than the diameter of the platinum cathode. The diffusion path to the cathode was relatively long from points not directly over the platinum surface so that the flux through the membrane was not constant across the well bottom. However, an approximate solution (10) showed that, for this modified geometry, $\overline{D_{eff}}$ could be expressed in a form analogous to Equation (13), substituting an appropriate geometric constant Φ_1 for Φ_1' ; Φ_2 remains the same. In order to obtain a measurement of $\overline{D_{eff}}$, it was necessary to measure the current, I ; $(n_{O_2})_a$ was assumed to be the oxygen concentration in equilibrium with the gas phase oxygen partial pressure and Φ_1 and Φ_2 were obtained by calibration runs with systems in which $\overline{D_{eff}}$ was known. It should be noted that it is not necessary to know the value of ν in order to calculate $\overline{D_{eff}}$.

Hemoglobin Preparation and Analysis

Hemoglobin solutions were prepared from whole human blood which had been in storage for 3 wk in the blood bank of The Johns Hopkins Hospital. The blood was centrifuged at approximately 3500 RPM for 25 min and the plasma discarded. The red blood cells were washed twice with isotonic saline and hemolyzed by repeated freezing and thawing in a salt-ice slush at -15°C . After hemolysis the hemoglobin solution was again centrifuged to separate out the cell stroma.

Hemoglobin concentrations of approximately 30 g/100 ml were obtained in this manner. They were diluted with 0.05 M, pH 7.4 phosphate buffer to obtain other solution concentrations. Normally six dilutions were prepared ranging from approximately 5 g/100 ml to 30 g/100 ml.

A Bausch and Lomb Spectronic 20 colorimeter was used to measure concentrations. The absorption maxima at 542 $m\mu$ and 576 $m\mu$ were determined. Reproducibility of readings on this instrument was approximately ± 0.4 g/100 ml. The instrument was calibrated by the pyridine hemochromagen technique; i.e., the actual hemoglobin concentration of a sample was determined by converting part of it to reduced alkaline pyridine hemochromagen and measuring its optical density at 557 $m\mu$ (16); this value was used to establish a calibration point on the oxyhemoglobin transmittance-concentration plot. The sample was also checked for the presence of methemoglobin by the technique described by Benesch et al. (2). The preparation was treated with a solution containing methemoglobin reductase and DPNH, reducing any methemoglobin present to oxyhemoglobin. A recheck of the transmittance following this treatment indicated that the methemoglobin concentration in the initial sample was less than 2%.

Hemoglobin preparations were stored at 4°C and checked before use to insure that no significant oxidation had occurred. Preparations were discarded after 4 or 5 days when evidence of denaturation appeared.

EXPERIMENTAL RESULTS

Calibration Runs

To determine the constants, Φ_1 and Φ_2 , two sets of calibration experiments were run; in the first set, the test solution well contained no liquid, so that it

was filled with the test chamber gas, and in the second set, the well contained distilled water. In both sets of experiments the partial pressure of oxygen was varied over a wide range. The data from the gas and water runs are plotted in Figs. 2 and 3 as $(p_{O_2})_a$ vs. I . A Henry law constant of 1.669×10^{-6}

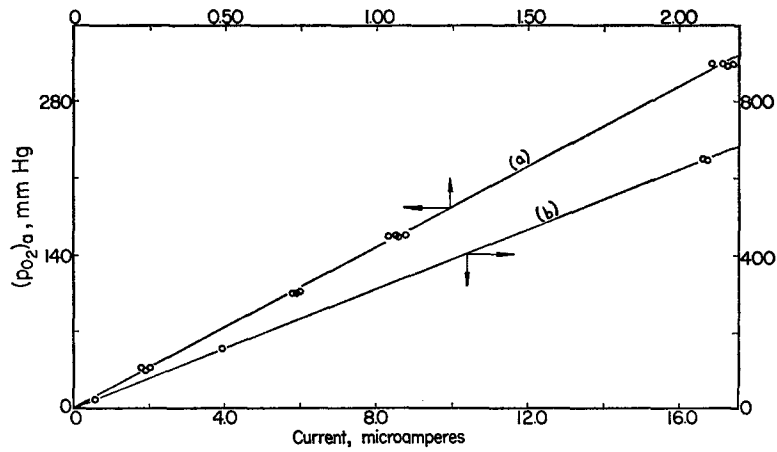


FIGURE 2. Gas calibration curves for oxygen electrode at 25°C: (a) 0.001 in. polyethylene membrane on electrode; (b) 0.001 in. Teflon membrane on electrode.

