HYDRODYNAMIC AND DIFFUSION CONSIDERATIONS OF RAPID-MIX EXPERIMENTS WITH RED BLOOD CELLS

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ABSTRACT From studies of the oxygenation rate of red blood cells (RBC) using rapid-mix techniques, it has been suggeted that RBC are surrounded by a stagnant layer of water that does not (or cannot) mix with the rest of the water. A consideration of the appropriate hydrodynamics and convective diffusion rates shows that a mixer can reduce the resolution time to ~1 ms (or possibly less) and give a diffusion layer around the RBC that is ~1 μm thick. In stopped flow equipment it expands to ~4 μm over ~10 ms, whereas in continuous flow work the diffusion layers expands slightly less rapidly and less far. Thus the rate of oxygenation of RBC should be slower when measured by stopped flow techniques than by continuous flow apparatus for which the rate will depend weakly on the Reynolds number of the flow in the interrogation tube.

1. INTRODUCTION

Many measurements of the rate of oxygenation of hemoglobin (Hb) solutions have been made. In dilute stirred solutions, the characteristic time is $\lesssim 1$ ms (1–4). By contrast, similar measurements on dilute suspensions of red blood cells (RBC) have shown that the time scale of oxygenation is $\gtrsim 10$ ms (1–4). Since these early studies of Hartridge and Roughton, many contributions to the unravelling of complexities of RBC have been made. Notable articles by Roughton (4), Kutchai (5, 6), and Coin and Olson[1] have developed the theoretical and experimental aspects. Yet the emphasis of studies has changed over the last 10 yr from the physicochemical parameters of RBC to the facility of oxygen transport to the cell membrane. This reflects the growing awareness that the rate-limiting step of oxygenation of RBC is extracellular oxygen transport. To the author's knowledge there have been no attempts to discuss the hydrodynamics appropriate to the rapid mixing of a cellular suspension with a solution of gas in liquid. Recently Milgram (7) considered the mixing of two solutions containing molecular reactants.

Recently Gad-El-Hak et al. (8), Sha'afi et al. (9), and Coin and Olson[1] have drawn attention to the likelihood that RBC are surrounded by a "stagnant" layer of water that does not mix with the oxygenated solution after mixing in stopped flow experiments. Gad-El-Hak et al. measured the eddy length scale of water flowing in 4-mm-diam tubes containing either 500-nm polystyrene spheres or RBC. The eddy scales were sufficiently large that they suggested the discrepancy between the observed rate of RBC oxygenation and the (faster)

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[1] Coin, J. T., and J. Olson. Work accepted for publication.

rate calculated by one (or three) dimensional Roughton models could be reconciled if the cell was surrounded by a "stagnant" layer of water 1.4 μm thick. Sha'afi et al. measured the induction time of dilation of RBC when they were rapidly mixed with an hypertonic solution, and obtained a stagnant layer thickness of 5.5 μm. Coin and Olson measured the rate of RBC oxygenation and then observed that a one dimensional Roughton model would mimic the experimental data if the stagnant layer was initially absent, and increased to $\sim$1.6 μm after 5 ms and to $\sim$5 μm after 300 ms.

It is the purpose of this article to consider some of the more important aspects of the hydrodynamics of oxygen transport into RBC when studied by rapid-mix techniques. To actually model this problem even in laminar flow regions is a matter of great complexity, involving solution of two partial differential equations (Navier-Stokes and convective diffusion) in three space dimensions and in time, with very awkward boundary conditions. For turbulent mixing, even stopped flow experiments will be difficult to model, and continuous flow experiments yet more difficult, since there are no satisfactory theories of turbulent flow for mixing. However, unless some effort is made to study oxygen mass transport to a RBC, experiments on the rate of RBC oxygenation can yield only limited information on the physiological properties of RBC in oxygen transport. Though previous studies have been made of stagnant water layers around RBC (8–10),[1,2] these have been phenomenological, and have no a priori justification or predictive ability.

2. ROUGHTON'S MODEL: ITS EXTENSIONS AND MODIFICATIONS

Following the pioneering work of Hartridge and Roughton (1–3) in measuring rates of oxygenation of RBC in suspension, Roughton and co-workers (4, 11–13) have developed simple but descriptive accounts of the transport of oxygen from the solution surrounding RBC through the membrane into the cell interior and reacting with hemoglobin. There are many assumptions in this picture and many have been probed. The most important assumptions are discussed below.

