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NUMERICAL STUDY OF OXYGEN UPTAKE BY LAYERS OF HEMOGLOBIN SOLUTION

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Abstract. Numerical solutions have been obtained for the equations describing O_2 uptake by layers of concentrated hemoglobin solution 0.25, 0.5, 1, 1.6, 2, 3.6, 5, 10, and 20 μ thick. The results indicate that the diffusion of oxyhemoglobin has almost no effect on the rate of oxygenation of the 0.25 μ layer, but its influence increases with increasing layer thickness, so that it shortens the time required to reach 50% saturation in the 1.6 μ layer by 23% and in the 5μ layer by 34%. For the 1.6 μ layer, which might be considered a model red blood cell, the results suggest that the rate at which O_2 reacts with hemoglobin is of primary importance early in the uptake process, but that later the diffusion of O_2 into the deeper parts of the layer becomes rate-limiting.

Facilitated diffusion Oxygen diffusion
Hemoglobin Oxyhemoglobin

The partial differential equations that describe the diffusion and chemical reaction of O_2 in Hb solution are nonlinear and thus not solvable by analytical methods. For this reason much of the theoretical work done in this area has involved approximate solutions to the diffusion equations or solutions to approximations of the diffusion equations (see ROUGHTON (1959) for a thorough review). Most of this work is due to Roughton, Forster, and their associates, who have been extremely inventive in wresting important insights from seemingly intractable equations. The electronic digital computer has made it practical to solve the diffusion equations numerically. The advantages of this approach are that it can deal with the nonlinearities present in the equations and theoretically solutions may be had with whatever accuracy is desired.

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NICOLSON and ROUGHTON (1951) and KLUG, KREUZER and ROUGHTON (1956) employed numerical approaches to solving the diffusion equations. FORSTER (1964) has reported on the numerical integration of the diffusion equations in a biconcave disc. MOLL (1968) used numerical solutions of the diffusion equations to model the uptake and release of O_2 by 1.6 and 3.6 μ layers of Hb solution. He found significant effects of HbO_2 diffusion on the uptake and release processes in both layers, but computed larger effects for the 3.6 μ layer. He also found that while the time course calculated for deoxygenation of 1.6 μ layers matched quite closely experimental determinations on human RBCs, that computed for oxygenation was much faster than RBCs have been observed to take up O_2.

I have obtained numerical solutions for the equations describing O_2 uptake by layers of concentrated Hb solutions 0.25, 0.5, 1, 1.6, 2, 3.6, 5, 10, and 20 μ thick. The solutions indicate that the diffusion of HbO_2 has almost no effect on the rate of oxygenation of the 0.25 μ layer, but its influence increases quickly with layer thickness, so that it shortens the time required to reach 50% saturation in the 1.6 μ layer by 23% and that in the 5 μ layer by 34%. For the 1.6 μ layer I attempted to estimate the relative importance of diffusion and reaction parameters in determining the overall rate of O_2 uptake. The results suggest that the rate at which O_2 reacts with Hb is of primary importance early in the uptake process, but that later the diffusion coefficient of O_2 becomes the most important parameter.

The discrepancy between observed and calculated rates of O_2 uptake by RBCs is discussed. I argue that the O_2 diffusion resistance of the RBC membrane, which is generally regarded as the cause of the discrepancy, is probably not large enough to explain the difference between theory and experiment.

Methods

THE MODEL

I have used a mathematical model to investigate the behavior of a layer of concentrated Hb solution, initially free of O_2, when exposed to 100 mm Hg O_2 tension at both surfaces (fig. 1). The mathematical formulation of the model consists of the one-dimensional diffusion equations for O_2 and HbO_2 plus 6 conditions on the solution functions $C_{O_2}(x, t)$ and $C_{HbO_2}(x, t)$, the concentrations of O_2 and HbO_2, respectively.