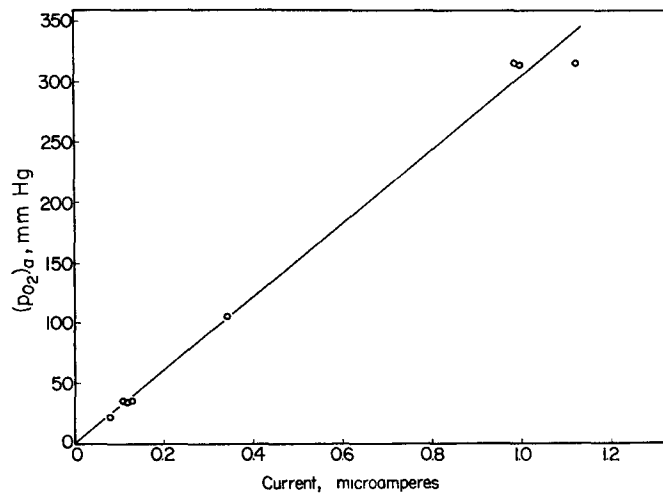


FIGURE 3. Oxygen electrode calibration curve for distilled water at 25°C with a 0.001 in. polyethylene membrane on electrode.

moles/liter/mm Hg was determined from the data of Douglas (4). Equation (13) can be rearranged to

$$(n_{O_2})_a = \left[\frac{\Phi_1}{D_{eff}} + \Phi_2 \right] I \quad (14)$$

The term in brackets can be determined from the slopes of Figs. 2 and 3. In the absence of augmentation, $\overline{D_{eff}}$ is equal to D_{O_2} , which, in the gaseous mixture is approximately 0.2 cm²/sec, and for oxygen in water is 2.3×10^{-5} cm²/sec (19). For the gas runs Φ_1/D_{O_2} is negligible compared to Φ_2 , so that the slopes of Fig. 2 are equal to the values of Φ_2 . Φ_2 is a function of the electrode membrane material. Both 0.001 in. polyethylene and 0.001 in. Teflon were used yielding the following values of Φ_2 :

For the 1 mil polyethylene membrane, $\Phi_{2P} = 0.242$ g mole sec/coulomb cm³

For the 1 mil Teflon membrane, $\Phi_{2T} = 0.0659$ g mole sec/coulomb cm³

From the slope of Fig. 3 and the value of Φ_{2P} , Φ_1 was determined to be $6.33 \times 10^{-6} \frac{\text{g mole}}{\text{coulomb cm}}$. From the definition of Φ_1 , its value can be calculated as $2.24 \times 10^{-5}/\nu \frac{\text{g equiv}}{\text{coulomb cm}}$. If this is equated to the experimental

value, one finds a value of ν of 3.54. In view of the possible experimental error and a mathematical simplification used in obtaining the analytical form of Φ_1 , ν can be approximated as 4, the value which other workers have postulated for the over-all reduction of oxygen at this operating voltage (12).

$\overline{D_{eff}}$ Measurements

Data with oxyhemoglobin solutions in the test well were first obtained with oxygen partial pressures of 315 mm Hg and 105 mm Hg. These data are tabulated in Table I. Since the lowest oxygen tension (at the membrane surface) is above the oxyhemoglobin saturation value, the oxyhemoglobin could be assumed to be fully saturated throughout the sample and, by Equation (9), $\overline{D_{eff}} = D_{O_2}$. Values of D_{O_2} were thus determined from these runs and the data plotted in Fig. 4. Note that the data from both the 315 mm Hg runs and the 105 mm Hg runs fall along the same line, corroborating the hypothesis that as long as no gradient in oxyhemoglobin exists, the diffusivity of oxygen is independent of the absolute level of oxygen concentration. The line drawn through the data is the least squares fit. The points shown at a hemoglobin concentration of zero were obtained using phosphate buffer. These points were not included in the least squares fit, although the extrapolated line passes through them as expected.

