The Transport of Oxygen and Carbon Dioxide in Hemoglobin Systems

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ABSTRACT

A linearized theory of multicomponent diffusion accompanied by an arbitrary number of reversible, chemical reactions is developed for the study of simultaneous transport of oxygen and CO_2 through *in vitro* hemoglobin solutions. The special case of the scheme containing five reactions that have been thought to be dominant in this system at low oxygen tension and low hemoglobin concentration is considered in detail. This simple theory yields results that are consistent with certain previously observed phenomena, and also yields additional results that apparently have not been suggested previously.

The reaction facilitated augmentation of O_2 transport in Hb solution is calculated to be at a maximum near the physiological pH range, based on the available physical constants, and it is suggested that such augmentation may play an increased role in O_2 transport through concentrated Hb solutions.

The bicarbonate reaction and the carbamino reaction involving CO_2 are predicted to have a significant effect on the reaction-augmented transfer of O_2 both in the equilibrium and the near equilibrium reaction domains, the reactions involving CO_2 reinforcing the O_2 transfer when the CO_2 moves countercurrent to the O_2, but suppressing the O_2 transfer when moving parallel with the O_2.

It is suggested that the simple theory may provide a useful framework in which experimental results such as those designed to provide kinetic characterization of abnormal and impaired hemoglobin as well as normal Hb, may be interpreted.

The topic of gaseous transport through hemoglobin systems has received considerable attention since Scholander [22] and Wittenberg [29]

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discovered that the diffusive transport of oxygen at low tensions through a hemoglobin paste is decidedly augmented by the oxyhemoglobin reaction. The original hypothesis advanced by Scholander was that oxygen was passed along from one hemoglobin molecule to another in bucket brigade fashion.

An alternative to this mechanism was offered by Fatt and LaForce [5] when they suggested that the product of reaction, oxyhemoglobin, diffuses in parallel with the dissolved oxygen and is therefore responsible for this facilitated transport. The existence of the latter mechanism in dilute hemoglobin solutions was confirmed by Keller and Friedlander [11, 12]. The analysis and data of Keller and Friedlander were subsequently employed by Spaeth and Friedlander [23] in an interpretation of the extraction rate of oxygen from whole blood.

The foregoing explanations of hemoglobin's role in gaseous transport were all limited to the consideration of a single chemical reaction taking place in the system, and that one reaction was restricted to thermodynamic equilibrium throughout the system. In reality, however, gaseous components of interest may undergo a myriad of reactions in hemoglobin systems [20], and it may be that the circumstances are such that equilibrium may not be universally achieved, especially in systems as small as the red blood cell. The purpose of this communication is to present the results of an analysis based on a model that includes any number of simultaneous reactions which are allowed to deviate slightly from equilibrium.

The approach is based on the developments during the past decade in the theory of multicomponent diffusion in multireaction systems such as those of Byers-Brown [1], Wei and Prater [27], and Toor [24]. In extending the theory to the heterogeneous, two-phase problem, procedures were developed for numerically analyzing the solutions to the mathematically "stiff" equations that describe a linearized, multicomponent, multireaction problem [26]. These developments in combination have provided an apparatus for the analysis of simultaneous transport of oxygen and carbon dioxide in hemoglobin systems, and subsequent interpretation of the results in physical terms.

THEORETICAL CONSIDERATIONS

A special linearized theory may be developed for the O_2-CO_2-Hb system that reveals certain features of the system that are in agreement with previous observations, and which also provides predictions that (qualitatively) explain experimental results that have recently become available. The simple theory may therefore provide a useful framework for interpreting kinetic information about various hemoglobin systems.

A complete, exact theory apparently is not available for this system at this time because the complete reaction scheme, or mechanism, has not been clearly established, and the form of the concentration dependence of the transport coefficients in this multicomponent system is unknown. Even if this information were available, it would appear that one could expect only very special solutions to the resulting system of nonlinear equations, at most. What we have sought to do here is to provide a simple, but moderately complete description of a well-defined model of the system in order to provide a quantitative basis against which hypotheses dealing with reaction and transport mechanisms may be tested.

The approach is to restrict consideration here to a system that is perturbed slightly from equilibrium, and which is operating in the steady state in a convection-free environment. The isothermal, isobaric system consists of $n + 1$ species. Any given species may react chemically with any or all of the remaining species. In brief, we are considering a multicomponent, multireaction system with diffusive transport. One of the species, the "solvent phase," is present in preponderance over all the rest, and the molar fluxes of the remaining n species are measured with respect to it. The equations of conservation of the remaining n species may be written as a single n-dimensional vector equation:

$$\nabla \cdot (J) = (R). \tag{1}$$

The reaction rates of the n diffusing species are related to the reaction velocity through the stoicheiometric coefficient matrix,

$$(R) = [\nu](\omega). \tag{2}$$

The mass fluxes and the reaction rates are generalized thermodynamic forces, and in the neighborhood of thermodynamic equilibrium they may be expressed as linear combinations of their respective thermodynamic driving forces (see [2], for example),

$$(J) = -[\mathfrak{D}]\nabla(\mu), \tag{3}$$

$$(\omega) = [L](a), \tag{4}$$

where the affinities of the various reactions are themselves linear combinations of the chemical potentials [17],

$$(a) = -[\nu]^T(\mu). \tag{5}$$

Using (2), (3), (4), and (5) to rewrite (1) and assuming that the Onsager coefficients of mass diffusion are independent of distance* gives

$$\nabla^2(\mu) = [W](\mu), \tag{6}$$

* This implies that the concentrations do not vary widely with distance, which is consistent with the near-equilibrium restriction.

where

$$[W] = [\mathfrak{D}]^{-1}[\nu][L][\nu]^T. \tag{7}$$

The $n \times n$ matrix $[W]$ is positive, semidefinite and possesses rank r. The r eigenvalues have the dimensions L^{-2}; thus characteristic reaction-diffusion lengths can be obtained from them.