(*a*) Roughton approximated the biconcave discoid shape of the normal RBC to a semi-infinite layer. Other authors have extended these calculations to squat discs and spheres (5, 6).[3] Although a semi-infinite layer model is simple, it is inappropriate. Not only can it not be used to discuss the hydrodynamic flow of water arond RBC (even the flow of fluid about an infinite cylinder and about a sphere require totally different treatments and lead to significantly different results), but also convective diffusion is grossly miscalculated. Even if there is no fluid motion after mixing ceases, the nature of particle diffusion into a sink in one or two dimensional space is totally different from that in three dimensions (consider, for instance, the Pólya problem [14]). If, as is usual, RBC are treated as one dimensional, the concentration gradient of oxygen outside the cell tends to zero with time. The situation is saved by the ad hoc imposition of a boundary at which the oxygen concentration is maintained constant. This leads to the stagnant layer. In three dimensions no such approach is necessary, since the concentration gradient remains nonzero even at infinite time. This is the basis of

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[2] Weingarden, M., H. Mizukami, and S. A. Rice. Unpublished work.

[3] Mochizuki, M. Unpublished work.

Smoluchowski's (15) celebrated analysis of colloidal growth, which has been extended to diffusion-controlled molecular reactions (16).

(*b*) $Hb(O_2)_n$ is formed, $n = 1, 2, 3, 4$, with a single association and dissociation rate constant:

$$Hb(O_2)_{n-1} + O_2 \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} Hb(O_2)_n. \tag{1}$$

Since it is considered that the equilibrium constants, $K_n = k_n/k_{-n}$, increases with n (17, 18), this assumption cannot be entirely adequate. It is probably reasonable if operational rate constants for association and dissociation are measured from experiment and used in calculations. To relax this assumption measured values of k_n and k_{-n} will be required (19).

(*c*) The dissociation rates are slow compared to association rates. If oxygenation continues to at least 50% saturation, then the forward and backward rates in (Eq. 1) will be comparable if $K_n \sim 1/[O_2] \sim 10^4\ M^{-1}$. From Winslow et al. (17) $K_1 \sim 10^4\ M^{-1}$, and $K_{2,3,4} \gtrsim 10^5\ M^{-1}$. Thus, except nearer to the membrane, dissociation of $Hb(O_2)_n$ may be disregarded for oxygen saturation <50%. Both Nicolson and Roughton (20) and Kutchai (5) have incorporated oxyhemoglobin dissociation in their numerical modeling, and reported small effects at low saturation, but above 50% saturation it is an essential factor.

(*d*) Neither deoxy-hemoglobin nor oxy-hemoglobin undergo significant diffusion during oxygenation. Were these molecules to move more than 0.5 μm during the 100 ms or so that it takes for oxygenation of RBC to occur, this assumption would be unjustified. That requires D_{Hb} (and D_{HbO_2} should be almost identical) $< (0.5 \times 10^{-6})^2/2(0.1) \sim 10^{-12}\ m^2\ s^{-1}$. However, $D_{Hb} \sim 10^{-11}\ m^2\ s^{-1}$. (21) Again, Kutchai (5, 6) has observed that the diffusion of hemoglobin partially offsets the reduction in rate of oxygenation due to the depletion of hemoglobin by reaction with oxygen.

(*e*) During oxygenation there is little alteration to the deoxy-hemoglobin concentration. This assumption, like those in (*b*), (*c*), and (*d*) above can only be avoided if numerical studies are made. Again, Nicolson and Roughton (20) and Kutchai (5, 6) have found that the rate of oxygenation is reduced at high saturation due to the reduction in hemoglobin concentration.

(*f*) Throughout the cell interior, hemoglobin is uniformly distributed, and the cell membrane is considered uniform and structureless.

(*g*) No account is taken of the different solubility of oxygen in the cell membrane, cell interior, or surrounding water. This can be easily incorporated (4), and Weingarden et al.[2] found that the probable greater solubility (by a factor of ~4) of oxygen in the membrane than in the aqueous phases enhanced the rate of oxygenation, because it reduced the oxygen gradient in the membrane, and so increased the oxygen concentration gradient in the stagnant layer.

(*h*) Oxygen dissolved in water is present at the cell membrane's exterior immediately after mixing the RBC with oxygenated isotonic solution. Furthermore the transport of oxygen in the oxygenated solution was believed to be much faster than across the membrane or cell interior, at least initially (22). This assumption is related to (*a*). It will be seen later that even were mixing complete, with oxygen present up to the surface of the RBC, such a situation would not remain for long. Oxygen would be depleted near the RBC, and the subsidence of eddy currents after mixing will accentuate this formation of a diffusion layer around a RBC.

(*i*) Transient effects arising from the time taken to diffuse across the membrane are ignored, so that oxygenation occurs in dynamic equilibrium between (convective) diffusion and reaction (Eq. 1). Although these effects are small,[2] they are nevertheless important at short times and explain the results of Coin and Olson.[1]

Of these nine points only (*f*) cannot be assessed. It is difficult to improve on (*b*), but all the rest have been considered or are considered in this article. In particular (*a*), (*h*), and (*i*) are especially sensitive to parameters at short times, but crucial at all times for a deeper understanding of rapid-mix experiments.