The data agree closely with those obtained by Pircher (19) in pig methemoglobin. They also agree with the predictions of Longmuir and Roughton (13) based on their study of nitrogen diffusion in sheep hemoglobin.

After establishing the values of D_{O_2} , the oxygen partial pressure was reduced and runs made at 35 mm Hg and at 23.1 mm Hg. A polyethylene membrane was used during the 35 mm Hg runs and during the first set of runs at 23.1 mm Hg. It was then replaced with a Teflon membrane and

TABLE I
THE DIFFUSIVITY OF OXYGEN IN HUMAN
HEMOGLOBIN SOLUTIONS AT 25°C

HbO ₂ concentration	Gas phase pO ₂	Membrane O ₂ tension	DO ₂ × 10 ⁶
<i>g/100 ml</i>	<i>mm Hg</i>	<i>mm Hg</i>	<i>cm²/sec</i>
0	315	145.8	1.98
0	315	142.0	2.02
0	105	46.2	2.08
5.0	105	44.4	1.85
7.1	315	124.9	1.79
9.9	105	42.1	1.74
9.9	105	39.6	1.49
14.2	315	110.8	1.47
14.9	105	38.9	1.46
20.3	315	99.1	1.25
24.8	105	28.8	0.93
24.8	105	35.9	1.25
29.8	105	31.2	0.99
29.8	105	19.9	0.58
28.4	315	88.3	1.02

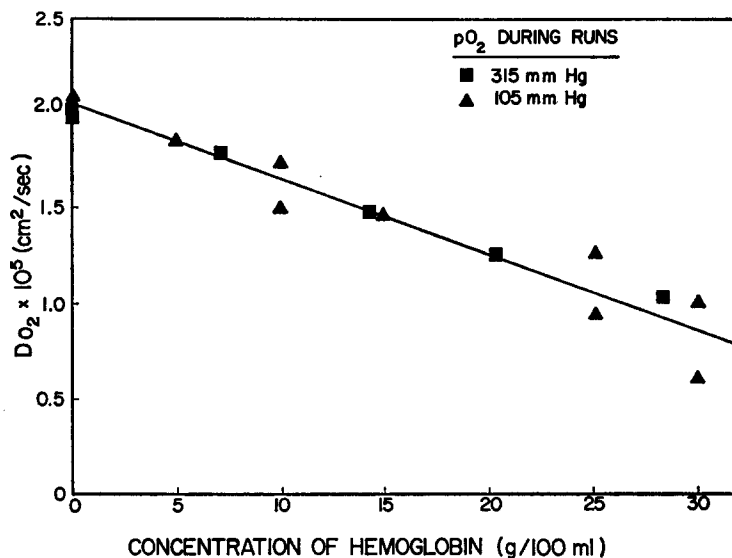


FIGURE 4. Diffusivity of oxygen in hemoglobin solutions at 25°C. Measurements were made at high oxygen tensions corresponding to negligible augmentation. Over the concentration range 0 to 30 g/100 ml the diffusivity is approximately linear in the concentration.

another set of runs made. Values of $\overline{D_{eff}}$ were calculated from these data and are tabulated in Table II.