The coupled set of differential equations can be uncoupled by a suitable transformation,

$$(u) = [S](\mu). \tag{8}$$

Use of (8) in (6) yields

$$\nabla^2(u) = [\lambda^2](u), \tag{9}$$

where

$$[\lambda^2] = [S][W][S]^{-1}, \tag{10}$$

and $[S]$ is subject to the normalization constraint:

$$\frac{[S]^{-T}[\mathfrak{D}][S]^{-1}}{l} = [I]. \tag{11}$$

The matrix $[\lambda^2]$ is diagonal with the eigenvalues arranged along the diagonal in descending order.

The answers to particular questions, such as the extent to which the flux of a given species is augmented by a given chemical reaction scheme, are contained in the system of Eqs. (9) which are written in terms of the modified chemical potential vector (u). We seek to explore the physical character of the system represented by the solution to these equations, and many aspects of interest are largely independent of the geometry of the system chosen for the exploratory study. A convenient geometry from an *in vitro* experimental standpoint, and one which also may provide a (crude) model of a red blood cell, is that of a one-dimensional phase, a film, in which the diffusion and reaction are taking place. This film is bounded on one side at $z = 0$ by a semipermeable barrier allowing only the gaseous species to pass. Further into the solution at $z = l$ the chemical potentials of the components are specified, and all reactions are taken to be in equilibrium there. The situation, illustrated in Fig. 1, is essentially the classical film transfer model with semipermeable barrier at one side.

For a one-dimensional rectangular configuration, (9) becomes

$$\frac{d^2}{dz^2}(u) = [\lambda^2](u) \tag{12}$$

and has a general solution,

$$(u) = [\sinh \lambda z](A) + [\cosh \lambda z](B), \tag{13}$$

where the matrix $[\sinh \lambda z]$ is diagonal, and its first r diagonal terms are

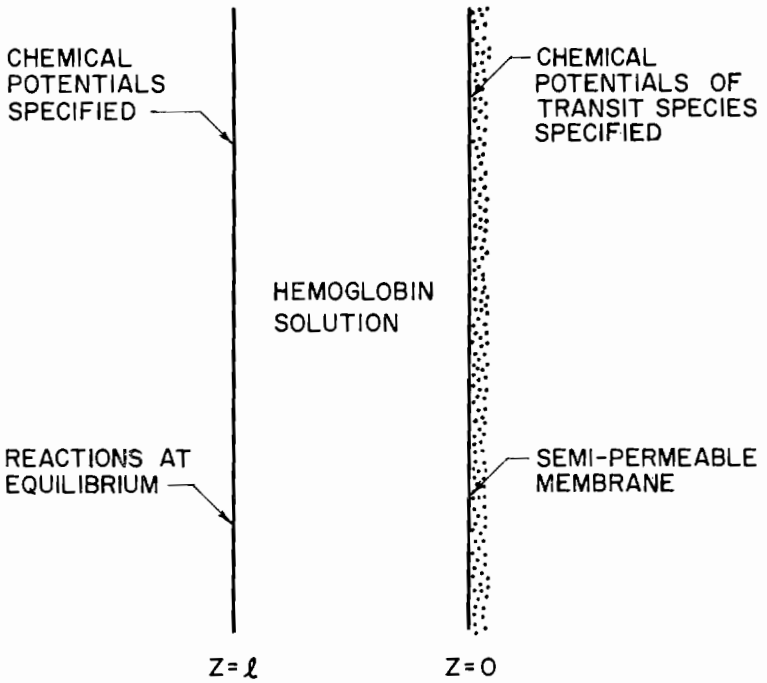

FIG. 1. Sketch of hemoglobin film, with boundary conditions.

the entries $\sinh \lambda_i z$, while the remaining $n - r$ terms are simply z. Similarly, the diagonal matrix $[\cosh \lambda z]$ has as its first r elements the functions $\cosh \lambda_i z$ but, unlike $[\sinh \lambda z]$, the last $n - r$ diagonal entries are simply unity.

Now the chemical potentials are specified at $z = l$ by a vector $(\mu)_l$ so that

$$(u)_l = [\sinh \lambda l](A) + [\cosh \lambda l](B), \tag{14}$$

where $(u)_l$ is formed from $(\mu)_l$ in accordance with Eq. (8).

The reactions at $z = l$ are specified to be at equilibrium; this means that the elements of $(\mu)_l$ will be in stoichiometric ratios to one another. When such conditions prevail, it can be demonstrated [25] that the first r components of $(u)_l$ are identically zeros.