3. COMMENTS ON STOPPED-FLOW AND CONTINUOUS-FLOW TECHNIQUES

Many of the studies on RBC and hemoglobin solutions have been made using stopped flow or continuous flow techniques. Berger (23) has discussed many of the latest developments in these techniques. To briefly describe the equipment is worthwhile at this juncture. The two reactant solutions (or suspensions) are contained in syringes and are simultaneously rapidly expelled into a mixing chamber. In stopped-flow the mixture flows into a collection tube and abruptly the flow is stopped. Continuous-flow allows the mixture to continue flowing out of the mixing chamber and down an interrogation tube. To monitor chemical reactions, optical absorbance of reactants or products are measured. In stopped flow this is at a fixed point close to the mixing chamber and optical density changes with times are followed, whereas in continuous flow techniques the time after mixing is varied by measuring the absorption at several points down the exit tube and knowing the mean flow rate in this tube.

Because RBC are a little larger than the wavelength of light, they scatter light very effectively. Consequently, studies of RBC oxygenation rates are affected by this scattered light, and during the turbulence subsidence the degree of scattering changes. Coin and Olson[1] described a dual wavelength spectrophotometer to minimize such interference.

Miyamto and Moll (24) have shown that no change to the shape of RBC occurs in flow up to a Reynolds number, ~500. However, Coin and Olson found that RBC were ruptured at too high a mixing rate (Reynolds number not discussed).

4. MIXING OF RBC WITH OXYGENATED SOLUTION

Laminar and Turbulent Flow

As the velocity with which a fluid moves increases relative to the walls of a tube or some other part of the fluid, the even, continuous (laminar) flow ceases to be so regular. It undergoes a transition to turbulent flow, characterized by eddies of various sizes. The Reynolds number,

$$\mathrm{Re} = uL/\nu, \tag{2}$$

characterizes this transition, with u the mean flow velocity, L a characteristic distance of the scale of the tube or flowing liquid, and ν the kinematic viscosity (for water $\nu \sim 10^{-6}\ \mathrm{m^2 s^{-1}}$). In pipe flow below Re ~ 2000, flow is laminar, and above Re ~ 3000, it is usually turbulent, between these regions it is transitional (25, 26).

Turbulent motion is a state of high energy, the energy being distributed mainly in the larger eddies of (approximately) size ℓ (the macroscale $\ell \sim L/2$[8]). The smaller the eddies, the less energy they store (8, 25, 27, 28); for eddies of scale λ_0 (the microscale) and velocity v_0,

$$\mathrm{Re}_{\lambda_0} \sim \frac{v_0 \lambda_0}{\nu} \sim 1 \tag{3}$$

will be strongly damped. On the length scale, λ_0, inertial momentum transfer is less effective than viscous momentum transfer. Consequently, energy from the largest eddies is transferred to the smaller eddies (via intermediate size eddies) which are viscous damped. Finally one other Reynolds number is required, that of the largest eddies,

$$\mathrm{Re}_m \sim \frac{\Delta u \cdot \ell}{\nu} \gtrsim 0.01\ \mathrm{Re}, \tag{4}$$

where Δu is the mean fluctuation of velocity in the flow (~2–10% of u), and the maximum size of an eddy is $\ell \sim L/2$ (8).

Gad-El-Hak et al. (8) have measured the macroscopic and Kolmogoroff microscopic turbulence length scale of RBC suspensions in 4-mm bore tubes as a function of Reynolds number, and found λ_0 decreases from ~70 to 50 μm as Re increases from 3,000 to 8,000. The characteristic velocity of an eddy of scale λ is:

$$v_\lambda \sim (\epsilon\lambda)^{1/3} \sim (\Delta u^2 \lambda t^{-1})^{1/3}, \tag{5}$$

where the rate of dissipation of energy per unit mass ϵ is $\sim \Delta u^2/t$, and ϵ is independent of λ. Hence $v_\lambda \sim \Delta u(\lambda/\ell)^{1/3}$. Combining this with (Eq. 3) shows (25, 26):

$$\lambda_0 \sim 10\ell/\mathrm{Re}^{3/4} \tag{6}$$

However, an alternative suggestion (27, 28) for $\epsilon \sim 15\nu(\Delta u^2/\ell^2)$ can be used with Eqs. 3 and 5:

$$\lambda_0 \sim \ell/2\mathrm{Re}_m^{1/2} \sim 5L/2\mathrm{Re}^{1/2}. \tag{7}$$

Both forms of λ_0 show stronger dependence on Re than observed experimentally (8); with $\ell \sim$ 4 mm and Re ~ 4,000 mm, Eq. 6 gives $\lambda_0 \sim 50$ μm and Eq. 7 gives $\lambda_0 \sim 100$ μm.