In Fig. 5, the data taken with the polyethylene membrane at 35 mm Hg

TABLE II
 \overline{D}_{eff} OF OXYGEN IN HEMOGLOBIN
 SOLUTIONS AT 25°C

HbO ₂ concentration	Gas phase pO ₂	Membrane O ₂ tension	$\overline{D}_{eff} \times 10^5$
<i>g/100 ml</i>	<i>mm Hg</i>	<i>mm Hg</i>	<i>cm²/sec</i>
4.8	35.0	17.2	2.43
4.9	35.0	19.4	3.09
4.8	23.1	13.1	3.11
5.0	23.1	7.8	4.87
9.8	35.0	21.2	3.62
9.8	23.1	16.1	5.49
10.0	23.1	11.2	9.09
10.2	23.1	10.8	8.50
14.9	35.0	21.9	4.13
14.9	35.0	19.9	3.26
14.7	35.0	20.5	3.36
14.6	23.1	16.2	5.56
15.0	23.1	12.1	10.67
15.3	23.1	12.2	10.91
19.7	35.0	20.3	3.57
19.2	23.1	16.1	5.48
20.0	23.1	11.2	9.05
24.6	35.0	19.2	3.16
24.0	23.1	16.7	6.58
25.0	23.1	9.1	6.27
29.5	35.0	17.7	2.79
29.5	35.0	17.7	2.68
28.8	23.1	17.1	6.46
30.0	23.1	9.4	6.57
30.6	23.1	10.7	8.36

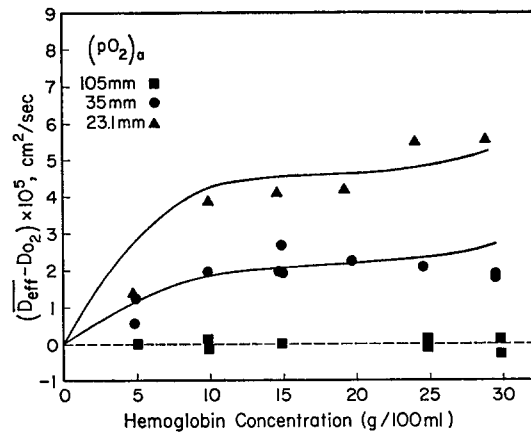


FIGURE 5. Augmentation of oxygen transport as a function of hemoglobin concentration and reservoir oxygen tension. Oxygen diffused from the reservoir through a thin film of hemoglobin solution and a 1 mil polyethylene membrane of known permeability. Values of the integral effective diffusivity were calculated from Equation (9). The lines shown were calculated from theory and are based on values of D_{MHb} measured independently (11).

and 23.1 mm Hg are plotted. The ordinate, $\overline{D_{eff}} - D_{O_2}$, was determined by subtracting a value of D_{O_2} obtained from Fig. 4 from the experimental value of $\overline{D_{eff}}$. This difference is a measure of the amount of transport augmentation. The amount of augmentation is plotted vs. hemoglobin concentration with oxygen tension at the upper boundary of the test solution as a parameter. While $\overline{D_{eff}}$ depends upon the oxygen tension at both boundaries of the solution, Equations (11), (12), and (13) determine a unique relation among $\overline{D_{eff}}$, $(pO_2)_a$, and $(pO_2)_o$ for constant membrane permeability. Since only the data for runs made with the polyethylene membrane are plotted (constant membrane permeability), the specification of $\overline{D_{eff}}$ and $(pO_2)_a$ is sufficient to describe each point unambiguously.

DISCUSSION

The data in Fig. 5 confirm the earlier experimental observations (9) on the effect of decreasing oxygen tension on the augmentation effect. The data from the 105 mm Hg runs, in which the hemoglobin was saturated throughout the test solution, are plotted to show that under these conditions there is no augmentation. For the 35 mm Hg runs, where the oxygen tensions in the test solution correspond to a section of the oxygenation curves with shallow, but finite slope (i.e., $\Delta y/\Delta n_{O_2} > 0$), augmentation appears and for the 23.1 mm Hg runs, where oxygen tensions in the test solution correspond to a section of the oxygenation curves with relatively steep slope, augmentation is even greater.

The data also show that as hemoglobin concentration increases, augmentation increases rapidly at first, but the magnitude of the effect then levels out. Equation (9) indicates that the augmentation effect is related to the magnitude of the term $D_{HbO_2} n_t \frac{(y_a - y_o)}{(n_{O_2})_a - (n_{O_2})_o}$. Since n_t increases with hemoglobin concentration, the leveling of the augmentation effect must result from a decrease in one of the other two terms in the product. $\frac{(y_a - y_o)}{(n_{O_2})_a - (n_{O_2})_o}$ is a function of the pH of the hemoglobin solution which decreased with increasing hemoglobin concentration. Examination of typical hemoglobin oxygenation curves shows that such a shift would tend to increase augmentation in the range of oxygen tensions studied. Thus the leveling of the enhancement effect with increasing hemoglobin concentration indicates that D_{HbO_2} decreases. In the lower concentration range it appears that this decrease is slow and the net effect of concentration increase is an increase in augmentation.

