NONEQUILIBRIUM FACILITATED OXYGEN TRANSPORT IN HEMOGLOBIN SOLUTION

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ABSTRACT We have used the quasi-linearization method to obtain numerical solutions to the equations which describe steady-state diffusion of oxygen through layers of hemoglobin solution. The numerical solutions show how the facilitated flux of oxygen depends upon the layer thickness, reaction-rate coefficients, and other parameters of the system. The results indicate that steady-state oxygen diffusion in layers of hemoglobin solution, similar to those studied by Scholander, should be adequately described by the models which assume chemical equilibrium exists throughout the layer, but for layers of concentrated hemoglobin solution about the thickness of a human erythrocyte, the facilitation of oxygen diffusion should be much less than the equilibrium models predict.

INTRODUCTION

Wittenberg (1) and Scholander (2) demonstrated facilitated steady-state oxygen diffusion through layers of hemoglobin solution and their results stimulated experimental and theoretical studies of the facilitation of oxygen diffusion by heme pigments. Work in this field has been reviewed by Hemmingsen (3) and Wittenberg (4).

A theoretical model of a system in which second order chemical reaction results in steady-state facilitated diffusion in a liquid layer was presented by Olander (5). Later, stimulated by Scholander's results, a number of basically similar models appeared (6–11). The most important assumption of these models is that chemical reaction processes everywhere in the layer are much faster than diffusion processes, so that chemical equilibrium exists at all points in the film. This assumption allowed linearization of the diffusion equations and their analytical solution.

Snell (12) proposed a reaction velocity correction and Fox and Landahl (13) proposed a pH gradient correction to the equilibrium model.

Friedlander and Keller (14) applied the techniques of nonequilibrium thermodynamics to this problem. They assumed that the reaction is near equilibrium (namely, that the thermodynamic affinity is small with respect to RT), which allowed them to linearize the reaction rate terms in the diffusion equations.

Simultaneous diffusion and chemical reaction (even in the steady state) is usually

difficult to deal with mathematically because, if the reaction is of second or higher order, the reaction-rate terms in the diffusion equations are nonlinear and analytical solutions to such problems are generally not available. Furthermore, numerical methods often produce unstable solutions (15, 16).

We have used the method of quasi-linearization to obtain numerical solutions to the equations which describe the steady-state diffusion of oxygen through layers of hemoglobin solution. The numerical solutions show how the facilitated flux of oxygen depends on the layer thickness, the reaction rates, and other parameters of the sytem. The similar problem of steady-state diffusion of oxygen through solutions of cobaltodihistidine has been solved by Bassett (16) using a different numerical method.

The results indicate that steady-state oxygen diffusion in layers of hemoglobin solution similar to those studied by Scholander (2) (150 μ layers of 15 g/100 ml hemoglobin solution) should be adequately described by the models which assume chemical equilibrium throughout the layer. However, for layers of concentrated hemoglobin solution about the same thickness as a human erythrocyte, substantial deviations from the predictions of the equilibrium model were observed.

METHODS

The Equations

Suppose a substance w is diffusing through a liquid layer in which it reacts with substance u to form substance v according to the reaction

$$\mathrm{w} + \mathrm{u} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{v} \tag{1}$$

where k_1 and k_2 are the forward and reverse reaction rate coefficients, respectively.

Applying Fick's second law of diffusion to each species and solving for the steady state, one obtains equations 2–4, in which u, v, and w represent the concentrations of u v, and w respectively and the D's are the diffusion coefficients.

$$0 = D_w \frac{d^2w}{dx^2} + k_2 v - k_1 uw \tag{2}$$

$$0 = D_v \frac{d^2v}{dx^2} - k_2 v + k_1 uw \tag{3}$$

$$0 = D_u \frac{d^2u}{dx^2} + k_2 v - k_1 uw. \tag{4}$$

Because each of these equations contains the nonlinear term k_1uw, analytical solutions to this system are not available.

We are interested particularly in the diffusion of oxygen through hemoglobin

solutions, so let w denote the oxygen concentration, u be the concentration of unoxygenated heme groups, and v be the concentration of oxygenated hemes. We shall suppose that the layer is bounded on both sides by well-mixed gas phases and that both forms of hemoglobin are confined to the liquid phase, but oxygen may cross the interfaces.

We have treated the oxygen-hemoglobin reaction as a single-step reaction, while it is believed to proceed in four steps with four distinct equilibrium constants. The principal justifications for this oversimplification are that it simplifies mathematical analysis and that, for most cases, the eight reaction-rate constants are not known accurately. Unfortunately, the sigmoid oxygen-hemoglobin equilibrium curve cannot be closely fitted by the rectangular hyperbola predicted by the single step reaction scheme.

$$V = \frac{Kw}{1 + Kw}, \tag{5}$$

where K is the equilibrium constant of the reaction ($K = k_1/k_2$) and V is the fraction of heme groups having a bound oxygen molecule. Nevertheless, the use of this one-step reaction scheme is almost universal in theoretical work on diffusion and chemical reaction in hemoglobin-containing systems. In most cases we have adjusted K so that the hyperbolic curve has the same half-saturation oxygen concentration as the true equilibrium curve.