Mixing Times

To initiate the reaction of interest, the two reactants are mixed at very high Reynolds numbers, by using small orifices and high velocities. The experimental resolution time is such that diffusion occurs to a significant degree over the scale λ_0 (7). Since the diffusion distance is δ,

$$\delta \sim (Dt)^{1/2} \sim \lambda_0 \sim \nu^{3/4} \Delta u^{-1/2} t^{1/4},$$

then the mixing time,

$$\tau_m \sim \nu^3/D^2\Delta u^2. \tag{8}$$

With well-designed mixers Δu may approach u. Taking $u \sim 100\ \mathrm{ms}^{-1}$, and $D \sim 2.10^{-9}\ \mathrm{m}^2\,\mathrm{s}^{-1}$, gives $\tau_m \gtrsim 25$ μs; though a figure of 10 times this is more probable for τ_m.

Such an analysis is appropriate for molecular reactants, since the solutions may be regarded as homogeneous. When considering the oxygenation of RBC, it is important to compare the Kolmogoroff microscope, λ_0, of turbulence with the RBC radius, R. If $\lambda_0 > R$, the RBC is surrounded by a quasilaminar region, whereas if $\lambda_0 < R$, the eddies penetrate, to varying degrees, the viscous sublayer around the RBC. The viscous sublayer is caused by the necessary continuity of normal and tangential velocity components of the RBC and surrounding liquid at the RBC surface (stick boundary conditions).

There are two forms of convective mixing when $\lambda_0 > R$. Levich (26) pointed out that the energy flux from large to small eddies dictates that the smallest eddies are continually being formed and decaying. Local regions of the fluid are constantly being buffeted. The characteristic acceleration of $f_{\lambda_0} \sim \nu^2/\lambda_0^3$. Since the density (29) of RBC and of solution (serum) are $\rho \sim 1.10$ kg dm^{-3} and $\rho_0 \sim 1.03$ kg dm^{-3}, a differential acceleration of RBC and serum occurs. Levich pointed out that the current of oxygen into a RBC was of the form:

$$I = DA\frac{\partial c}{\partial x} \sim DA\frac{C_o}{\delta}, \tag{9}$$

where A is the RBC surface area, C_o is the bulk oxygen concentration, and δ is the diffusion-layer thickness. For spherical particles of radius R, Levich suggests

$$\delta \sim \left(\frac{9}{2}\right)^{1/3}\frac{\pi}{2}\left(\frac{\rho}{\rho - \rho_0}\right)^{1/3} Pr^{-1/3}\lambda_0, \tag{10}$$

where $Pr = \nu/D \sim 10^3$ is the Prandtl number and measures the ratio of momentum:mass transfer. Typically, $\delta \sim 0.5\ \lambda_0 \sim 25\ \mu$m, for Re $\sim$ 5,000, and the diffusion layer is at least $\sim$10 μm in thickness. Since 25 μm corresponds to a diffusion time $\tau_d \sim \delta^2/2D \sim 300$ ms, such a mode of convective mixing is ineffective, especially when compared to the thickness of the diffusion layer in the absence of convection ($\sim$4 μm, see section 6).

The other mode of convective mixing for $\lambda_0 > R$ is when the RBC is surrounded by liquid that is undergoing a shearing stress, shown schematically in Fig. 1. The RBC plane is in x-z plane, and the y axis is the cylindrical axis. At $y = 0$ the liquid and the RBC are at rest relative to one another. However, a y increases or decreases, the liquid velocity tends to $v_z = (\partial v_z/\delta y)y$, regardless of x and z, and the shear velocity gradient $(\partial v_z/\partial y)$ is constant far from the RBC. In Fig. 1 the x-axis is out of plane. Streamlines are shown for the Reynolds number of particle flow, Re $\sim$ 0.1 and $(\partial v_z/\partial y) \sim 10^3$ s^{-1}. In general, if the liquid velocity is v, the convective diffusion equation is:

$$\frac{\partial c}{\partial t} = \nabla[D\nabla c - vc], \tag{11}$$

where c is the oxygen concentration gradient. In the steady state, $\partial c/\partial t = 0$. Providing the velocity is known, the oxygen concentration can be evaluated, and hence the thickness of the diffusion layer. An estimate of δ may be made by expanding Eq. 11 and recalling $\nabla \cdot v = 0$ (that is, the liquid is incompressible):

$$D\left\{\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right\} = v_x\frac{\partial c}{\partial x} + v_y\frac{\partial c}{\partial y} + v_z\frac{\partial c}{\partial z}.$$

