

# TURBULENT FLOW OF RED CELLS IN DILUTE SUSPENSIONS

## EFFECT ON KINETICS OF O<sub>2</sub> UPTAKE

MOHAMED GAD-EL-HAK, J. B. MORTON, AND HOWARD KUTCHAI,  
*Department of Engineering Science and Systems and Department of  
Physiology, University of Virginia, Charlottesville, Virginia 22901*

**ABSTRACT** The turbulent flow properties of dilute (0.06% by volume) suspensions of human red blood cells in 4-mm-bore glass tubing were estimated by laser anemometry. The flow properties of the dilute red cell suspension were similar to those of a dilute suspension of polystyrene spheres (0.5  $\mu$ m diameter) in isotonic NaCl solution. Flow was found to be laminar when the Reynolds number was below 2,000, transitional in the range of Reynolds numbers from 2,000 to 3,000, and fully turbulent above Reynolds number 3,000. These results differ from previous studies of more concentrated red cell suspensions. The length scales of the turbulence were also estimated: at a Reynolds number near 4,000 the macroscale is about 1.25 mm, the Taylor microscale is about 0.85 mm, and the Kolmogoroff scale is near 0.075 mm.

The results are discussed in relation to previous measurements of the rate of oxygen uptake by dilute red cell suspensions in the flow-type rapid reaction apparatus. Our results suggest that under the conditions of most of these oxygen uptake measurements, the turbulent flow is characterized by eddies about 1 mm across, mixing with each other on a time scale of about 45 ms. Since most of the reported oxygen uptake measurements involve a similar time scale, it is possible that an effective "unstirred layer" influenced the reported rate of oxygen uptake.

### INTRODUCTION

Hartridge and Roughton (1), using their flow-type rapid reaction apparatus, showed that a dilute suspension of sheep red blood cells takes up oxygen some 20 times slower than a hemoglobin solution of similar oxygen-combining capacity. Hartridge and Roughton attributed the difference to the resistance offered by the red cell membrane to oxygen diffusion, and the resistance to diffusion of oxygen of the red cell interior.

Calculations by Roughton (2) showed that the resistance to oxygen diffusion within the red cell could not account for all the difference in oxygen uptake rates of hemoglobin solutions and red cell suspensions. He concluded that the red cell membrane is an important barrier to the passage of O<sub>2</sub> into the red cell. This conclusion was strengthened by the theoretical work of Nicolson and Roughton (3) on diffusion and

---

Dr. Gad-el-Hak's present address is: Flow Research Corporation, Seattle, Wash.

chemical reaction of oxygen in layers of hemoglobin solution with and without limiting membranes. Without attributing significant diffusion resistance to the red cell membrane, it was not possible to reconcile their calculations with experimental data.

Strong experimental evidence in support of the role of the red cell membrane in oxygen uptake comes from the work of Carlsen and Comroe (4). They measured the rates of uptake of CO and NO by normal human red cells and red cells converted to a spherical shape without volume change by brief heat treatment. The uptake of CO by normal and spherical red cells followed the same kinetic curve. If factors relating to diffusion inside the red cell were important, the spherical cells should have taken up CO much more slowly than the disks. Furthermore, there was no significant difference in the uptake rates of CO and NO, which differ in their rates of reaction with hemoglobin by a factor of 10. This rules out the rate of combination of the gas with hemoglobin as a rate-limiting factor. All of this is consistent with the passage of the gas through the red cell membrane being the rate-limiting step in gas uptake.

There are experiments apparently not consistent with the view that the red cell membrane limits  $O_2$  uptake. Kreuzer and Yahr (5) found the rate of oxygen uptake by thin layers of concentrated hemoglobin solution to be the same as for similar layers of packed erythrocytes. Kutchai and Staub (6) measured the same steady-state oxygen diffusion rate through a 165- $\mu m$  layer of packed erythrocytes as through a layer of concentrated hemoglobin solution of the same thickness.