For purposes of comparison, it might be of interest to try to use the kinetic formulation which leads to Hill's equation, equation 6,

$$V = \frac{Kw^n}{1 + Kw^n}, \tag{6}$$

because it is well-known that Hill's equation gives a much better approximation to the oxyhemoglobin dissociation curve than does equation 5. However, it is the reaction kinetics which we must approximate, not the dissociation curve, and equation 1 gives a better approximation for the reaction kinetics. The model solved here should be exact for myglobin, which does react with O_2 according to the reaction scheme of equation 1. Hemmingsen (17) has demonstrated facilitated O_2 transport through layers of sea lion myoglobin, but we could not simulate his experiments because the values of the reaction-rate and diffusion coefficients are not known.

The problem can be further simplified by assuming that $D_u = D_v$. This has not been experimentally verified, but seems reasonable (6–8). Adding equations 3 and 4 and integrating gives

$$D_v \frac{dv}{dx} + D_u \frac{du}{dx} = a. \tag{7}$$

Physically, equation 7 represents the sum of the fluxes of hemoglobin and oxy-

hemoglobin. Since this sum must be zero at the layer boundaries, then $a = 0$. Integrating equation 7 and setting $D_u = D_v$, $a = 0$ gives

$$v + u = b. \tag{8}$$

Thus, the total hemoglobin concentration is constant throughout the layer as a consequence of the equality of D_u and D_v. (A case in which $D_u \neq D_v$ is treated by Bassett [16]).

Equation 8 defines u as a function of v, reducing by one the number of dependent variables; thus we need only consider equations 2 and 3. Substituting $(b - v)$ for u in equations 2 and 3 they become

$$0 = D_w \frac{d^2w}{dx^2} + k_2 v - k_1(b - v)w \tag{9}$$

$$0 = D_v \frac{d^2v}{dx^2} - k_2 v + k_1(b - v)w. \tag{10}$$

Before equations 9 and 10 can be solved, appropriate boundary conditions must be found. LaForce and Fatt (18) pointed out that assuming chemical equilibrium exists at each point in the layer places too many constraints on the equations (an infinite number). It can also be shown that this assumption leads to a contradiction. Since $k_1(b - v)w$ is the rate of formation of oxyhemoglobin and k_2v is the rate of its breakdown, then at equilibrium $k_1(b - v)w - k_2v = 0$. Thus, at equilibrium the kinetic terms in equations 9 and 10 drop out, leaving $d^2w/dx^2 = 0$ and $d^2v/dx^2 = 0$, the solutions to which are linear functions of x. But it is not possible for the concentration profiles of w and v to be linear and at the same time for w and v to be in chemical equilibrium at every value of x, for the equilibrium relation between w and v is not linear.

The boundary conditions that we employed were derived from the assumptions that (*a*) in the steady state the total flux of oxygen is independent of x, (*b*) molecules of oxyhemoglobin cannot cross the interfaces, and (*c*) the oxygen pressure drops across the interfaces are negligible.

The constancy of the total oxygen flux can be established as follows. Addition of equations 9 and 10 and integration gives

$$D_w \frac{dw}{dx} + D_v \frac{dv}{dx} = c. \tag{11}$$

The physical interpretation of equation 11 is that the sum of the fluxes of oxygen and oxyhemoglobin is invariant. Thus c equals the negative of the total flux of oxygen $(-J_t)$ in free and bound forms at every value of x (5–8).

Since oxyhemoglobin cannot penetrate the film boundaries (at $x = 0$ and $x = L$), the oxygen flux at the boundaries must be due solely to the passage of molecular

oxygen; thus,

$$D_w \frac{dw}{dx}\bigg|_{x=0} = D_w \frac{dw}{dx}\bigg|_{x=L} = c = -J_t. \tag{12}$$

From equations 11 and 12 it follows that

$$\frac{dv}{dx}\bigg|_{x=0} = \frac{dv}{dx}\bigg|_{x=L} = 0. \tag{13}$$

We shall also assume that the oxygen pressure drops across the interfaces are negligible, so that the oxygen tension at a boundary is in equilibrium with the neighboring gas phase. If this assumption is incorrect, then the solutions we obtain will be valid for slightly different gas phase partial pressures of oxygen (P_w). Assuming Henry's Law holds,

$$\begin{aligned} w(0) &= \alpha_w P_w(0) = w_0 \\ w(L) &= \alpha_w P_w(L) = w_L, \end{aligned} \tag{14}$$

where α_w is the solubility of oxygen in the hemoglobin solution. α_w is assumed to be equal to the solubility of oxygen in water at the same temperature (19).

We have solved equations 9 and 10 subject to the boundary conditions of equations 13 and 14.