Multiplying (14) on the left by the matrix $[\cosh \lambda l]^{-1}$ simplifies it to

$$(u)_l = [\tanh \lambda l](A) + (B), \tag{15}$$

where the assumption of equilibrium at $z = l$ has allowed one to write

$$[\cosh \lambda l]^{-1}(u)_l = (u)_l. \tag{16}$$

Attention is now focused on the boundary at the semipermeable membrane. In general, using the transformation (8) and the normalization constraints (11), the expression for the vector of fluxes, Eq. (3), may be rewritten as

$$(J) = -[S]^T \nabla(u). \tag{17}$$

In particular, at $z = 0$ this flux-force relation takes the form

$$(J) = -[S]^T[\lambda](A), \tag{18}$$

where the matrix $[\lambda]$ is diagonal, having as its first r terms the positive square roots of the eigenvalues of matrix $[W]$, and unity in the remaining $n - r$ entries.

Only certain of the species may pass the membrane at $z = 0$ (e.g., the gaseous components). Without loss of generality these may be designated the first k species. The last $n - k$ fluxes will then be zero at the boundary and may be written

$$(0) = [P](A), \tag{19}$$

where $[P]$ is an $(n - k) \times n$ matrix whose rows are identically the last $(n - k)$ rows of the matrix $[S]^T[\lambda]$.

The expression for the chemical potential at $z = 0$ is

$$(\mu)_0 = [S]^{-1}(B), \tag{20}$$

which may be rewritten with the help of (14) as

$$(\mu)_0 = (\mu)_l - [S]^{-1}[\tanh \lambda l](A). \tag{21}$$

The chemical potentials of the first k species are specified at $z = 0$. This leads us to write the first k component equations of (21) in the form

$$(\Delta\mu) = [Q](A), \tag{22}$$

where $(\Delta\mu)$ is a k-dimensional vector having as its entries the first k elements of the chemical potential difference vector $(\mu)_l - (\mu)_0$, and the rows of the $k \times n$ matrix $[Q]$ are the first k rows of the matrix $[S]^{-1}[\tanh \lambda l]$.

Combining the $n - k$ equations of (19) with the k component equations in (22), we may write the n-dimensional matrix relation,

$$(U) = [T](A), \tag{23}$$

where the first k rows of $[T]$ are the k rows of $[Q]$, and the last $n - k$ rows of $[T]$ are the $n - k$ rows of $[P]$. The first k entries of (U) are the k entries of $(\Delta\mu)$, and the last $n - k$ entries are zeros. We may invert (23) to solve for the constants of integration, (A), obtaining

$$(A) = [T]^{-1}(U), \tag{24}$$

and the fluxes at the membrane according to (18) become

$$(J) = -[S]^T[\lambda][T]^{-1}(U). \tag{25}$$

Equation (25) thus provides us with the (perturbation) fluxes of the gaseous components, and other components that may be permeable to the membrane, that are caused by small differences in chemical potentials across the film. Required for a given computation are a reaction scheme, the chemical potentials, or their ratios, of an equilibrium distribution of the n species at one boundary, and both the reaction and the diffusion parameters. These properties provide the entries for the matrices in Eq. (25) and, in principle, any number of independent reactions and components may be included in the reaction scheme. The form (25) therefore provides a means for testing proposed reaction mechanisms, and of establishing the relative importance of individual reactions in a given mechanism, for example. We now have a framework for exploring the transport characteristics of O_2 and CO_2 in the hemoglobin system as affected by a realistic reaction mechanism.

APPLICATION TO THE Hb-O_2-CO_2 SYSTEM

Oxygen and carbon dioxide are known to undergo a large number of reactions with hemoglobin in its various states [20]. Certain of the reactions have been subjected to considerable quantitative study, and their role in O_2 and CO_2 transport is reasonably well understood [20]. As a test of the simple linear theory developed above, we thus select from the larger set for our (trial) reaction mechanism five of the better-known reactions which are thought to be dominant under certain conditions. These reactions are

Reaction 1: $$H^+ + Hb \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} HHb^+$$

Reaction 2: $$H^+ + HbO_2 \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} HHbO_2^+$$

Reaction 3: $$O_2 + Hb \underset{k_3'}{\overset{k_3}{\rightleftharpoons}} HbO_2$$

Reaction 4: $$H^+ + HCO_3^- \underset{k_4'}{\overset{k_4}{\rightleftharpoons}} CO_2$$

Reaction 5: $$CO_2 + Hb \underset{k_5'}{\overset{k_5}{\rightleftharpoons}} HbCOO^- + H^+$$

The equilibrium constant for the ith reaction is given by $K_i = k_i/k_i'$.

Reaction 3 represents the reversible combination of oxygen with hemoglobin to yield oxyhemoglobin. Reactions 1 and 2 show reduced hemoglobin and oxyhemoglobin acting as buffers, since it is the difference in pK of these two reactions which is responsible for the well-known Bohr effect [20]. Carbon dioxide may be hydrolyzed into the bicarbonate anion via reaction 4, with the zinc-containing enzyme, carbonic anhydrase, as catalyst. Finally, in this scheme, carbon dioxide may react with one of the free amyl groups on the hemoglobin moiety to yield carbamino-hemoglobin as represented by reaction 5. We restrict our consideration here to an abundance of water as the solvent phase, which is not shown explicitly in the reaction mechanism above. The system represented by the five reactions ($r = 5$) thus consists of nine species ($n = 9$) which are free to diffuse relative to the aqueous phase.