Nicolson and Roughton (3) showed that the ratio of the permeabilities to oxygen of the red cell membrane to that of the cell interior ( $\lambda_{rbc}$ ) could be determined by comparing the initial rate of oxygen uptake by a red cell suspension with the predictions of their equations. The variation in values of  $\lambda_{rbc}$  obtained in various ways is considerable. Nevertheless, Roughton (2) points out that for four different processes— $O_2$  uptake, CO uptake, NO uptake, and displacement of  $O_2$  by CO—a value of about 1.5 for  $\lambda_{rbc}$  was obtained. If, however, the additional resistance to oxygen uptake resides not in the red cell membrane, but in an unstirred layer around the cell, we calculate (see Discussion) that an unstirred layer 1.4  $\mu m$  thick would account for the effect attributed to the cell membrane. In stopped-flow measurements of osmotic water flux across the red cell membrane, Sha'afi et al. (7) found an unstirred layer 5.5  $\mu m$  thick.

The influence of an effective unstirred layer on the rate of  $O_2$  uptake by red cells in the rapid reaction apparatus has not been given much attention because it has been assumed (*a*) that the flow is turbulent and (*b*) that this implies effectively complete mixing of the red cells with the suspending medium. Neither of these assumptions has been critically tested.

It is established that in flows in tubes at Reynolds numbers exceeding the minimum value at which turbulence *can* occur, three types of flows are possible: the flow can remain laminar; it can become fully turbulent; or it can be intermittent (i.e., laminar part of the time and turbulent part of the time). Which type of flow occurs depends in a complicated manner on the Reynolds number and the details of the flow apparatus. Most of the studies of gas uptake by red cells in the rapid reaction apparatus have used very dilute suspensions of red cells (about 0.1% red cells by volume after mixing). We

are aware of no studies of turbulent pipe flow by such dilute suspensions of red cells. In particular, the ranges of Reynolds numbers in which flow is laminar, transitional, and turbulent have not been directly determined.

Even if it were known that flow of red cell suspensions in the rapid reaction apparatus is unequivocally turbulent at the Reynolds numbers previously employed in studies of gas uptake, the question of whether the red cells are effectively completely mixed with suspending medium remains. In turbulent flow, the fluid is composed of eddies with a spectrum of sizes. Whether or not red cells within such eddies are effectively completely mixed with respect to the diffusion of oxygen into the red cells depends on the size of the eddies and the time scale in which they exist relative to the distance and time-dependence of the  $O_2$  uptake by the cells.

In the present study we have used a laser anemometer to determine the conditions under which dilute suspensions of red blood cells exhibit turbulent flow in a rapid reaction apparatus. Turbulent flows have been characterized by their root-mean-square (RMS) velocity fluctuations and by the different turbulence scales. The influence of turbulence-induced convection on the uptake of  $O_2$  by red cells in the rapid reaction apparatus is discussed. Our results show that it is possible that an effective "unstirred layer" influences that rate of oxygen uptake observed in the rapid reaction apparatus.

## METHODS

The flow system is shown in Fig. 1. A dilute suspension of red blood cells (5 ml of freshly drawn human blood in 4 liters of isotonic [0.9%] NaCl) is driven at known flow rates through precision-bore glass tubing having an inside diameter of 4 mm. A magnetic stirrer in the storage tank prevents the cells from settling. The pump used is a variable-speed Varistaltic standard model, catalog 72-310-000, Manostat Corp. (New York), with flow rate from 75 to 350 ml/min.

The laser anemometer measures the velocity of either the red cells or small, light-scattering polystyrene particles added to the flow. These particles are very small ( $0.5\ \mu\text{m}$  in diameter) and neutrally buoyant, so they follow the motion of the fluid extremely well, even in highly turbulent flows.

The general layout for the laser anemometer is similar to that described by Morton and Clark (8) and shown schematically in Fig. 2. Light from the laser is split into two parallel, equally intense beams by a beam-splitter and focused in the flow with a lens. At the intersection

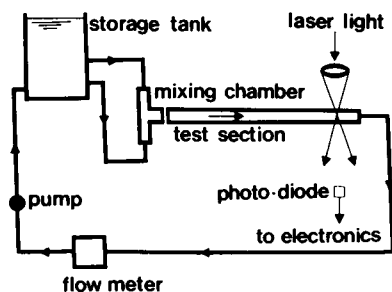


FIGURE 1 Schematic diagram of the flow system. The mixing chamber is a Pyrex t-tube with 3.9 mm inside diameter. The test section is precision-bore glass tubing with 4 mm internal diameter.