The Normalized Equations

Equations 9 and 10 were normalized with use of the transformations

$$\begin{aligned} W &= w/w_0 \\ V &= v/b \\ y &= x/L, \end{aligned} \tag{15}$$

where b is the total heme concentration and the boundary at $x = 0$ is chosen to be the high oxygen side of the layer. In terms of the normalized variables, equations 9 and 10 become

$$\begin{aligned} 0 &= \frac{d^2V}{dy^2} + \alpha\gamma(1 - V)W - \alpha V \\ 0 &= \frac{d^2W}{dy^2} - \alpha\beta\gamma(1 - V)W + \alpha\beta V, \end{aligned} \tag{16}$$

where the dimensionless constants α, β, and γ are given by

$$\alpha = k_2 L^2/D_v$$

$$\beta = D_v b/D_w w_0$$

$$\gamma = k_1 w_0/k_2 . \tag{17}$$

The boundary conditions of equations 13 and 14 become

$$\left.\frac{dV}{dy}\right|_{y=0} = \left.\frac{dV}{dy}\right|_{y=1} = 0$$

$$W(0) = 1, \quad W(1) = w_L/w_0. \tag{18}$$

The Numerical Method

The quasi-linearization method (20, 21) has proved valuable in solving nonlinear multipoint boundary value problems. The references should be consulted for the details of the method, in which a linear approximation to the problem is solved iteratively, but its essential features can be illustrated by an example.

Consider the set of two simultaneous first order nonlinear differential equations

$$\frac{dy_1}{dx} = f_1(x, y_1, y_2)$$

$$\frac{dy_2}{dx} = f_2(x, y_1, y_2) \tag{19}$$

with the boundary conditions $y_1(0) = a$, $y_2(1) = b$.

Initial guesses $z_1(x)$ and $z_2(x)$ are made for y_1 and y_2, respectively, which satisfy the boundary conditions. The right hand sides of equations 19 are then expanded in Taylor's series about z_1 and z_2 and terms of order greater than one are discarded, giving the *approximations*

$$\frac{dy_1}{dx} = f_1(x, z_1, z_2) + \left.\frac{\partial f_1}{\partial y_1}\right|_{y_1=z_1} (y_1 - z_1) + \left.\frac{\partial f_1}{\partial y_2}\right|_{y_2=z_2} (y_2 - z_2)$$

$$\frac{dy_2}{dx} = f_2(x, z_1, z_2) + \left.\frac{\partial f_2}{\partial y_1}\right|_{y_1=z_1} (y_1 - z_1) + \left.\frac{\partial f_2}{\partial y_2}\right|_{y_2=z_2} (y_2 - z_2). \tag{20}$$

Equations 20 are linear differential equations which are an approximation to the ones with which we began. Essentially, the remainder of the method involves solving equations 20 iteratively by a finite difference method. After each solution the resulting y_1 and y_2 are substituted for z_1 and z_2, respectively, giving progressively better guesses in convergent cases.

Bellman and Kalaba (20) showed that when the method converges, it converges quadratically. In convergent cases five iterations were more than sufficient. In nonconvergent cases improving the initial guesses often resulted in convergence.

We used this method to solve equations 16 with boundary conditions of equations 18 and α, β, and γ appropriate for oxygen diffusion through hemoglobin solutions under various conditions. The method was programmed in FORTRAN IV and was run on the IBM 360/67 at the University of Michigan Computer Center using double precision arithmetic. The linearized approximation to the problem was solved by a finite difference method with a step size of 1/32.

For initial guesses for small values of L we chose $W(x/L)$ to be a straight line between its boundary values and $V(x/L)$ to be in chemical equilibrium with W. This procedure gave convergence only for relatively small values of L. We used the results of such a computation as initial guesses to the solutions for a somewhat larger thickness. Working up to larger values of L in this way we were able to obtain convergence for a considerable range of L ($1\ \mu \leq L \leq 1000\ \mu$).

LaForce (15) and Bassett (16) found it difficult to generate stable solutions to equations resembling equations 16 with standard numerical techniques owing to extreme sensitivity of the solutions to the boundary conditions. LaForce used a fifth order Runge-Kutta starting formula and continued the integration with a finite difference formula correct to sixth order, but was unable to obtain convergence. Bassett employed a fourth order Runge-Kutta method and was able to obtain convergent solutions by iteratively varying the initial carrier concentration.

RESULTS AND DISCUSSION

Effects of Film Thickness and Reaction Rate Constants

Effects of Varying Film Thicknesses. Equations 16 were solved for values of the constants[1] close to those for a 15 g/100 ml solution of human hemoglobin (at room temperature in the absence of CO_2) with $P_w(0) = 200$ mm Hg and $P_w(1) = 2$ mm Hg. K was chosen to fit the *upper third* of the oxygen-hemoglobin equilibrium curve. The solutions $W(x/L)$ and $V(x/L)$ for various values of the layer thickness L are shown in Fig. 1.