Other reactions that might be included in the preceding scheme are those involving the higher hemoglobin oxides as hypothesized by Adair (in [20]), for example, and the framework of the theory easily lends itself in principle to such an expanded mechanism. One reason why the complete Adair scheme was not used in this study is that property values for all the species involved in that mechanism are not known at this time, but we do have a framework now for eventual testing of that mechanism under dynamical conditions. By including only one of the oxygen-hemoglobin reactions in the scheme chosen here we restrict applicability of the results to situations of moderately low oxygen tensions.

In order to apply the result of the theory developed in the preceding section to the scheme of reactions 1 to 5, we need an estimate of the values of the physical properties of this system. The conditions selected for this study are experimentally accessible, and they simulate to some extent certain physiological features, although we do not restrict consideration here only to conditions expected in living organisms. The parameters and conditions used are discussed in the following paragraphs.

The Onsager phenomenological coefficients of diffusion can be estimated from the pseudobinary diffusion coefficients by [17]

$$\mathfrak{D}_i \approx \frac{\bar{C}_i D_i}{RT}, \tag{26}$$

and the Onsager coefficients of reaction by [17]

$$L_i \approx \frac{v_{fi}}{RT}, \tag{27}$$

where v_{fi} is the forward reaction rate of the ith reaction expressed in terms of the law of mass action. Thus we must find or estimate nine diffusion

coefficients and five reaction rate constants. Also required is the equilibrium concentration distribution. Since there are nine species and five reactions, there are four degrees of freedom which may be varied by an experimentalist along with the geometric parameter, the film thickness. These four degrees of freedom were chosen to be the pH of the solution, the equilibrium tensions of oxygen and carbon dioxide, and the total hemoglobin concentration. A specification of these quantities along with the five equilibrium constants sufficed to determine the equilibrium concentrations.

Unless otherwise specified, the four degrees of freedom are taken as

$$\begin{aligned} \text{pH} &= 7.4 \\ P_{O_2} &= 5 \text{ mm Hg} \\ P_{CO_2} &= 40 \text{ mm Hg} \\ \text{Hb}_{\text{total}} &= 5 \text{ g/100 ml} \\ T &= 37.5^\circ\text{C} \end{aligned}$$

Finally, two Henry's law constants are needed to relate the gaseous O_2 and CO_2 tensions with the aqueous concentrations.

A summary of the parameters used in the following simulations is provided in Table I. Whenever possible, the parameters are based on experimental observations; where no data were available, estimates were made from existing correlations and theory. When necessary, the values of the parameters found in the literature were corrected for temperature and hemoglobin concentration. For a detailed discussion of the evaluation and selection of the parameters, see [25]. There are some inconsistencies in the literature for the value of the diffusion coefficient of O_2 in Hb, and the analysis developed here may provide a broader basis for the interpretation of diffusion data.

THE SIMULATION

A useful property that characterizes in part a reactive system such as that considered here during gaseous transfer is the augmentation coefficient. This coefficient is defined as the ratio of the flux of oxygen, for example, J_{O_2}, while reacting with the hemoglobin to the value the oxygen flux would have, J'_{O_2}, if the oxygen were to diffuse through the system as an inert species. Thus J_{O_2}/J'_{O_2} is the oxygen augmentation coefficient. The behavior of this property can now be determined as affected by the relatively complicated reaction mechanism above and the associated system properties.

In particular, the behavior of the augmentation coefficient may now be elucidated under conditions of nonequilibrium reaction. It should be

TABLE I

VALUES OF PARAMETERS USED IN THE SIMULATION[a]

Reaction no.	Equilibrium constant, K_i K_i[=] liter/mole	Ref.	Forward rate coefficient, k_i k_i[=] liter/(mole-sec)	Ref.
i	5.36×10^7	30	5×10^{10}	28
ii	3.01×10^6	30	5×10^{10}	28
iii	1.53×10^4	21	3×10^6	9
iv	2.00×10^6	15	1.69×10^3	13, 20
v	2.00×10^{-5}[b]	7	1×10^4	6

Species i	Diffusion coefficient, D_i D_i[=] cm²/sec	Ref.	Henry's law constants, K_i K_i[=] mole/(liter-mm Hg)	Ref.
O_2	2.4×10^{-5}	12	1.43×10^{-6}	10
CO_2	1.85×10^{-5}	8	5.54×10^{-6}	10
H^+	8.78×10^{-5}	18		
HCO_3^-	1.11×10^{-5}	15		
Hb[c]	8.38×10^{-7}	12		

[a] Values corrected for temperature or Hb concentration, as applicable.

[b] K_5 is nondimensional.

[c] Diffusion coefficients of all Hb species taken the same.

recalled that a set of r characteristic lengths can be formed from the eigenvalues of matrix $[W]$. These characteristic lengths depend on the reaction stoichiometries and velocities, and the transport coefficients. When the phase length of the system is much greater than the largest of the characteristic lengths, all reactions will be essentially at equilibrium throughout the system. As the physical dimensions of the system are reduced to within the order of these characteristic lengths, at least some of the reactions will be displaced from equilibrium. This knowledge of the system may be used both to clarify possible ambiguities in postulated reaction mechanisms and to interpret experimental information in terms of the system properties, for example.