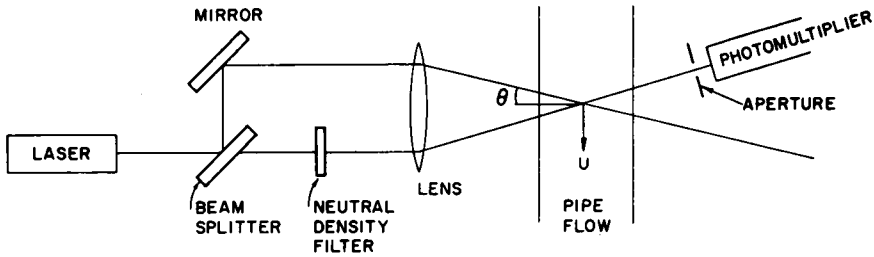


FIGURE 2 Schematic depiction of the optics of the laser anemometer system.

of the two beams, an interference pattern (a series of light and dark regions) is established. As the scattering particles cross this pattern, more light is scattered from the bright areas than the dark areas. This scattered light is focused by a second lens (not shown in Fig. 2) on the photodetector, which then sees alternating periods of light and dark.

The distance between bright areas (fringes) is given by  $\phi/2\sin\theta$ , where  $\phi$  is the wavelength of light and  $\theta$  is half the angle between the beams. The time  $T$  between peak outputs from the photodetector is related to the velocity,  $u$ , by  $T = \phi/2u\sin\theta$ . Thus the frequency of the output from the photodetector is  $f_D = 2u\sin\theta/\phi$ .

For a typical arrangement, the fringe spacing is approximately  $1\ \mu\text{m}$ . For velocities typically present in a rapid reaction apparatus,  $u \sim 100\ \text{cm/s}$ ,  $T$  is approximately  $10^{-6}\ \text{s}$  and  $f$  is about 1,000 KHz. In laminar flow the frequency of the photodetector output is constant, while turbulent flow is characterized by rapid fluctuations of the frequency. The transition between laminar and turbulent flow is thus readily detected. Measurements are made about 120 mm (30 tube diameters) downstream from the mixing chamber, so that events in the mixing chamber should not influence the observed turbulent flow.

The red blood cell diameter is approximately an order of magnitude larger than the fringe spacing, while the polystyrene particles are of the same order of magnitude as the fringe spacing. In practice, however, the relatively large size of the red blood cells has little effect on the signal.

### *Determination of the Length and Time Scales of the Turbulence*

The length scales are estimates of the sizes of the fluid domains in motion relative to one another in the turbulent flow. The turbulent scales were calculated from the auto-correlation function (8). Let  $u = u(\mathbf{x}, t)$  at a point  $\mathbf{x}$  and time  $t$  in the direction of the flow. This velocity can be written as the sum of the average or mean velocity and the fluctuating part of the velocity. Thus,  $u(\mathbf{x}, t) = \bar{u}(\mathbf{x}) + u'(\mathbf{x}, t)$  where  $\bar{u}$  is the mean velocity and  $u'$  is the fluctuating velocity. The auto-correlation function is defined  $R(\mathbf{x}, \tau) = \langle u'(\mathbf{x}, t)u'(\mathbf{x}, t + \tau) \rangle / \langle u'^2(\mathbf{x}, t) \rangle$  where  $\langle \rangle$  indicates a time average. From this function, two scales are generally defined. The integral scale,  $\lambda$ , also known as the macroscale, is given by  $\lambda = \int_0^\infty R(\mathbf{x}, \tau) d\tau$ , and is the area under the auto-correlation function. This scale indicates the size of the turbulent structure that contains most of the energy in the flow. For a detailed discussion see Batchelor (9).

Another scale measurable from the auto-correlation function is the Taylor microscale,  $\lambda$ :  $\lambda^2 = d^2R/d\tau^2 | \tau = 0$ . The quantity  $1/\lambda^2$  is proportional to the rate of dissipation of turbulent energy.