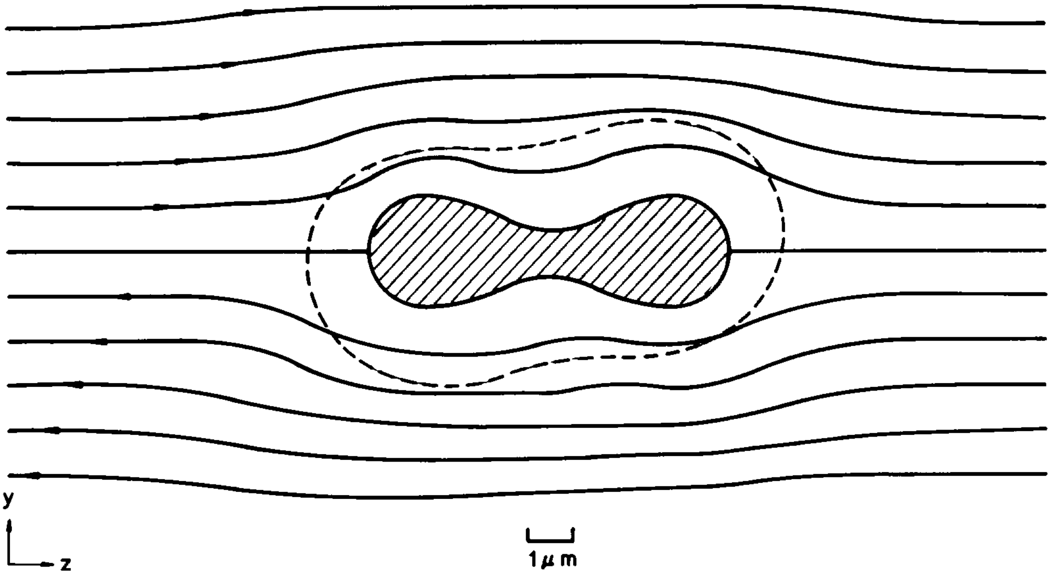

FIGURE 1 Schematic diagram of a RBC in a shear velocity gradient. The z axis is horizontal, y axis vertical, and x axis out of plane, so that the RBC plane lies in the $y = 0$ plane. Approximate streamlines are shown (relative to the $y = 0$ plane), for Re ~ 0.1 and $(\partial v_z/\partial y) \sim 10^3\ \mathrm{s}^{-1}$. The dotted line is an approximate diffusion-layer boundary.

At a point a diffusion layer above the RBC surface, convection and molecular diffusion are of equal facility. Estimates for the magnitude of these derivatives are:

$$\frac{\partial c}{\partial x} \sim \frac{\partial c}{\partial y} \sim \frac{C_o}{\delta},\ \frac{\partial c}{\partial z} \sim \frac{C_o}{R};\ \frac{\partial^2 c}{\partial x^2} \sim \frac{\partial^2 c}{\partial y^2} \sim \frac{C_o}{\delta^2},\ \frac{\partial^2 c}{\partial z^2} \sim \frac{C_o}{R^2};\ v_x \sim v_y \sim \left(\frac{\partial v_z}{\partial y}\right)\delta^2/R$$

and

$$v_z \sim \left(\frac{\partial v_z}{\partial y}\right)\delta.\ \text{Hence}\ DC_o/\delta^2 \sim \left(\frac{\partial v_z}{\partial y}\right)\delta^2/R \cdot C_o/\delta$$

and

$$\delta \sim \left[DR\Big/\left(\frac{\partial v_z}{\partial y}\right)\right]^{1/3} \qquad (12)$$

(unless the shear velocity gradient is $<10^2\ \mathrm{s}^{-1}$, and then convective mixing becomes relatively inefficient, with $\delta \gtrsim 4\ \mu\mathrm{m}$). The expression (Eq. 12) for the diffusion layer thickness may be obtained in a different manner. To diffuse through the diffusion layer takes a time $\sim\delta^2/D$. During this time, liquid a distance δ from the RBC surface may travel a distance $\sim(\partial v_z/\partial y)\delta \cdot \delta^2/D$. If this distance is comparable to the radius of a RBC, diffusive and convective motions are of approximately equal importance; that is, depletion of oxygen concentration a distance δ from a RBC surface at the leading edge by diffusion is similar to the rate at which this liquid sweeps the oxygen across the RBC surface. Setting $(\partial v_z/\partial y)\delta^3/D \sim R$ again yields Eq. 12. This estimate may be a little too small in turbulent flow, because not all RBC are entrained in eddies of scale λ_0, where the shear velocity gradients $(\partial v_z/\partial y) \sim (v_{\lambda_0}/\lambda_0) \sim \nu/\lambda_0^2$ are greatest.