The reaction of Hb with O_2 has been assumed to be the one step reaction

$$(1) \qquad Hb + O_2 \underset{k}{\overset{k'}{\rightleftharpoons}} HbO_2$$

where k′ and k are the forward and backward reaction rate coefficients, respectively. This representation is not consistent with the widely accepted concept of a four step reaction of O_2 with Hb. In addition, eq. (1) predicts a hyperbolic Hb-O_2 equilibrium curve that cannot closely fit the sigmoid curve of whole blood. The principal justification for my using this oversimplified reaction scheme is that it can fairly closely

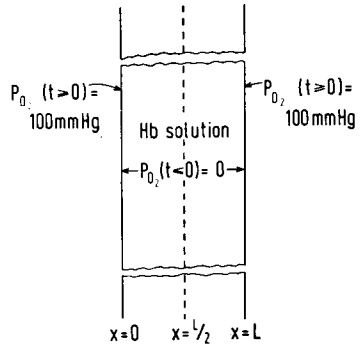

Fig. 1. Schematic diagram of the physical situation considered.

represent the kinetics of O_2 uptake and release (see ROUGHTON, 1959).

The total Hb concentration (C_t) is assumed to be the same everywhere in the layer, so that

$$C_{Hb}+C_{HbO_2}=C_t \tag{2}$$

holds for all values of x. Thus C_{Hb} can be expressed in terms of C_{HbO_2} and need not be considered separately.

The diffusion equations for the diffusion and chemical reaction of O_2 and HbO_2 in a one-dimensional system are

$$\frac{\partial C_{O_2}}{\partial t} = D_{O_2}\frac{\partial^2 C_{O_2}}{dx^2} - k' C_{O_2}(C_t - C_{HbO_2}) + kC_{HbO_2} \tag{3}$$

$$\frac{\partial C_{HbO_2}}{\partial t} = D_{HbO_2}\frac{\partial^2 C_{HbO_2}}{\partial x^2} + k' C_{O_2}(C_t - C_{HbO_2}) - kC_{HbO_2} \tag{4}$$

where the D's are the diffusion coefficients of the species denoted by subscripts. Note that C_{Hb} has been expressed as $C_t - C_{HbO_2} \cdot C_{HbO_2}$ and C_t are in mole heme/ml.

To specify unique solutions to eqs. (3) and (4) appropriate initial conditions and boundary values must be imposed. Because the O_2 and HbO_2 concentration profiles must be symmetrical about the center of the layer we need only consider the range $0 \leqslant X \leqslant L/2$.

It is assumed that initially the layer is completely free of O_2 and at $t=0$ its surfaces are exposed to an O_2 tension of 100 mm Hg, so

$$C_{O_2}(x, 0)=0, \quad x>0 \tag{5}$$

$$C_{HbO_2}(x, 0)/0, x \geqslant 0. \tag{6}$$

Since the O_2 tension at the boundaries is 100 mm Hg for $t \geqslant 0$

$$C_{O_2}(0,t) = \alpha_{O_2}^{\text{Hb soln}} \cdot 100 \tag{7}$$

where $\alpha_{O_2}^{Hb\ soln}$ is the solubility of O_2 in the Hb solution expressed in mole/(ml·mm Hg).

Because HbO_2 cannot cross the boundaries of the layer

$$\left.\frac{\partial C_{HbO_2}}{\partial x}\right|_{x=0} = 0,\ t \geqslant 0\,. \tag{8}$$

For reasons of symmetry the minimum concentrations of O_2 and HbO_2 must occur in the middle of the layer, so

$$\left.\frac{\partial C_{O_2}}{\partial x}\right|_{x=L/2} = 0,\ t \geqslant 0 \tag{9}$$

$$\left.\frac{\partial C_{HbO_2}}{\partial x}\right|_{x=L/2} = 0,\ t \geqslant 0\,. \tag{10}$$

The mathematical model is then eqs. (3) and (4) subject to the constraints of eqs. (5)–(10). The effects of HbO_2 diffusion were investigated by comparing numerical solutions of the model for $D_{HbO_2}=0$ to those for reasonable values of D_{HbO_2}. The relative importance of D_{O_2}, D_{HbO_2}, k′, and k was estimated from the effects of changing these constants one at a time.