The augmentation coefficient has been computed for this system for two types of boundary value problems for a wide range in the system thickness l. The first is that of countercurrent transfer of oxygen and carbon dioxide. This is in the sense of a physiological situation. The second boundary value problem is that of cocurrent transfer of both gases. These two problems are of interest, as they are both feasible in an *in vitro* experimental setup. The information concerning the system that

can be obtained from a comparison of the results of the analysis of these two cases is discussed below.

A computer was used in the evaluation of the augmentation coefficient, after special procedures were developed [25] for handling the highly singular matrices contained in Eq. (25).

A plot of the oxygen augmentation coefficient as a function of the film thickness l over several orders of magnitude is shown on Fig. 2.

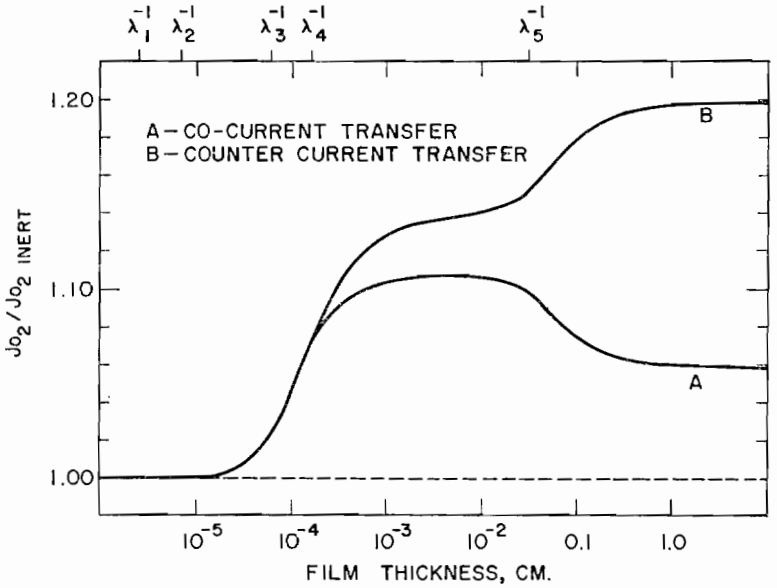

Fig. 2. Effect of CO_2 transfer on the oxygen augmentation coefficient.

Curve A represents the cocurrent flux of O_2 and CO_2; curve B is that case when the two species are diffusing against one another. If the oxyhemoglobin reaction were the only one occurring, such a plot would yield a sigmoidal curve leveling out at about $J_{O_2}/J'_{O_2} = 1.13$. Thus the presence of a carbon dioxide flux and the concomitant reactions definitely affect the oxygen transfer for l greater than the order of 10^{-4} cm. The effect of the countercurrent CO_2 flux, curve B, is to further enhance the oxygen flux, while the cocurrent transfer of CO_2 with oxygen provides an interference. All reactions in this model are displaced from equilibrium by the fluxes until the film thickness is increased to the order of a centimeter or greater on the basis of the available property data.

Two regions of O_2-CO_2 interaction are discernible in Fig. 2. The first region begins around 10^{-4} cm, where the characteristic lengths from the

third and forth eigenvalues, λ_3 and λ_4, appear. These eigenvalues are determined predominantly by the parameters involved in the oxyhemoglobin and the carbamino-hemoglobin reactions. A second interaction occurs in the region of the fifth characteristic length (about 0.1 mm), which is determined mainly by the parameters of the bicarbonate reaction.

The interference of the carbon dioxide reactions with the oxygen augmentation seems to stem from a straightforward mechanism. The reactions of CO_2 to form bicarbonate or carbamino-hemoglobin both tend to lower the pH. The Bohr Effect (operative at low to moderate pH's) then implies that HbO_2 will dissociate. The general sequence of the augmentation is: the reaction of the transit species to form a compound; the diffusion of this compound under its own gradient; and the release of the transit species from its compound. In the case of countercurrent diffusion of O_2 and CO_2, the oxyhemoglobin diffuses into progressively higher concentrations of CO_2, and the rate of dissociation of HbO_2 is thus increased. When the dissolved gaseous species diffuse cocurrently, this mutual facilitation which the two fluxes lend one another is replaced by a competition for the carrier.

The physiological range of interest for the hemoglobin reactions with O_2 and CO_2 is on the order of the dimensions of the red blood cell, i.e., $10^{-4} - 10^{-3}$ cm. The simple model considered here suggests that the bicarbonate reaction becomes important only at larger phase lengths. However, this need not preclude the bicarbonate role in augmentation since the corpuscle membrane is permeable to HCO_3^-, and the system length associated with the bicarbonate reaction is thus effectively increased.