Inserting this estimate for $(\partial v_z/\partial y)$ into Eq. 12 gives:

$$\delta \sim (\lambda_0^2 R)^{1/3} Pr^{-1/3}. \tag{13}$$

With $\lambda_0 \sim 50\ \mu\text{m}$, $\delta \sim 3\ \mu\text{m}$ or for $\lambda_0 \sim 20\ \mu\text{m}$, $\delta \sim 1.5\ \mu\text{m}$. Such diffusion layer thicknesses have associated diffusion times of ~20 and 5 ms. These are still larger than the desired resolution time of $\lesssim 1$ ms. It is necessary to conclude that to obtain such a resolution, $\lambda_0 \lesssim R$, for which neither of the estimated diffusion-layer thicknesses (Eq. 10 or 13) is appropriate, since the flow near the RBC is far from laminar.

Levich (26) has also discussed the case of $\lambda_0 \lesssim R$. He pointed out that around the RBC a layer of liquid is entrained (viscous sublayer) into which eddies, of scale λ_0, move but which are efficiently damped. These eddies carry oxygen with them and the exchange of oxygenated and deoxygenated liquid is efficient because oxygenated liquids is brought to rest by viscous damping within the viscous sublayer and so near the RBC surface. Since $\lambda_0 \lesssim R$, the RBC may be approximated to a plane surface, and the viscous sublayer depth is $\delta_0 \sim \lambda_0$, and the diffusion layer has thickness (26):

$$\delta \sim \lambda_0 Pr^{-1/3} \lesssim R\, Pr^{-1/3}. \tag{14}$$

Since $Pr \sim 10^3$, $\delta \lesssim 0.5\ \mu\text{m}$. $\tau_d \sim 0.1$ ms, while the microscale eddy time $\tau_{\lambda_0} \sim \lambda_0^2/\nu \sim 10\ \mu\text{s}$, hence the resolution time is determined by the diffusion time. Mixing is rapid and efficient if the microscale can be reduced to $\lambda_0 \sim R$, which may require $\text{Re} \sim 10^5$ utilizing turbulence creating devices to increase Δu to nearly u. However, mixing with cellular reactants cannot be as efficient as using molecular reactants.

5. DECAY OF TURBULENCE AFTER MIXING

To reduce the resolution time of rapid mix apparatus beneath 1 ms requires the length microscale in the mixer to be $\lesssim 10\ \mu\text{m}$, and needs high Reynolds number. On leaving the mixer this highly turbulent fluid passes into the observation tube. In stopped-flow equipment the mean flow is abruptly halted by a stop syringe, whereas in continuous-flow the mixture passes down an observation tube at 1–10 ms^{-1}. The eddies die out with time and the flow ultimately reverts to laminar with small Reynolds number.

Kolmogorov showed that at short times the largest eddy scale increases with time as $t^{2/7}$ and the velocity decreases as $t^{-5/7}$ (hence, $\text{Re}_m \propto t^{-3/7}$), but at long times all eddies increase in scale as $t^{1/2}$ and velocity decreases at $t^{-5/4}$ (due to the Navier-Stokes equation reducing to a diffusion equation) (25, 27). To estimate the short-term behavior of the microscale λ_0, using Eq. 5 with $\epsilon \sim v_\ell^2/t$, where v_ℓ is the macroscale eddy velocity (the majority of the energy is in large eddies) shows $\lambda_0 \sim (\nu^3 t v_\ell^{-2})^{1/4}$, *but* $v_\ell \propto t^{-5/7}$, and so:

$$\lambda_0 \propto t^{17/28}. \tag{15}$$

The microscale expands more rapidly than the macroscale. When they are nearly the same scale they lose their distinguishability and increase as $t^{1/2}$.

Now consider a stopped-flow experiment where the resolution time is 1 ms. This implies a diffusion layer ~1 μm, and a microscale of 10 μm as discussed above, and a microscale time $\sim\lambda_0^2/\nu \sim 100\ \mu\text{s}$. If the flow in a stopped-flow apparatus can be terminated within 1 ms, the eddies will decay such that after 10 ms, the microscale is ~60 μm. From (Eq. 13) the diffusion layer $\delta \sim 3$ μm, whereas that based on differential acceleration (Eq. 10) is much larger. In section 6, it is shown that even in the absence of convection the diffusion layer attains a thickness ~4 μm after ~10 ms, and so convection is relatively unimportant after ~10 ms. Although large Reynolds numbers are essential to obtain the efficient and rapid mixing required to give short resolution times, even 10 ms after cessation of mixing turbulence has decayed so much that it has little effect on molecular oxygen diffusion to RBC.

In an analogous continuous-flow, the eddies again subside after mixing, but a little less rapidly than in stopped-flow equipment, because further mixing occurs along the interrogation tube. If Re ~ 5,000 in the interrogation tube, $\lambda_0 \sim 50$ μm, and the diffusion layer (Eq. 13) in $\delta \sim 3$ μm. This mixing continues for the whole course of RBC oxygenation; the diffusion layer being comparable to or slightly less than that for no convective mixing (section 6) of $\delta \sim 4$ μm. Consequently the rate of oxygenation of RBC should be weakly dependent on the Reynolds number flow in the interrogation tube, and should be faster in continuous-flow than in stopped-flow.