THE NUMERICAL METHOD

Because of the nonlinear chemical reaction term in eqs. (3) and (4) analytical methods of solution cannot be applied. I have used a finite difference method to obtain numerical solutions to eqs. (3) and (4) subject to the conditions imposed by eqs. (5)–(10). The method, originally due to SAUL'YEV (1964) and modified by CARNAHAN, LUTHER and WILKES (1969), uses the following finite difference forms as approximations to $\partial C/\partial t$ and $\partial^2 C/\partial x^2$:

$$\left.\frac{\partial C}{\partial t}\right|_{x_i,t_j} = \frac{C(x_i,t_{j+1})-C(x_i,t_j)}{\Delta t} \tag{11}$$

$$\left.\frac{\partial^2 C}{\partial x^2}\right|_{x_i,t} = \frac{C(x_{i-1},t_{j+1})-C(x_i,t_{j+1})-C(x_i,t_j)+C(x_{i+1},t_j)}{(\Delta x)^2} \tag{12}$$

$$\left.\frac{\partial^2 C}{\partial x^2}\right|_{x_i,t_j} = \frac{C(x_{i+1},t_{j+1})-C(x_i,t_{j+1})-C(x_i,t_j)+C(x_{i-1},t_j)}{(\Delta x)^2}\,. \tag{13}$$

The method is explicit in that concentrations are computed directly without solving systems of algebraic equations. Knowing all concentrations at $t=t_0$ one calculates the concentrations at the next time level ($t=t_0+\Delta t$) by using (12) to represent the second

derivatives and proceeding in the +x direction. Upon reaching a boundary (in our case the center of the layer) one calculates the concentrations at the next time level ($t = t_0 + 2\Delta t$) by proceeding in the −x direct in using eq. (13) to represent the second derivatives. The references should be consulted for details of the method.

Equations (11)–(13) were used to approximate $\partial C_{O_2}/\partial t, \partial C_{HbO_2}/\partial t$, $\partial^2 C_{O_2}/\partial x^2$, and $\partial^2 C_{HbO_2}/\partial x^2$ for $t \geqslant 0$, $0 < x < L/2$. For approximating the chemical kinetic terms in the partial differential equations I assumed that over the time step from t_i to t_{i+1}, $C_{O_2} = C_{O_2}(t_i)$ and $C_{HbO_2} = C_{HbO_2}(t_i)$ for each value of x.

The method was programmed in FORTRAN-IV using double precision arithmetic for the IBM 360/67 at the University of Michigan Computing Center and in FORTRAN-II for the Nord 1 computer at the Physiological Institute of the University of Oslo. The results from the two computers were nearly identical, which indicates that roundoff error was negligible. The results reported here are from the Nord 1.

The half-thickness of the layer was divided into 10 subintervals, so that $\Delta x = L/20$. The value to be used for Δt was determined by trial and error. If Δt was made too large the solutions failed to converge. Values of Δt ranged from 1×10^{-7} sec for 0.25 μ layers to 2×10^{-5} sec for 20 μ layers. Most methods for numerically integrating partial differential equations use fairly large amounts of computing time. In general larger times were required for larger values of L, the solutions for 5 μ layers taking about 2 min computing time on the IBM 360/67.

CONSTANTS USED IN THE CALCULATIONS

Table 1 gives the values of constants used in the calculations. Most of them are identical to the values used by MOLL (1968) which I believe to be the most reliable values currently available for physiological temperature and pH.

TABLE 1

Constants used in the calculations.

Constant	Value	Reference
D_{O_2}	8×10^{-6} cm²/sec	GROTE and THEWS (1962) KELLER and FRIEDLANDER (1966)
D_{HbO_2}	4.5×10^{-8} cm²/sec	MOLL (1966)
k′	3.5×10^{9} ml/(mole sec)	GIBSON *et al.* (1955)
k	44 sec^{-1}	GIBSON *et al.* (1955)
$\alpha_{O_2}^{\text{Hb soln}}$	1.51×10^{-9} $\frac{\text{mole}}{\text{ml mm Hg}}$	SENDROY *et al.* (1934)
C_t	2×10^{-5} mole heme/ml (=32.25 g Hb/100 ml)	————

D_{O_2} has been measured in Hb solutions up to a concentration of 30 g/100 ml by KELLER and FRIEDLANDER (1966). The value obtained by extrapolating their results to 33 g/100 ml Hb concentration agrees well with the estimation of D_{O_2} in human RBCs by GROTE and THEWS (1962).