Figure 2 shows the oxygen augmentation coefficient for a given, fixed value of the thermodynamic driving forces. To show how the fluxes of O_2 and CO_2 mutually affect one another, it is useful to plot the combinations of percentage augmentations of CO_2 and O_2, using the ratio of the thermodynamic driving forces as a parameter. When this is accomplished for a film thickness sufficiently large that the reactions proceed essentially at equilibrium, for example, the points lie along a rectangular hyperbola as shown in Fig. 3. The upper cusp corresponds to a negative ratio of forces, i.e., countercurrent fluxes, where mutual augmentation is effected, whereas the lower cusp is the result of possible combinations of cocurrent forces, where a mutual deaugmentation prevails. The asympotes have the distinction of being the augmentations each of the two species would experience if the reactions its companion engaged in were absent; for example, if only the oxyhemoglobin reaction occurred, the O_2 would undergo an augmentation at infinite film thickness of 12.8%, the horizontal asymptote, for the particular value of the oxygen tension used here.

It is interesting to note that on the lower cusp of Fig. 3, ratios of forces

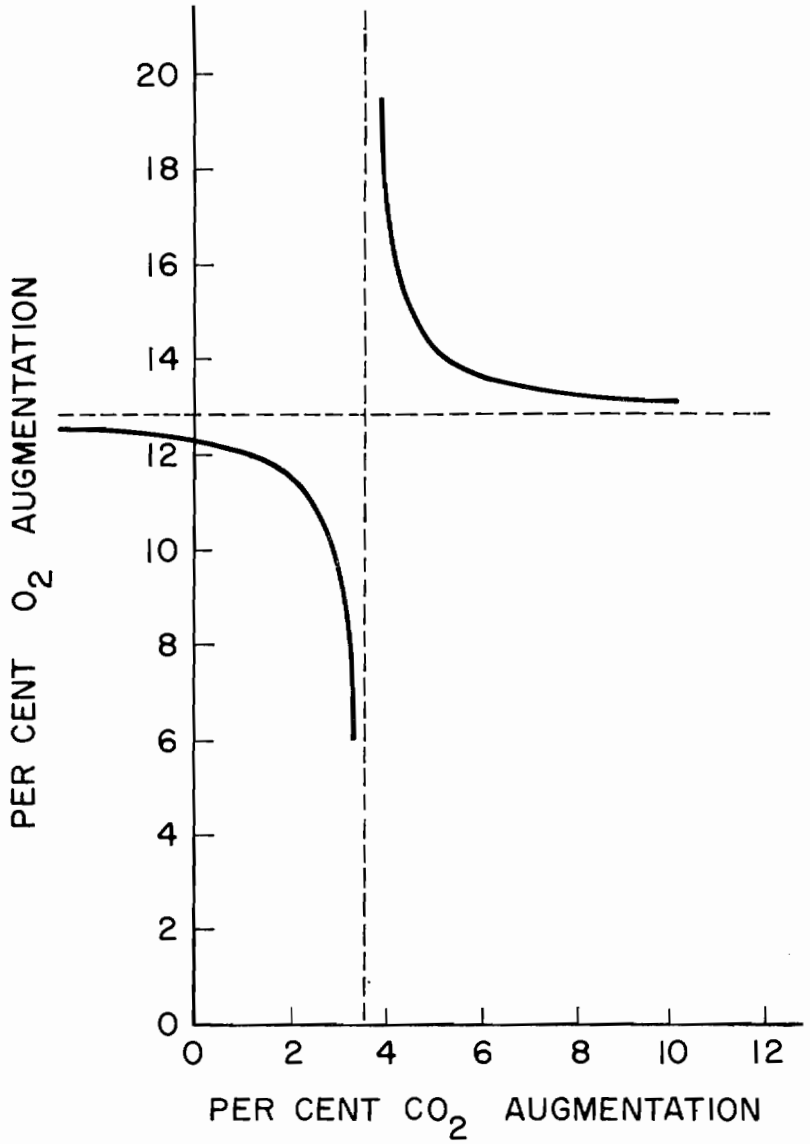

FIG. 3. Possible combinations of both the O_2 and the CO_2 augmentation for the system at "average conditions."

exist for which one of the components experiences an added resistance to transfer rather than an augmentation (a "negative" augmentation). For further increases of the force ratio in this direction this linearized model predicts contragradient diffusion.

Another feature of interest in this system is the effect of change in pH of the system on the transport. This effect is shown in Fig. 4, again for

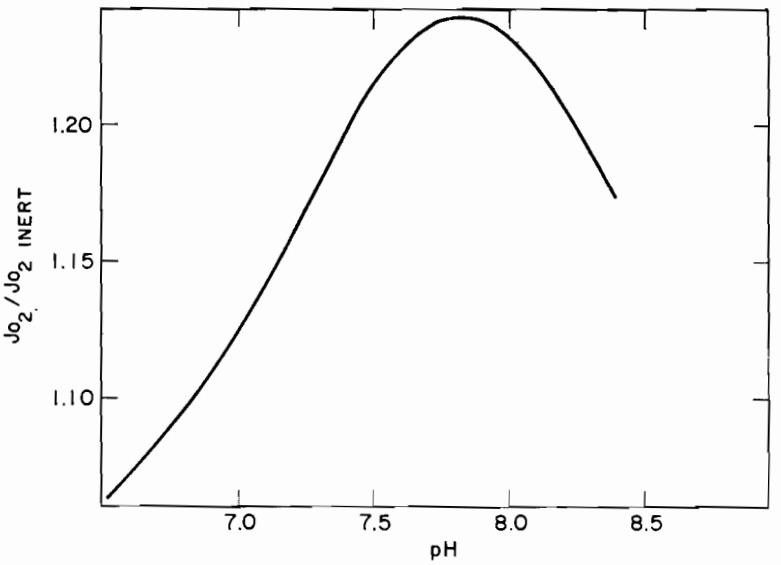

FIG. 4. Oxygen augmentation rates for large phase length as a function of the pH of the hemoglobin solution.