6. RBC OXYGENATION WITHOUT CONVECTION

So far we have discussed the oxygenation of RBC by simultaneous convection and molecular diffusion of oxygen. In the mixer, the oxygen approaches to within a few micrometers of the RBC. The decay of the eddies after the mixing necessarily reduces the importance of convective diffusion, yet as was remarked in section 2, molecular diffusion still continues. To estimate the efficacy of molecular diffusion of oxygen to the RBC surface, Eq. 11 must be solved with $v = 0$, and the boundary conditions:

$$C(\mathrm{r} \rightarrow \infty, t) \qquad \rightarrow C_o \tag{16a}$$

$$C(\mathrm{r} \rightarrow \text{surface}, t) \rightarrow \quad 0 \text{ for } t \lesssim 10 \text{ ms.} \tag{16b}$$

That is there is an infinite source of oxygen at infinity (Eq. 16*a*) and at early times the RBC surface is an almost perfect sink (Eq. 15*b*). The initial condition is:

$$c(\mathrm{r} > \text{surface}, 0) \rightarrow C_o \tag{16c}$$

for complete mixing. Although this boundary value problem has not been solved for a RBC shape, for spheres of radius R the solution has been known for a long time. Smoluchowski (15) showed that:

$$C(r, t) = C_o\left[1 - \frac{R}{r}\, erfc\left(\frac{r - R}{(4Dt)^{1/2}}\right)\right],$$

and that the current of oxygen into the RBC is

$$I = 4\pi R^2 D \cdot \frac{C_o}{R}\,[1 + R(\pi Dt)^{-1/2}].$$

By comparison with Eq. 9, the diffusion layer has a thickness:

$$\delta \sim R[1 + R(\pi DT)^{-1/2}]^{-1}. \tag{17}$$

A similar expression might be expected for a RBC with suitably scaled long and short time values of δ. Taking $R \sim 4\ \mu m$, $D \sim 2.10^{-9}\ m^2\ s^{-1}$, at $t \sim 1$ ms indicates $\delta \sim 1.4\ \mu m$, and as $t \longrightarrow \infty$, $\delta \longrightarrow 4\ \mu m$. The time scale over which the diffusion layer expands appreciably is $t_{ss} \sim R^2/\pi D \sim 3$ ms, a time very similar to the time scale over which the turbulence eddies increase in scale and reduce the efficacy of convection mixing. To make allowance for the nonsphericity of RBC, the diffusion layer will be thicker than for a sphere (by ~20%) because of less effective penetration by eddies and the obvious geometric factor, and the time dependence in Eq. 16 will be more pronounced (again by ~20%). Hence t_{ss} may be nearer 5 ms. (At short times, little aggregation has occurred and the oxygen-diffusion current is, approximately, proportional to surface area, which for a RBC is ~20% greater than that of an equivolume sphere. The estimates of diffusion-layer thickness and time development are based on a comparison of one dimensional and spherically symmetric sink problems and on intuition. Certainly deviations of the RBC oxygenation rates from that of an equivolume sphere should be in the range of 10–50%.)

The previous discussion of convective mixing (sections 4 and 5) has tacitly assumed that convective diffusion is much faster than molecular diffusion. Providing oxygen and RBC are mixed sufficiently well that the initial condition (Eq. 16*c*), $c(\mathrm{r} > \text{surface}, t) = C_o$, is a good approximation, convective mixing is important if the diffusion layer (Eq. 10, 13, 14) is comparable to or less than that for molecular diffusion (Eq. 17).

As oxygenation proceeds for longer than ~10 ms, the surface boundary condition (Eq. 16*b*) is no longer satisfactory. Concentration gradients decrease with time, and the diffusion layer expands. The rate-limiting step in the oxygen transport changes from transport in the liquid up to the cell membrane to transport within the RBC. The importance of the points discussed above diminishes with this change.

7. COMPARISON WITH EXPERIMENT

To summarize the expected rate of oxygenation, mixing may be completed within ~1 ms (possibly a little faster in the best designed mixer and stopping syringe), and the diffusion layer will be ~1–2 μm thick (see Mixing Times in section 4). Over the next 10 ms or so, the eddies will decay and reduce the efficiency of convective mixing (section 5). Furthermore, the oxygen concentration in the immediate vicinity of RBC is depleted by diffusion into the RBC, and so the oxygen concentration gradient relaxes to a steady state. After ~10 ms molecular diffusion of oxygen occurs through an effective diffusion layer ~4 μm thick, and convection is relatively less important (section 6).