The concentration profiles depend markedly on the thickness in the range $1\ \mu \leq L \leq 25\ \mu$ and more subtly for larger L. For all values of L observed, oxygen and hemoglobin are nearly in chemical equilibrium at the high oxygen boundary, but the concentration of oxyhemoglobin at the low oxygen boundary depends on L. For a thickness of $1\ \mu$, $V(1)$ is 1.8 times its equilibrium value and as L increases $V(1)$ approaches the equilibrium value, so that for $L = 100\ \mu$ $V(1)$ is 1.08 times the equilibrium values.

The values of W and V at the boundaries of the film are especially important because they determine the oxygen flux across the film. This can be seen (6–8) by

[1] See Appendix.

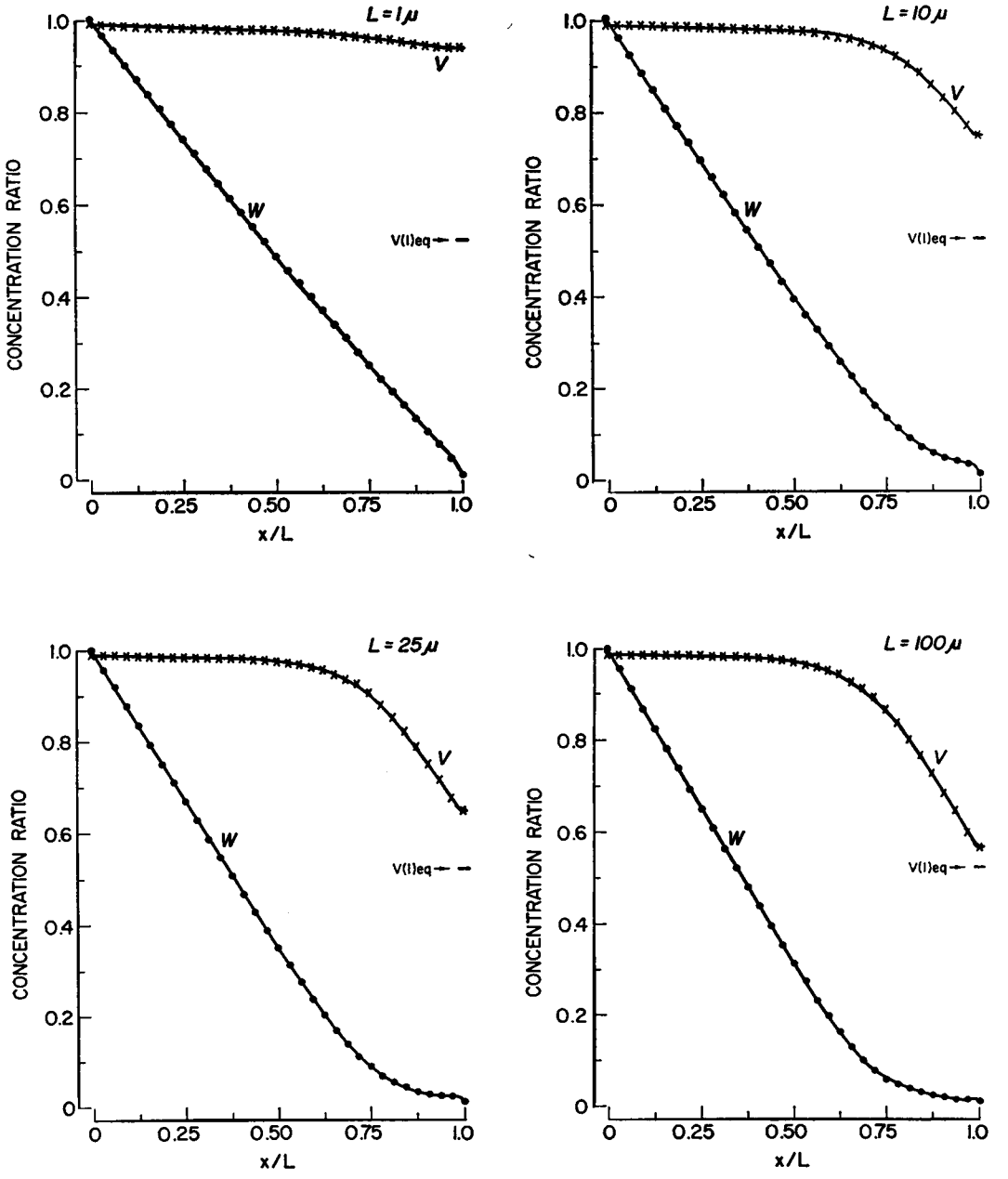

FIGURE 1 Concentration profiles of oxygen and oxyhemoglobin for layers of hemoglobin solution of different thickness (L). $P_{O_2}(0)$ = 200 mm Hg, $P_{O_2}(1)$ = 2 mm Hg.

integrating equation 11 from $x = 0$ to $x = L$ which gives

$$J_t = D_w \frac{w_0 - w_L}{L} + D_v \frac{v_0 - v_L}{L}. \quad (21)$$

In this relation the first term is the flux of oxygen which would be observed in the absence of any chemical reaction and the second term is the facilitated flux or the hemoglobin-mediated flux of oxygen.