The diffusion coefficient of Hb has been measured in Hb solutions more concentrated than 30 g/100 ml by MOLL (1966).

The problems involved in treating the Hb–O_2 reaction as a single step reaction are discussed above. I chose $k' = 3.5 \times 10^9$ ml/(mole sec) and $k = 44$ sec^{-1} (GIBSON *et al.*, 1955).

All values chosen are intended to apply to solutions of human Hb at physiological temperature and pH.

Results and discussion

EFFECTS OF LAYER THICKNESS

The model was solved for layer thickness L=0.25, 0.5, 1, 1.6, 2, 3.6, 5, 10, and 20 μ.

TABLE 2

Effect of layer thickness on oxygen uptake by layers of hemoglobin solution.

Avg. saturation	time (msec)								
	0.25 μ	0.5 μ	1 μ	1.6 μ	2 μ	3.6 μ	5 μ	10 μ	20 μ
10%	0.28	0.47	0.90	1.38	1.68	2.76	3.64	7.08	20.99
20%	0.59	0.99	1.99	3.32	4.26	8.94	14.68	49.68	189.70
30%	0.94	1.56	3.28	5.86	7.90	19.48	34.72	129.51	508.77
40%	1.33	2.19	4.79	9.09	12.70	34.74	64.12	246.84	977.96
50%	1.79	2.91	6.53	13.04	18.82	54.32	101.88	398.04	1582.68
60%	2.35	3.76	8.56	17.75	26.08	77.72	147.00	578.40	2304.16
70%	3.10	4.81	11.01	23.37	34.72	105.22	199.88	789.36	3147.52
80%	4.25	6.32	14.20	30.46	45.48	138.82	264.24	1045.20	4169.77
90%	7.35	9.98	20.56	43.03	63.84	193.24	367.04	1449.27	6280.92

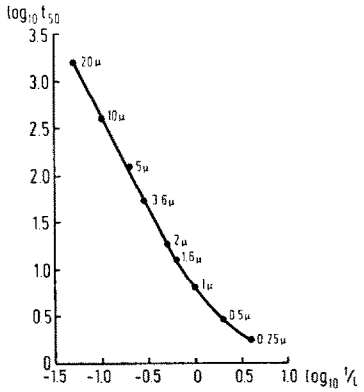

Fig. 2. The dependence of the time required for half-saturation (t_{50}) on layer thickness (L). $Log_{10}(t_{50})$ is plotted against $log_{10}(1/L)$. The figures next to the data points give the layer thicknesses.

The results are presented in table 2. Figure 2 is a graph of log t_{50} (t_{50} is the time required to reach 50% O_2 saturation) vs log (1/L). For L greater than 1.6 μ the graph is linear with slope -2, so that in this region t_{50} is proportional to L^2. This result might be expected if the diffusion of oxygen were the limiting process. For L less than 1.6 μ the graph is curvilinear and it is tempting to speculate that in this range of L diffusion of O_2 is so rapid that chemical reaction rates are important in determining the overall rate of O_2 uptake.

TABLE 3

The effects of changing D_{O_2} or k′ and k in 0.25 μ and 5 μ layers on their rates of O_2 uptake.