If the diffusion of HbO_2 occurs by a collisional mechanism rather than by Brownian motion of the hemoglobin molecule, D_{HbO_2} should increase with increasing concentration because of the increased collision rate. Perutz has pointed out that even at the red blood cell concentration, hemoglobin mole-

cules can rotate freely about any axis (17) which indicates that restricted rotation would not offset the effect of increased collision rate. Thus the data indicate that the collisional mechanism is not of significant importance in the augmentation of oxygen transport.

It is then necessary to determine whether the diffusion of HbO_2 by Brownian motion is of sufficient magnitude to account for the augmentation effect. In a separate set of experiments reported elsewhere (11), the diffusivity of methemoglobin was determined over a wide range of hemoglobin concentration. Because of the similarity in the molecular architecture of methemoglobin and oxyhemoglobin (18), their Brownian motion diffusivities should be the same. Therefore the Brownian motion hypothesis of augmentation can be checked by using the D_{MHb} data and Equation (9) to predict values of $\overline{D_{\text{eff}}}$, provided hemoglobin oxygenation curves are available. In the

TABLE III
pH MEASUREMENTS OF OXYHEMOGLOBIN SOLUTIONS

Oxyhemoglobin concentration	pH
<i>g/100 ml</i>	
5.0 ± 0.03	7.1 ± 0.05
10.0	7.0
15.0	6.9
19.7	6.8
24.6	6.72
29.5	6.65

absence of experimental data, oxygenation curves were calculated from the Hill equation:

$$y = \frac{K(p\text{O}_2)^n}{1 + K(p\text{O}_2)^n} \quad (15)$$

The ionic strength of the salts in the hemoglobin solutions used varied from 0.1 to 0.15. In this range n has a value of approximately 2.6 (20). Values of K as a function of pH were obtained from Wyman's data for the log of the oxygen pressure at half-saturation, $\log p_{1/2}$, vs. pH at 25°C and ionic strength 0.15 (24). Although Wyman's data are for horse hemoglobin, the similarity in the Bohr effect in horse and human hemoglobin has been shown and commented upon frequently (5, 1). Since the amount of buffer used in preparing each of the hemoglobin solutions varied, pH values as a function of the hemoglobin concentration were measured (Table III) and from these, appropriate values of K were determined for each hemoglobin concentration. In the unbuffered, 30 g/100 ml solution, a rise in pH of 0.3 during deoxygenation was assumed and a K value based on the average pH for each experimental point was determined.

Values of $D_{eff} - D_{O_2}$ were calculated from the experimentally measured values of D_{MHb} for $(pO_2)_a = 35$ mm Hg and $(pO_2)_a = 23.1$ mm Hg and for the range of hemoglobin concentrations studied. The calculations were made by a trial and error procedure for each hemoglobin concentration and upper oxygen tension using Equations (9), (11), (12), (13), and (15).

Curves based on these calculations are shown on Fig. 5. The agreement between the calculated curves and the experimental data is good. The largest deviation occurs for the 5 g/100 ml run at $(pO_2)_a = 23.1$ mm Hg; this may