If the facilitation (F) is defined as the carrier-mediated oxygen flux divided by the flux of oxygen in the absence of the carrier, then

$$F = \frac{D_v(v_0 - v_L)}{D_w(w_0 - w_L)} = \frac{D_v\, b[V(0) - V(1)]}{D_w\, w_0[W(0) - W(1)]}. \quad (22)$$

Since $V(0)$ is always slightly smaller and $V(1)$ is always larger than their respective equilibrium values, the maximum value for F (F_{max}) for given $W(0)$ and $W(1)$ can be obtained by using the equilibrium values of $V(0)$ and $V(1)$ in equation 22. Table I shows the dependence of F/F_{max} on layer thickness for this problem. The decreased facilitation for small values of L is due to high values of $V(1)$ and consequent reductions in the values of $V(0) - V(1)$.

The shapes of the oxygen and oxyhemoglobin profiles are also of interest. Both profiles have inflection points rather near the low oxygen boundary. Keller (22) showed that assuming the coupling coefficients (Dc/RT) to be constant for all species implies that the profiles have inflection points in the center of the layer.

TABLE I

THE DEPENDENCE OF THE FACILITATION OF OXYGEN DIFFUSION THROUGH 15 G/100 mL HEMOGLOBIN SOLUTION LAYERS ON LAYER THICKNESS FOR $P_{O_2}(0) = 200$ MM HG AND $P_{O_2}(1) = 2$ MM HG

L	$V(0)$	$V(1)$	F	F/F^*_{max}
μ				
1	0.990	0.939	0.052	0.111
2	0.991	0.906	0.086	0.183
10	0.991	0.749	0.244	0.519
25	0.991	0.647	0.347	0.738
50	0.991	0.597	0.398	0.847
75	0.991	0.577	0.418	0.889
100	0.991	0.567	0.428	0.911
150	0.991	0.555	0.440	0.936
200	0.991	0.549	0.446	0.949
500	0.991	0.538	0.457	0.972
1000	0.991	0.536	0.458	0.974

* $V(0) = 0.991$ and $V(1) = 0.526$ for chemical equilibrium. These values were used in calculating F_{max} from equation 22.

TABLE II

THE EFFECTS OF REACTION-RATE CONSTANTS ON OXYGEN SATURATION OF HEMOGLOBIN AT THE FILM BOUNDARIES; $P_{O_2}(0)$ = 200 MM HG, $P_{O_2}(1)$ = 2 MM HG

K	k_1^*	k_2	$V(0)$	$V(1)$	$V(0)/V(0)_{eq}$	$V(1)/V(1)_{eq}$
10^4	10^4	1	0.0034	0.00023	0.950	6.39
	10^6	10^2	0.0036	0.000056	0.997	1.56
	10^8	10^4	0.0036	0.000038	1.000	1.06
10^6	10^6	1	0.254	0.0234	0.958	6.69
	10^8	10^2	0.264	0.0057	0.996	1.63
	10^{10}	10^4	0.264	0.0038	0.996	1.09
10^7	10^7	1	0.776	0.159	0.992	4.58
	10^9	10^2	0.782	0.050	0.999	1.44
	10^{11}	10^4	0.783	0.037	1.000	1.07
10^8	10^8	1	0.973	0.503	1.000	1.90
	10^{10}	10^2	0.973	0.302	1.000	1.14
	10^{12}	10^4	0.973	0.274	1.000	1.04

* Units of k_1 are ml/(mole·sec).

(See also reference 23.) With respect to predicting the shapes of the concentration profiles this assumption can be misleading. Note that dv/dx must equal zero at both boundaries and that dw/dx must have the same value at both boundaries (see equations 12 and 13).[2] As shown in Fig. 1 the inflection points move closer to the boundary at $x/L = 1$ as L increases.

Effects of Reaction Rate Coefficients. In the steady state any tendency for a species to accumulate due to diffusion must be exactly balanced by its disappearance due to chemical reaction, and vice versa. The rate of change of the concentration of oxyhemoglobin due to diffusion at any point is equal to $D_v d^2v/dx^2$, and the rate of its concentration change due to chemical reaction is $k_1uw - k_2v$. In the steady state these two rates must sum to zero (equation 3). Thus where d^2v/dx^2 is negative there must be a net formation of oxyhemoglobin and where d^2v/dx^2 is positive there must be a net dissociation of oxyhemoglobin. So where d^2v/dx^2 is negative the burden of keeping up with the tendency of v to diminish falls on the forward reaction of equation 1 and where d^2v/dx^2 is positive the backward reaction is of principal importance.

Fig. 1 shows that the largest deviations from equilibrium occur near the low O_2 boundary where d^2v/dx^2 is positive. This is to be expected because the backward reaction to equation 1 is much slower than the forward reaction ($k_2 \doteq 50$ sec^{-1},

[2] This has been a source of confusion in the literature. Papers on diffusion with simultaneous chemical reaction have shown profiles with different slopes of the permeating species at the two boundaries and nonzero boundary slopes of the species confined to the film. See Bassett (16) for further discussion of this point. We have benefitted from discussing this matter with Drs. K. H. Keller, R. J. Bassett, and J. S. Schultz.