Avg. saturation	time (msec)					
	0.25 μ layer			5 μ layer		
	Values of table 1	20% incr. in D_{O_2}	20% incr. k′ and k	Values of table 1	20% incr. in D_{O_2}	20% incr. k′ and k
10%	0.28	0.27	0.25	3.64	3.36	3.28
20%	0.59	0.56	0.52	14.68	13.08	14.20
30%	0.94	0.90	0.82	34.72	30.72	34.20
40%	1.33	1.28	1.16	64.12	56.64	63.56
50%	1.79	1.72	1.56	101.88	90.08	101.36
60%	2.35	2.27	2.04	147.00	129.96	146.48
70%	3.10	3.00	2.68	199.88	176.36	199.32
80%	4.25	4.14	3.66	264.24	232.16	263.56
90%	7.35	7.21	6.28	367.04	320.00	366.00

To test these ideas the model was solved for L=0.25 and 5 μ with a 20% increase in either D_{O_2} or k′ and k. The results given in table 3 suggest that for the 0.25 μ layer reaction rates are more important than D_{O_2} and the reverse is true for the 5 μ layer. It should be noted that for both thicknesses changing the less important parameter has a significant effect on the t_{50}. In the 5 μ layer the reaction rates have an important effect effect on the initial rate of O_2 uptake: I expect that for layers thicker than 5 μ the primacy of diffusion coefficients becomes more clear cut and that for layers thinner than 0.25 μ the limiting effects of reaction rates are more definite.

THE EFFECT OF HbO_2 DIFFUSION

It has been shown that under some conditions O_2 diffuses at a faster rate through hemoglobin solutions than through comparable layers of plasma or Hb solution saturated with CO (see HEMMINGSEN, 1965 and WITTENBERG, 1966 for reviews). The facilitated diffusion of O_2 in Hb solutions has most often been attributed to HbO_2 diffusion. Facilitated steady-state O_2 diffusion through layers of intact human erythrocytes has been demonstrated by MOLL (1969) in 300 μ layers and by KUTCHAI and STAUB (1969) in 165 μ layers. The role of facilitated diffusion in the *uptake* of O_2 by hemoglobin solutions and RBCs has received relatively little attention.

TABLE 4

Effect of HbO_2 diffusion on O_2 uptake by layers of hemoglobin solution of various thicknesses.

Avg. saturation	% by which HbO_2 diffusion shortens time to reach indicated saturation								
	0.25 μ	0.5 μ	1 μ	1.6 μ	2 μ	3.6 μ	5 μ	10 μ	20 μ
10%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.2	31.3
20%	0.0	0.0	0.0	0.9	4.0	16.6	23.5	31.2	33.6
30%	0.0	0.0	2.9	9.6	14.3	26.1	29.8	33.0	33.9
40%	0.0	1.8	7.9	17.3	21.9	29.9	31.9	33.7	34.2
50%	0.0	3.0	12.8	22.9	26.6	32.2	33.6	34.7	35.0
60%	0.9	4.6	16.9	26.4	29.5	33.9	35.0	35.9	36.2
70%	0.6	6.0	19.5	28.0	30.6	34.3	35.2	36.0	36.2
80%	0.7	6.9	20.8	27.6	29.6	32.2	32.8	33.2	33.3
90%	0.8	6.9	19.3	24.3	25.7	27.5	27.9	28.3	22.2

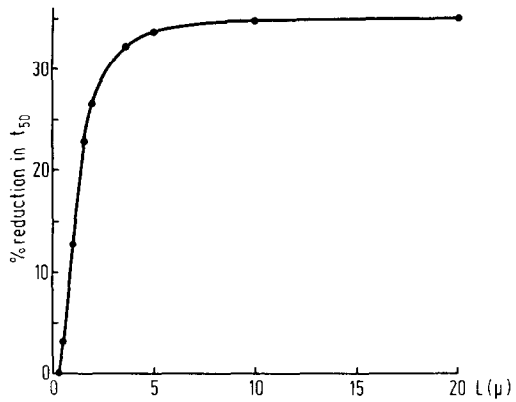

Fig. 3. The effect of HbO_2 diffusion on half-saturation time (t_{50}) as a function of layer thickness (L).

I have investigated the role of HbO_2 diffusion in O_2 uptake by comparing solutions of the model for which $D_{HbO_2}=0$ to solutions for which $D_{HbO_2}=4.5\times10^{-8}$ cm^2/sec. The results are shown in table 4 and fig. 3 shows the percent reduction in t_{50} attributable to HbO_2 diffusion. For $L=0.25\mu$ the effect of HbO_2 diffusion is small, the reduction in t_{50} being 0 and the reduction in t_{80} being 0.7%. The influence of HbO_2 diffusion rises with increasing L so that in the 5 μ layer the reduction in t_{50} is about 35%. I think this reflects the importance of reaction rates in the thinner layers and the primacy of diffusion processes in the thicker ones.