the (representative) case of large film thickness such that the reactions proceed at quasi-equilibrium. As can be seen, the oxygen augmentation goes through a maximum near the physiological range of pH values, an effect which apparently has not been suggested previously. This effect can be explained in terms of the reaction scheme above. The diminished augmentation at low pH seems straightforward in that such conditions are unfavorable for the formation of oxyhemoglobin as in the case of the Bohr effect. The drop in augmentation at high pH apparently is the result of a tendency for the carbamino reaction to oppose the normal Bohr effect, i.e., raising the pH tends to shift the oxyhemoglobin reaction to the left [19]. In this there are aspects of the reverse Bohr effect.

The position of the maximum in Fig. 4 depends on the system properties, and may be shifted slightly one way or the other as better values become available. Additional properties not considered here, such as total

ionic strength, may also influence this curve, but the existence of the maximum does seem clear, and comparison of experiment with this prediction is suggested as a test of this model.

The one remaining degree of freedom to be considered in this system is that of the total hemoglobin concentration. Figure 5 provides the transport profiles calculated for three different values of the total hemoglobin

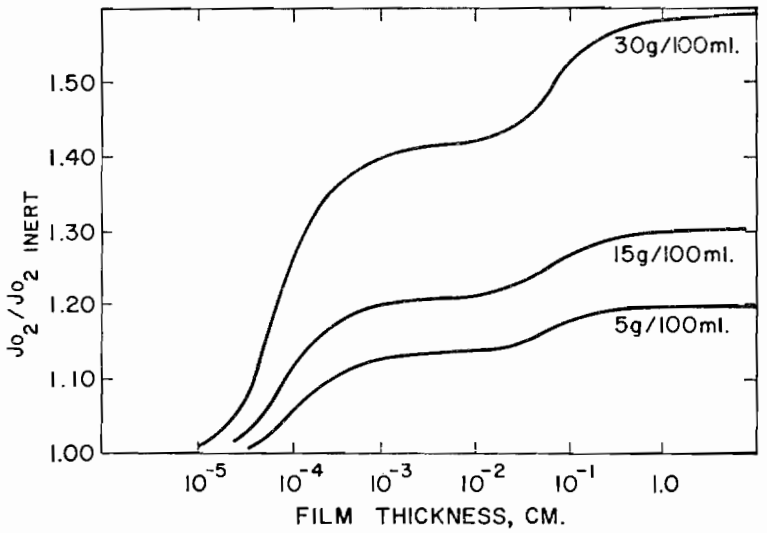

FIG. 5. Effect of the phase thickness on the oxygen augmentation for various values of the total hemoglobin concentration.

concentration in order to reveal the trend with increasing Hb concentration above that used in the previous analyses. The results indicate that at higher Hb concentrations a greater portion of the total oxygen flux is attributable to the reaction-diffusion mechanism. The values of the appropriate diffusivities at the indicated concentration levels were taken from [12]. Actually, the diffusivitives of both O_2 and hemoglobin decrease with increasing Hb concentration; hence the total oxygen transport decreases as Hb concentration is raised. At the higher concentration the data of Keller [11] show the diffusivity of oxygen decreasing faster than that of Hb, and a greater relative amount of transport should therefore be accomplished by the oxyhemoglobin route; this is consistent with the results shown in Fig. 5. Also, it is noted that a greater concentration of Hb implies a greater proportion of reduced Hb ready to enter into the diffusion reaction-augmentation mechanism.

DISCUSSION

A simple, linearized, steady state model has been proposed for the transfer of various reactive species through hemoglobin films. The case of the O_2-$C\dot{O}_2$-Hb system, with five reactions which are thought to be dominant in this system, is considered in some detail. Results of this analysis indicate that CO_2 flux can further augment or suppress the O_2 flux, depending on whether it moves countercurrent to or cocurrent with the O_2. This effect is due to reaction-diffusion coupling via the oxyhemoglobin and carbamino-hemoglobin. Whether or not this effect is appreciable in the red blood cell may be questionable, however, as the diffusivity of Hb and its compounds is reduced considerably at those concentrations [7]. This effect may be useful, however, as a means of interpreting experimental data in terms of basic physical and chemical parameters as discussed below.

When O_2 and CO_2 are diffusing simultaneously, there exist ratios of the driving forces for which the O_2 transfer suppresses the CO_2 flux, and may even force contragradient CO_2 diffusion. This prediction must be viewed in the framework of this linearized model, but contragradient diffusion has been suggested in other systems [14] and this would be of special interest if it could occur under physiological conditions.

The O_2 augmentation coefficient passes through the maximum as the pH is increased through the physiological range. This effect apparently has not been suggested before, but can be explained in terms of the Bohr and reverse Bohr effects [20]. Experimental confirmation of this effect, along with observation of the suggested contragradient CO_2 diffusion, would provide considerable confidence in the linearized model as a realistic representation of this system.