$k_1 \doteq 10^{10}$ ml/(mole.sec)). Since k_2 is a fixed quantity, the only way that the rate of dissociation of oxyhemoglobin (k_2v) can be increased is by an increase in the concentration of oxyhemoglobin. Thus before the steady state is established, should $D_v d^2v/dx^2$ be sufficiently large (positive) to exceed ($k_2v - k_1uw$), v must increase until the dissociation reaction can keep up with the tendency of oxyhemoglobin to accumulate due to diffusion.

We have solved equations 16 with the same parameter values as above and $L = 100\ \mu$ for several values of k_1 and k_2. Some of the results of our calculations are shown in Table II. The data are arranged in groups for which K is constant. This has been done because changing k_1 or k_2 alone (which changes K) has a profound effect on the boundary values of V. The percentage deviations from equilibrium at the high O_2 boundary are quite small, so attention has been focused on the low O_2 boundary ($x/L = 1$). Increasing k_1 and k_2, keeping k_1/k_2 constant, results in a decrease in $V(1)/V(1)_{eq}$ for the cases studied.

Comparison with Experimental Results

Variation of Facilitation with Oxygen Pressure at the High Oxygen Boundary. We solved equations 16 for parameter values[1] which correspond closely to the experimental conditions of Scholander (2). L was fixed at 150 μ, while $P_w(0)$ was varied. Table III shows some of the results of these calculations. For all values of $P_w(0)$ tried the value of $V(0)$ calculated was within 0.003 of $V(0)_{eq}$, while the largest deviation of $V(1)$ from equilibrium was 0.04. Thus the equilibrium models discussed above should describe Scholander's results fairly well.

Fig. 2 compares our predictions of the facilitation with Scholander's experimental results. The experimental values of F were calculated from Scholander's Fig. 2. Note that the values of F from Table III are consistently less than those observed by Scholander. We believe this is partly due to the difference between the hyperbolic oxygen-hemoglobin equilibrium curve we have assumed and its true sigmoid

TABLE III

RESULTS OF OUR CALCULATIONS FOR THE EXPERIMENTAL CONDITIONS OF SCHOLANDER (2); $P_{O_2}(1) = 0$

$P_{O_2}(0)$	$V(0)$	$V(1)$	F	F^*_{max}
mm Hg				
10	0.577	0.014	6.61	6.82
12.5	0.611	0.016	5.81	5.99
25	0.760	0.021	3.60	3.70
50	0.864	0.029	2.03	2.10
100	0.927	0.040	1.08	1.13

* F_{max} calculated from equation 22 using equilibrium values of V computed from equation 5.

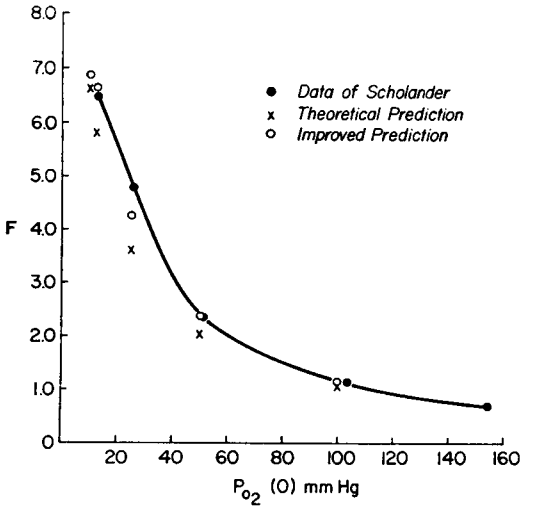

FIGURE 2 Facilitation (F) versus P_{O_2} on the high oxygen side of the layer, the P_{O_2} on the low oxygen side being zero. Compares the theoretical predictions with the results of Scholander (2).

shape. We have tried to compensate for this difference by multiplying F/F_{max} from Table III by the correct values of F_{max} for Scholander's conditions, which were obtained from equation 22 and the oxygen-hemoglobin equilibrium curve given by Hemmingsen and Scholander (24). The values of F obtained by this correction procedure fit Scholander's results quite well. (The closeness of fit may be fortuitous because D_w and D_v are not known precisely for these conditions; we have used what we believe to be the most reliable values available.)

Variation of Facilitation with P_{O_2} at the low O_2 Boundary. Hemmingsen and Scholander (24) studied the effects of backpressure on the facilitation of oxygen diffusion by hemoglobin by measuring the oxygen flux across layers of hemoglobin solution for several sets of values of P_{O_2} at the boundaries for which the oxygen pressure difference across the film was constant.

We solved equations 16 for parameter values[1] that correspond to the experimental conditions of Hemmingsen and Scholander for some values of $P_w(0)$ and $P_w(1)$ for which $P_w(0) - P_w(1) = 20$ mm Hg. Table IV shows some of the results of our calculations. As the backpressure increases, the facilitation decreases.