Similar computations were performed by MOLL (1968) for 1.6 and 3.6 μ layers and my results agree well with his in spite of the facts that he used a different numerical method and recomputed k during the course of the calculations in order to match the Hb-O_2 equilibrium curve of human blood. I believe the close agreement is due to k being a relatively unimportant determinant of the rate of O_2 uptake, an assertion supported in the next section.

The thickness dependence of facilitation of O_2 uptake may be compared to facilitation of steady-state O_2 diffusion which, according to the calculations of KUTCHAI, JACQUEZ and MATHER (1970), also increases with layer thickness and asymptotically approaches a theoretical maximum. As layer thickness is increased, the facilitation of O_2 uptake approaches its maximum value more quickly than does facilitation of steady-state O_2 diffusion. For layers of the same thickness, however, the percent by which the t_{50} is decreased by HbO_2 diffusion is less than the percent by which steady-state O_2 flux is increased due to HbO_2 diffusion.

THE RELATIVE IMPORTANCE OF D_{O_2}, D_{HbO_2}, k', AND k

Evidence has been presented that for thin layers the chemical reaction rates are the principal determinants of the O_2 uptake rate and the influence of diffusion parameters increases with layer thickness. Because the effective thickness of the human RBC is probably close to 1.6 μ I chose the 1.6 μ model to make a more detailed study of the relative importance of diffusion and reaction parameters. For this purpose the model was solved for 20% increases in D_{O_2}, D_{HbO_2}, k', and k, one at a time and k' and k together.

TABLE 5

The effects of diffusion and reaction parameters on oxygen uptake by 1.6 μ layers of 32.25 g/ml hemoglobin solution.

	time (msec)					
Avg. saturation	Values of table 1	20% incr. in D_{O_2}	20% incr. in D_{HbO_2}	20% incr. in k'	20% incr. in k' and k	20% incr. in k
10%	1.38	1.27	1.39	1.25	1.25	1.39
20%	3.32	3.01	3.31	3.04	3.06	3.34
30%	5.86	5.26	5.79	5.44	5.51	5.93
40%	9.09	8.11	8.90	8.55	8.67	9.23
50%	13.04	11.60	12.66	12.38	12.59	13.29
60%	17.75	15.75	17.14	16.94	17.27	18.14
70%	23.37	20.70	22.49	22.35	22.84	23.98
80%	30.46	26.93	29.30	29.00	29.83	31.54
90%	43.03	38.02	41.58	39.31	42.03	48.88

The results are given in table 5. For the initial rate of O_2 uptake as indicated by t_{10} the parameters, listed in order of decreasing importance, are k', D_{O_2}, D_{HbO_2}, and k. As the oxygenation process proceeds D_{O_2} becomes the most important parameter, k' taking second place. This probably reflects the fact that in the early stages of O_2 uptake the principal event is the reaction of Hb in the outer parts of the layer with O_2, but that later the diffusion of O_2 into the center of the layer is more important. In interpreting the effects of changing k' or k alone it should be kept in mind that the affinity of Hb for O_2 has been changed (this is important especially in the later stages of oxygenation when chemical equilibrium is approached).

It should be noted that for 1.6 μ layers of Hb solution none of the parameters is without significant influence on the rate of O_2 uptake.

COMPARISON OF O_2 UPTAKE BY LAYERS OF Hb SOLUTION AND RBCs

The uptake of O_2 by the RBC is a relatively simple biological transport process, yet it is not completely understood. Some experiments suggest that the O_2 diffusion resistance of the RBC membrane is insignificant and that the mobility of O_2 and Hb is the same in RBCs as in concentrated Hb solutions. However, calculations which assume the RBCs to be a bag of concentrated Hb solution surrounded by a membrane of infinite O_2 permeability invariably overestimate the rate of O_2 uptake that is measured in a rapid reaction apparatus. I would now like to consider possible explanations of this discrepancy.