Uses of the (verified) theory are suggested in areas such as the interpretation of experimental results in terms of physical and chemical properties of this system, and as a framework for the comparison and kinetic characterization of various classes of abnormal [16] and impaired [3] hemoglobins. As an example of the use of the theory in the study of the nonequilibrium kinetic properties of this system, consider reaction 4. Because the value of the forward rate coefficient, k_4, is apparently not well known under the influence of enzyme catalysis, to test the sensitivity of the O_2 augmentation coefficient to this parameter, Fig. 6 was constructed in which k_4 was changed parametrically through several orders of magnitude. The effect shows up mainly in the fifth eigenvalue, λ_5, and a three-order of magnitude change in k_5 thus produces almost a two-order of magnitude change in the film thickness required for the system to approach the equilibrium transfer asymptote; the slower the reaction,

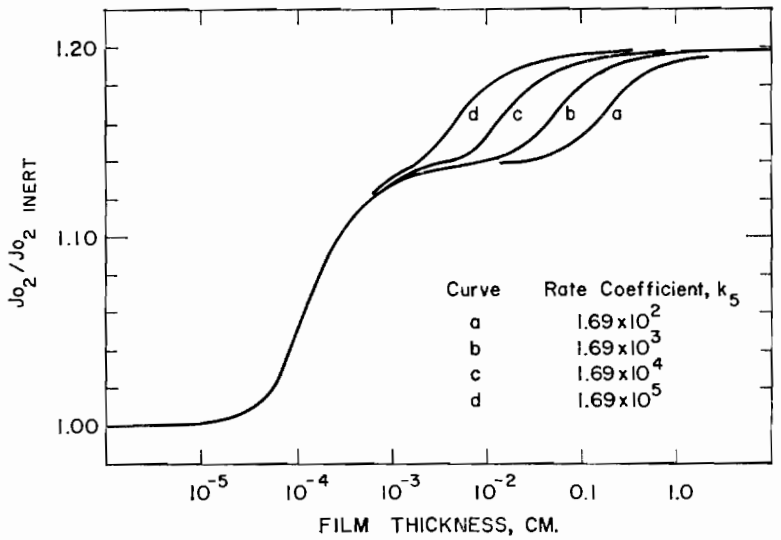

FIG. 6. Effect of the value of the bicarbonate reaction rate coefficient on the oxygen augmentation, with counter current CO_2 flux.

the larger is the film thickness required for quasi-equilibrium transfer. Thus we may evaluate this rate coefficient, for example, for various hemoglobins of interest with appropriate experimental data required to show J_{O_2}/J'_{O_2} as a function of film thickness.

Application of the simple theory presented here can be readily extended to other species of interest, such as alcohols, ethers, carbon monoxide, and various drugs, in both normal and abnormal hemoglobin systems.

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NOMENCLATURE

(A)	vector constant of integration	$[\cosh \lambda z]$	diagonal matrix defined after Eq. (13)
(a)	r-dimensional vector of reaction affinities	D_i	binary diffusion coefficient of species i
(B)	vector constant of integration	$[\mathfrak{D}]$	matrix of Onsager phenomenological coefficients of diffusion
$\bar{C}_i$	equilibrium concentration of species i	Hb	molecular hemoglobin

$[I]$	n-dimensional identity matrix
(J)	n-dimensional vector of molar fluxes
k	number of gaseous species
k_i, k_i'	reaction rate coefficient of the ith reaction, forward and reverse, respectively
K_i	equilibrium constant of the ith reaction; Henry's law constant of i^{th} species
l	length of the diffusion film
$[L]$	matrix of Onsager phenomenological coefficients of reaction
n	the number of species
P_{CO_2}	tension of the carbon dioxiode
P_{O_2}	tension of the oxygen
$[P]$	matrix defined after Eq. (19)
$[Q]$	matrix defined after Eq. (20)
r	number of independent reactions
(R)	species reaction rate vector
$[S]$	modal matrix of $[W]$
$[\sinh \lambda z]$	matrix defined after Eq. (13)
$[T]$	matrix defined after Eq. (23)
$[\tanh \lambda l]$	product matrix $[\cosh \lambda l]^{-1}$ $[\sinh \lambda l]$
(U)	vector defined by Eq. (23)
(u)	vector of transformed chemical potentials
$(u)_0$	value of (u) at $z = 0$
$(u)_l$	value of (u) at $z = l$
v_{fi}	forward reaction velocity of reaction i
$[W]$	matrix defined by Eq. (7)
z	independent variable of length

Greek Letters

$(\Delta\mu)$	vector defined after Eq. (22)
$[\lambda]$	matrix defined after Eq. (18)
$[\lambda^2]$	Canonical form of $[W]$
(μ)	vector of chemical potentials
$(\mu)_0$	vector (μ) evaluated at $z = 0$
$(\mu)_l$	vector (μ) evaluated at $z = l$
$[\nu]$	$n \times r$ matrix of stoichiometric coefficients
(ω)	vector of reaction rates

Matrix Superscripts

-1	matrix inversion
T	matrix transposition
$-T$	combined inversion and transposition

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