Hemmingsen and Scholander found that facilitation decreased to zero when the oxygen pressure on the low side was raised above 10 mm Hg. The data in Table IV show that the bimolecular reaction mechanism of equation 1 predicts a more gradual decline of facilitation with increasing $P_w(1)$.

Variation of Facilitation with Layer Thickness. Wittenberg (25) measured the diffusion of oxygen through hemoglobin solutions supported by millipore filters of different thicknesses. We solved equations 16 for parameter values[1] that correspond to the experimental conditions of Wittenberg for $L = 5$, 10, 25, 50, 100, and 300 μ.

Fig. 3 compares our calculations of F/F_{max} with the values calculated from Wittenberg's data. In order to estimate F from Wittenberg's data we used values of the hemoglobin-mediated flux from Wittenberg's Fig. 3 and values of the flux of oxygen in the absence of facilitation from his Fig. 1. (It was assumed that the simple flux of oxygen is inversely proportional to membrane thickness.) F_{max} was assumed to be equal to the facilitation measured or calculated for the 300 μ thickness. The theoretical curve fits Wittenberg's results fairly well.

Diffusion of Carbon Monoxide through Hemoglobin Solution. Mochizuki and Forster (26) measured the diffusion of CO through 150 μ layers of hemoglobin solution and found the facilitation to be much less than that predicted by the chemical equilibrium model. We solved equations 16 for parameter values[1] which correspond to the conditions of Mochizuki and Forster for various values of L. Table V shows that the facilitation is considerably less than F_{max} even for $L = 500\ \mu$. F_{max} for the conditions of Mochizuki and Forster was computed from equation 22 using the CO equilibrium curve of hemoglobin. They found a smaller facilitation than our calculations predict: i.e. for $P_{CO}(0) = 5$ mm Hg and $P_{CO}(1) = 0$ they found a facilitation near 2, while we predict $F = 7.84$ for the same conditions.

TABLE IV

THE EFFECT OF OXYGEN BACK PRESSURE ON FACILITATION FOR CONSTANT OXYGEN PRESSURE DIFFERENCE ACROSS THE LAYER

$P_{O_2}(0)$	$P_{O_2}(1)$	$V(0)$	$V(1)$	F	F^*_{exp}
mm Hg	*mm Hg*				
20	0	0.716	0.019	3.73	5.00
30	10	0.792	0.563	1.23	0
40	20	0.836	0.719	0.63	0
60	40	0.844	0.836	0.26	0
80	60	0.911	0.884	0.14	0

* From the data of Hemmingsen and Scholander (24).

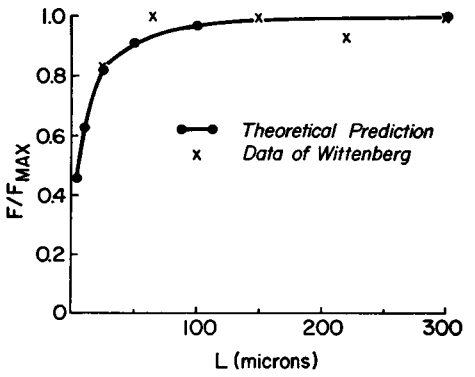

FIGURE 3 The dependence of facilitation (F) on layer thickness (L). Comparison of the results of our calculations with Wittenberg's (25) experimental results. $P_{O_2}(0) = 100$ mm Hg, $P_{O_2}(1) = 0$.

TABLE V

FACILITATION OF CARBON MONOXIDE DIFFUSION ACROSS A LAYER OF HEMOGLOBIN SOLUTION AS A FUNCTION OF LAYER THICKNESS; $P_{CO}(0) = 5$ MM HG, $P_{CO}(1) = 0$

L	$V(0)$	$V(1)$	F	F/F^*_{max}
μ				
10	0.954	0.930	0.750	0.025
50	0.964	0.844	3.75	0.123
100	0.967	0.771	6.13	0.202
150	0.968	0.717	7.84‡	0.258
300	0.970	0.604	11.44	0.376
500	0.971	0.509	14.44	0.475

* F_{max} calculated from equation 22 using values of V computed from equation 5.

‡ For this thickness and similar conditions Mochizuki and Forster (26) found F to be about 2.

Mochizuki and Forster attributed the small values of F/F_{max} that they measured to the slow rate of dissociation of CO from hemoglobin (k_2 is about 300 times smaller than for O_2). In support of this hypothesis they found that the presence of oxygen on the low CO side of the layer increased the facilitation (O_2 displaces CO from hemoglobin faster than HbCO dissociates in the absence of oxygen). We believe their explanation is correct.

Steady-State Facilitated Oxygen Diffusion in a Model Erythrocyte.

In considering the role of facilitated oxygen diffusion in red blood cells in the mammalian circulation, the relevant unit of function is a single erythrocyte. Steady-state facilitated diffusion of oxygen through a layer of intact erythrocytes has been demonstrated by Moll (27) in 300 μ layers and by Kutchai and Staub (28) in 165 μ layers, but in view of the marked thickness dependence of this phenomenon it is important to know the extent to which it would occur across a single red blood cell.