Theoretical calculation can be brought into agreement with experimental observations by assuming a sufficiently small O_2 permeability for the RBC membrane (ROUGHTON, 1959; FORSTER, 1964; MOLL, 1968).

KREUZER and YAHR (1960) spectrophotometrically measured the rate of O_2 uptake by 100–227 μ layers of packed RBCs and concentrated Hb solutions. They found no significant differences in O_2 uptake rates between the cells and the solutions. KUTCHAI and STAUB (1969) studied steady-state O_2 diffusion through 165 μ layers of packed RBCs and concentrated Hb solutions and found no difference in the O_2 permeability or the extent of facilitated O_2 transport in the two preparations. These experiments suggest that the resistance to O_2 diffusion due to the RBC membrane is negligible in comparison to that of the cell interior.

GROTE and THEWS (1962) extimated D_{O_2} in RBCs to be 0.8×10^{-5} cm^2/sec, which is quite close to the values obtained in concentrated Hb solutions by KELLER and FRIEDLANDER (1966). The results of KUTCHAI and STAUB support the conclusion that D_{O_2} in RBCs is similar to that in concentrated Hb solutions and also suggest that D_{HbO_2} in RBCs is similar to that in concentrated Hb solutions. Thus differences in D_{O_2} and D_{HbO_2} probably cannot explain the discrepancy in O_2 uptake rate between theory and experiment.

We must also consider the possibility that the rate of chemical reaction of Hb with O_2 is different in RBCs than in dilute solutions of Hb. (For technical reasons k′ and k have been determined only in dilute Hb solutions.) In order to fit my model to the experimentally observed initial rate of O_2 uptake by deoxygenated RBC suspensions k′ must be lowered to about 2×10^8 ml/(mole.sec), which is 17 times smaller than the value in dilute Hb solutions.

It has been shown that organic phosphates present in the RBC, especially 2,3-diphosphoglycerate, have a dramatic effect on the Hb-O_2 equilibrium curve (see review by BENESCH and BENESCH, 1969) and hence must effect k′, k, or both. Studies of the kinetic consequences of 2,3-disphosphoglycerate have not yet been reported. The discrepancy between observed and calculated rates of O_2 uptake by RBCs may be due to the effects of intracellular organic phosphates on the rate constants. However, the rates of CO and NO uptake by RBCs are also considerably less than theoreti-

cally predicted (see FORSTER, 1964) and it seems unlikely that the rates of reaction of Hb with O_2, CO, and NO should be affected to approximately the same extent by organic phosphates.

The possibility of an unstirred layer around the RBC in the rapid reaction apparatus has been considered by KOYAMA and MOCHIZUKI (1969). They studied the kinetics of O_2 uptake by RBCs at three different flow velocities and found an increase in the rate of O_2 uptake with increasing flow velocity.

Another possible explanation for the difference between theory and observation concerns the shape of the RBC in the rapid reaction apparatus. RBCs are known to be deformed in the flow in arterioles (BLOCH, 1962), thus they may be deformed in the rapid reaction apparatus.

CARLSEN and COMROE (1958) found the same rates of CO and NO uptake by human RBCs of normal discoidal shape and those made spherical by heating briefly to 49 °C. Since the biconcave disc should theoretically take up gases by diffusion much faster than the sphere, it was concluded that intracellular diffusion is not important in determining the rate at which RBCs take up gases.

The results of CARLSEN and COMROE might be explained by shape changes of the RBCs in the rapid reaction apparatus. If both discoidal and spherical forms were continually being deformed in the turbulent stream, the 'average' shape might lie between the disc and the sphere. This might explain the results of CARLSEN and COMROE as well as the discrepancy between observed and predicted rates of O_2 uptake by RBCs.

I conclude that we still cannot explain why RBCs take up gases considerably slower than theory predicts. An artefact of the rapid reaction apparatus may be involved or there may be some aspect of the gas uptake process that is poorly understood.

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