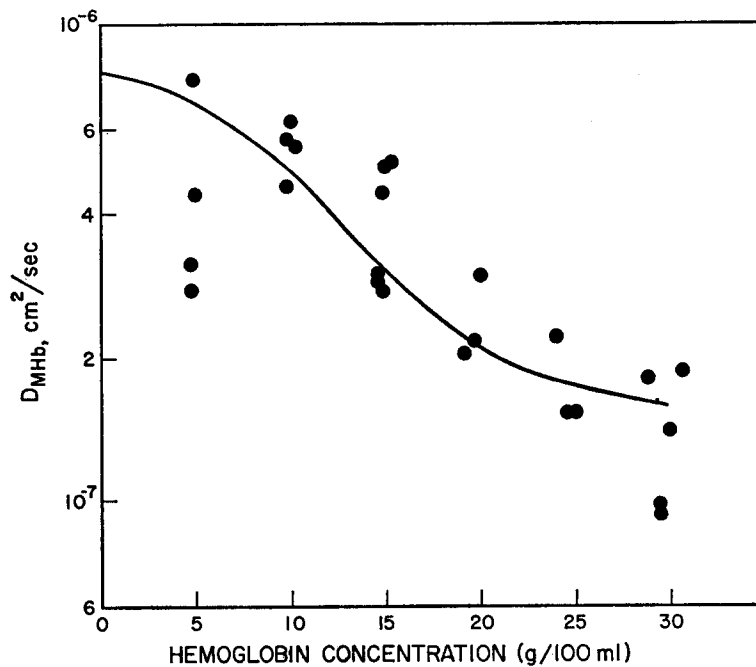


FIGURE 6. Comparison of D_{MHb} (solid line taken from Keller and Friedlander (11)) with predicted values of D_{HbO_2} (data points) for varying hemoglobin concentrations.

reflect some oxidation of the hemoglobin during the course of the run. The slight inflection in the curves which occurs at high hemoglobin concentration results from an increase in the magnitude of the variation of slope with pH in this region.

By inverting Equation (9), one obtains the following expression for D_{HbO_2} :

$$D_{HbO_2} = \frac{\overline{D_{eff}} - D_{O_2}}{n_t \frac{y_a - y_0}{(n_{O_2})_a - (n_{O_2})_0}} \quad (16)$$

Using this expression and the experimental values of $\overline{D_{eff}}$ and D_{O_2} , values of D_{HbO_2} were calculated and are plotted in Fig. 6. The curve drawn is a

plot of the separately measured D_{MHB} . As in Fig. 5, the data for the 5 g/100 ml solutions are least satisfactory. However, the data points fall quite close to the curve, indicating that $D_{\text{HbO}_2} = D_{\text{MHB}}$ and that the mechanism of augmented oxygen diffusion is the Brownian motion diffusion of the hemoglobin molecules.

The variation of D_{eff} , the differential effective diffusivity, is plotted in Fig. 7 as $(D_{\text{eff}} - D_{\text{O}_2})$ vs. oxygen concentration for a 10 g/100 ml hemo-

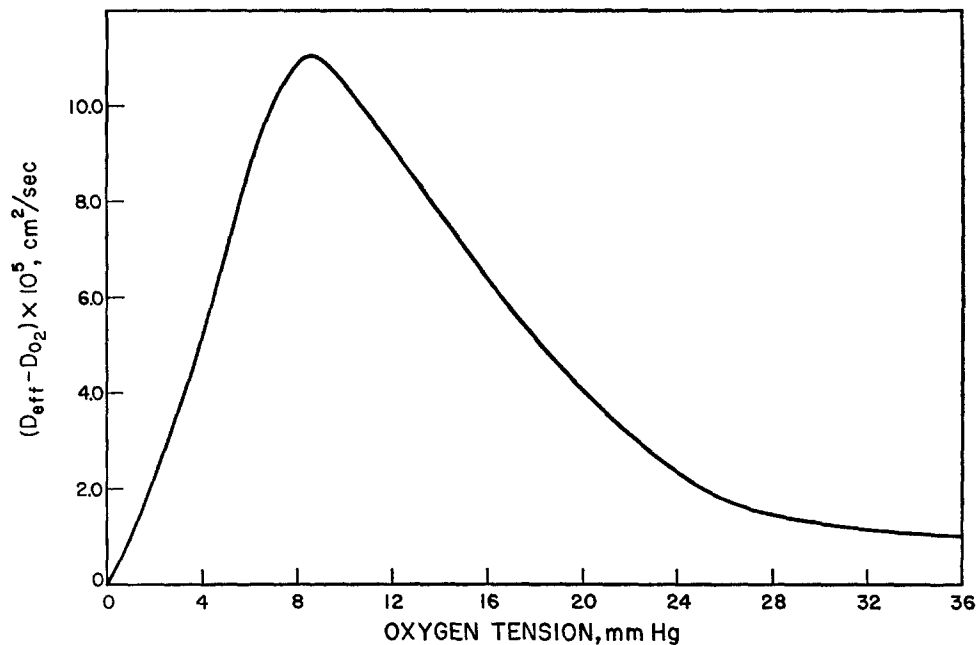


FIGURE 7. Predicted values of the differential effective diffusivity of oxygen for a 10 g/100 ml hemoglobin solution at pH 7.0. A maximum is predicted at an oxygen tension of about 9 mm Hg.