We treated the erythrocyte as a semi-infinite layer of 33 g/100 ml hemoglobin solution. The results of Kreuzer and Yahr (29) and Kutchai and Staub indicate that we are justified in neglecting the effect of the cell membrane of the erythrocyte. We solved equations 16 for parameter values[1] that are appropriate for physiological temperature and pH for various values of L with $P_w(0) = 125$ mm Hg and $P_w(1) =$ 0 and 50 mm Hg.

Tables VI and VII show the results of these calculations. The average path for diffusion in a human erythrocyte is probably between 1 and 2 μ. The data indicates that at these thicknesses the facilitation should be considerably less than that predicted assuming chemical equilibrium at the boundaries.

Moll (30) has numerically solved the partial differential equations for the time

course of the uptake and release of oxygen by thin sheets of hemoglobin solution of the same hemoglobin concentration as the human red cell. Assuming the diffusion coefficient of oxyhemoglobin to be 4.5×10^{-8}cm^2/sec he calculates that oxyhemoglobin diffusion reduces by 20% the time for an initially deoxygenated layer of hemoglobin (1.6 μ thick) to become fully saturated after being exposed to 100 mm Hg P_{O_2} on both sides, and reduces by about 30% the time required for an initially fully oxygenated layer to become 40% saturated after being exposed to zero P_{O_2}. Comparison of Moll's results with the results of our calculations on steady-state oxygen diffusion suggests that oxyhemoglobin diffusion makes approximately the same contribution to oxygen release and uptake by layers of hemoglobin solution similar in thickness to the human erythrocyte as it does to steady-state oxygen diffusion though such layers, for similar P_{O_2} differences.

TABLE VI

FACILITATION OF STEADY-STATE OXYGEN DIFFUSION THROUGH LAYERS OF 33 G/100 ML HEMOGLOBIN SOLUTION; $P_{O_2}(0) = 125$ MM HG, $P_{O_2}(1) = 0$

L	$V(0)$	$V(1)$	F	F/F^*_{max}
μ				
0.5	0.759	0.559	0.196	0.244
0.75	0.780	0.488	0.286	0.356
1	0.791	0.433	0.351	0.437
2	0.806	0.299	0.500	0.622
5	0.814	0.155	0.646	0.804
25	0.819	0.034	0.769	0.957

* Calculated from equation 22 using equilibrium values of V from equation 5.

TABLE VII

FACILITATION OF STEADY-STATE OXYGEN DIFFUSION THROUGH LAYERS OF 33 G/100 ML HEMOGLOBIN SOLUTION. $P_{O_2}(0) = 125$ MM HG, $P_{O_2}(1) = 50$ MM HG

L	$V(0)$	$V(1)$	F	F/F^*_{max}
μ				
0.5	0.793	0.716	0.126	0.442
0.75	0.802	0.699	0.168	0.589
1	0.807	0.689	0.193	0.677
2	0.813	0.670	0.234	0.821
5	0.817	0.656	0.263	0.923
25	0.819	0.648	0.279	0.979

* Calculated from equation 22 using the equilibrium values of V computed from equation 5.

Our results indicate that the equilibrium theory of facilitated oxygen transport should apply to layers of concentrated hemoglobin solution thicker than 25 μ, but that in thinner layers the facilitation will be less than predicted by the equilibrium theory.

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APPENDIX

CONSTANTS USED IN THE CALCULATIONS

(Reference number appears below the constant.)

Section starting on page	44	47	48	49	49	50	51
$D_v \times 10^7$, cm^2/sec	3.0	3.0	3.0	3.0	3.0	4.5	0.5
	(31)	(31)	(31)	(31)	(31)	(31)	(36)
$D_w \times 10^6$, cm^2/sec	1.5	1.5	1.45	1.45	1.45	1.8	0.6
	(32)	(32)	(32)	(32)	(32)	(32)	(32)*
$b \times 10^6$, $mole/ml$	1.8	1.8	1.0	0.88	1.0	0.67	2.0
	—	—	(2)	(24)	(25)	(26)	—
$w_0 \times 10^7$, $mole/ml$	3.6	3.6	—	—	1.7	0.0536	1.7
	—	—	—	—	—	—	—
$w_L \times 10^9$, $mole/ml$	3.6	3.6	0	—	0	0	0
	—	—	(2)	—	(25)	(26)	—
$k_1 \times 10^9$ $ml/mole \cdot sec$)	16	—	3.0	3.0	3.0	0.67	1.8
	‡	—	(34)	(34)	(34)	(35)	(35)
k_2, sec^{-1}	52	—	40	40	40	0.1	67.3
	(32)	—	(34)	(34)	(34)	§	§
L, μ	—	100	150	150	—	—	—
	—	—	(2)	(24)	—	—	—

* Obtained by extrapolating data in reference 32.
‡ Adjusted to fit the upper third of the oxygen-hemoglobin equilibrium curve.
§ Adjusted to fit the half-saturation oxygen pressure of the appropriate equilibrium curve for physiological conditions.

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