From experimental studies the importance of a stagnant layer of water around the RBC has been inferred and suggested to be ~5.5 μm by Sha'afi et al. (9) (which may be too large due to insufficient time response and slow mixing) and ~1.4 μm by Gad-El-Hak et al. (8). Coin and Olson[1] measured the rate of RBC oxygenation in stopped-flow equipment, and compared it to calculations based on the Roughton model (section 2), which incorporates a layer of water on either side of the (one dimensional) cell. By allowing this layer to expand from zero at zero time, to ~1.6 μm at 10 ms and ~5 μm at 300 ms good agreement between experiment and the model calculation was obtained. This one dimensional model requires a defined thickness to the stagnant layer to prevent diffusion layer thickness tending to infinity as time increases. It is not surprising that their estimates and those presented in this article differ by a factor ~2. In particular, Coin and Olson's assumption that the stagnant layer is absent initially cannot be correct. Nor can their model give correct predictions of diffusion-layer thicknesses and time scales. The analysis above has been based on semiquantitative treatments of hydrodynamics and diffusion, which can be accurate to a factor of 2 at best.

Hartridge and Roughton (22) considered the possibility that the RBC was surrounded by a diffusion layer, but discounted it on the basis that increasing the mixing rate in continuous flow only increased the rate of oxygenation slightly. A similar effect has been studied by Koyama and Mochizuki (30); a ~20% increase in oxygenation rate occurred on increasing Re from 3,000 to 6,000. Gad-El-Hak et al. (8) have remarked on the diminished time scale for eddies as the Reynolds number increases. However, it is probable that this increase in oxygenation rate arises both from better mixing (affecting early times before the diffusion layer expands) and subsequently down the interrogation tube used by Koyama and Mochizuki (section 5). In passing, it might be remarked that O'Loughlin et al.[4] observed the quenching of the fluorescence from pyrene that had been absorbed into chinese hamster ovary cells. They had used a continuous-flow apparatus and concluded that either any stagnant layer around the cells was 1 μm, or that pyrene was preferentially absorbed into the cell membrane. Since these cells are nearly spherical in shape and have a ~5-μm diam, the latter possibility seems more reasonable.

Carlsen and Comroe (31) has made an interesting study of the rate of RBC oxygenation both for the normal biconcave discoid shape and spherical cells. The rates were similar. Since the diffusion layer around normal cells is larger than around spheres (section 6), whereas oxygen transport within the cell is faster in the normal cell than the spherical cell, these effects are in opposition. Apparently their magnitudes are similar and so largely counterbalance each other.

8. CONCLUSIONS

By considering the turbulence in the mixer chamber of stopped- and continuous-flow experiments, it has been shown that an experimental resolution time for RBC oxygenation studies ~200 μs is the fastest feasible, whereas ~1 ms may be a more usual resolution time. At such times the diffusion layer around a RBC is ~1 μm thick. An analysis based on the decay of eddies and on convective diffusion suggests that the diffusion layer should expand to ~4 μm after 10 ms in a stopped-flow experiment. The further convective mixing in continuous-flow apparatus leads to a slightly thinner diffusion layer and which is smaller the greater is the Reynolds number.

The concept of a stagnant layer of defined thickness has been seen to be a necessary addition to a one dimensional model of RBC oxygenation to prevent the rate of oxygenation slowing to near zero. It has no theoretical justification. However, to further quantify the concepts discussed in this article is hampered by the lack of a satisfactory theory of turbulence. Even when such a theory becomes available, the problem will be very complex to solve. An irony remains: to reduce the effects of diffusion layers high Reynolds number turbulent flows are used in experiments. These hydrodynamic effects cannot yet be

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[4] O'Loughlin, M. A., D. A. Whillans, and J. W. Hunt. Presented at the Radiation Research Society meeting in Toronto, Canada, May 1978.

adequately described from a theoretical view, and RBC oxygenation cannot be interpreted free from such effects. In laminar flow, the mixing and resolution times are increased, and so more effectively mask the nature of RBC oxygenation, but the hydrodynamics can be relatively easily and accurately described.

Several important effects have not been discussed in this article that are especially important to the oxygenation of undiluted blood. It is well known that blood flow is non-Newtonian. In addition, when RBC come close to each other (within 10–20 μm), the entrained water of one RBC will affect the motion of another RBC and vice versa. This is the phenomenon of hydrodynamic repulsion (25, 32). Furthermore the viscosity and diffusion of RBC in serum will be affected by the local concentration of cells (33, 34). Finally, at high-volume fractions of RBC (>0.1) the concentratin gradient surrounding one RBC will influence that of surrounding RBC (35, 36). Although these effects may be ignored in dilute RBC suspensions, proper modeling of undiluted blood must give attention to these many body problems.

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