globin solution at pH 7.0. The experimental D_{MHB} values and the appropriate calculated oxygenation curve were used in Equation (6) to construct this curve. Unlike the integral diffusion coefficient, D_{eff} is a unique function of the oxygen tension at a given hemoglobin concentration. It is interesting to note that the augmentation effect passes through a maximum at about 9 mm Hg. This effect was not found experimentally because sufficient data in the low oxygen tension range were not obtained. The predicted maximum is a result of the sigmoidal shape of the oxygenation curve and the inflection point which occurs at approximately 9 mm Hg.

The importance of the phenomenon of augmentation in oxygen transfer in the red blood cell is not clear. Friedlander and Keller (7) showed that the augmentation effect is at a maximum in systems in which local equilibrium

can be assumed and decreases as the system departs from equilibrium. An approximate value of λ for the red cell conditions can be calculated from Equation 1. By extrapolating the experimental data to 37°C and 35.5 g/100 ml hemoglobin concentration, a value of 0.75×10^{-5} cm²/sec is obtained for D_{O_2} and a value of 1.75×10^{-7} cm²/sec for D_{Mhb} . Using these values, λ was calculated for various oxygen tensions. It was found that in the range of oxygen tensions of 10 mm Hg to 100 mm Hg, λ was fairly constant, varying from 1.8×10^{-5} cm to 2.6×10^{-5} cm. For a characteristic red cell dimension of about 5×10^{-4} cm, the ratio of cell size to λ is less than 100 and the system is not at local equilibrium. Moreover, since the in vivo system is an unsteady-state system, the actual characteristic length which should be used in applying the criterion of local equilibrium is probably not a red cell dimension, but the thickness of the hemoglobin-oxygen reaction zone. This reaction zone thickness is likely to be even smaller than the red cell diameter (or thickness), resulting in an even greater departure from local equilibrium.

NOMENCLATURE

a	thickness of layer of solution, cm.	p_{O_2}	oxygen tension; partial pressure of oxygen in equilibrium with solution, mm Hg.
D_i	diffusivity of the i^{th} species, cm ² /sec.	$p_{1/2}$	oxygen tension at which a hemoglobin solution is 50% saturated, mm Hg.
D_{eff}	effective diffusivity, defined by Equation (6), cm ² /sec.	r_m	effective radius of oxygen electrode surface, cm.
$\overline{D}_{\text{eff}}$	integral effective diffusivity, defined by Equation (9), cm ² /sec.	x	linear variable, cm.
F	Faraday's constant, 96,487 coulombs/g equiv.	y	fraction of hemoglobin present as oxyhemoglobin, dimensionless.
I	current through O ₂ electrode, amp.	λ	characteristic length in a reacting, diffusing system, defined for the hemoglobin-oxygen system by Equation (1), cm.
J_i	flux of species i , moles/cm ² /sec.	Φ_1	constant related to oxygen diffusion cell geometry, g mole/coulomb cm.
K	empirical coefficient in Hill equation, mm ⁻ⁿ	Φ_2	constant related to oxygen electrode permeability, g mole sec/coulomb cm ³ .
k	over-all reverse reaction rate coefficient, sec ⁻¹ .		
k'	over-all forward reaction rate coefficient, cm ³ /(g mole sec).		
k_e	oxygen electrode permeability, cm/sec.		
n	empirical exponent in Hill equation, dimensionless		
n_i	molar concentration of species i , g moles/cm ³ .		
n_t	total molar concentration of oxygenated and deoxygenated hemoglobin, g equivalents/cm ³ .		
		<i>Subscripts</i>	
		o	value at electrode membrane surface.
		a	value at upper boundary of test solution.

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