The smallest scale that can exist in a turbulent flow is the Kolmogoroff scale, defined by  $\eta = (\nu^3/\epsilon)^{1/4}$ , where  $\epsilon$  is the rate of dissipation per unit mass, and  $\nu$  is the kinematic viscosity. An estimate for  $\epsilon$  is given by the relation  $\epsilon = 15\nu(\bar{u}'^2/\lambda^2)$ , where  $\bar{u}'^2$  is the mean square turbulent fluctuation and  $\lambda$  is the Taylor (length) scale. This relation is strictly valid only for iso-

tropic turbulence. The value of  $\eta$  gives an estimate of the smallest unstirred domain possible in the turbulent flow and from it one can estimate the minimum unstirred layer around a red blood cell at any instant in time.

### *Determination of Degree of Hemolysis of Red Cells*

At the end of each experiment, a sample of the red blood cell suspension was subjected to centrifugation at 1,000 *g* for 15 min. The supernatant was removed with a Pasteur pipette and saved. The cell pellet was lysed in a volume of distilled water equal to the volume of the supernatant withdrawn. The degree of hemolysis was calculated from the optical density of the cell lysate and the supernatant at 420 nm (an isosbestic wavelength for oxyhemoglobin and reduced hemoglobin). The degree of hemolysis was always less than 1%.

## RESULTS

The Reynolds number was systematically altered in the range 700–8,000 by varying the flow rate. Laminar flow was observed for Reynolds numbers below 2,000, both with and without the red blood cells. Mean velocity profiles are parabolic, as predicted theoretically. Transition to turbulent flow was observed in the range 2,000–3,000, and fully turbulent flow was observed for Reynolds numbers above 3,000. It appears within our experimental accuracy that the addition of a small number of red cells does not affect the transition Reynolds number. The value of the Reynolds number at which the flow becomes fully turbulent depends strongly on the details of the apparatus. In some cases this value might be much larger than 3,000.

TABLE I  
THE FLOW PROPERTIES OF DILUTE SUSPENSIONS  
OF HUMAN ERYTHROCYTES.

$u'/\bar{U}$  represents the amplitude of the velocity fluctuation as a percentage of the mean flow velocity at various values of the Reynolds number (*Re*),  $\eta$  is the Kolmogoroff scale,  $\lambda$  is the Taylor microscale, and  $l$  is the macroscale.

<i>Re</i>	$\frac{u'}{\bar{U}}$ (%)	$\eta$ (mm)	$\lambda$ (mm)	$l$ (mm)
710	Laminar			
1065				
1420				
1686				
1908				
2219	Transition			
2929				
3506				
4083				
4837				
5059				
5858				
6169				
6967				
7367				
7544				
Averages:	2.25	0.069	1.00	1.33

Table I shows the variation of the turbulence levels, Kolmogoroff scale, Taylor scale, and macroscale with Reynolds numbers for the dilute suspensions of red blood cells. These measurements are made at the center of the tube, 160 tube diameters from the entrance region. Turbulence intensity levels are slightly smaller than previously measured in turbulent pipe flows. As seen from the table, the smallest scale ( $\eta$ ) that exists in the flow is almost an order of magnitude larger than the average diameter of the red blood cell ( $8\ \mu\text{m}$ ).

Table II shows the same parameters of the flow in the absence of red cells, but with  $1\ \mu\text{m}$ -diameter polystyrene spheres (0.02% by volume) added to scatter the laser light. Previous work in this laboratory has shown that at this concentration, the polystyrene spheres do not significantly alter the properties of the flow. The

TABLE II  
THE FLOW PROPERTIES OF DILUTE SUSPENSIONS OF  
POLYSTYRENE SPHERES IN ISOTONIC NaCl SOLUTION.  
The symbols are explained in the legend to Table I.

Re	$\frac{u'}{U}$ (%)	$\eta$ (mm)	$\lambda$ (mm)	$l$ (mm)
710				
888				
1420	Laminar			
1580				
1908				
2307				
2663	Transition			
2796				
3018	2.88	0.068	0.74	1.09
3062	3.31	0.055	0.56	1.36
3240	2.87	0.062	0.64	1.44
3550	2.50	0.068	0.71	1.06
3594	2.68	0.064	0.66	1.39
3683	1.64	0.073	0.94	0.72
4127	2.38	0.065	0.66	1.01
4260	2.88	0.060	0.74	1.40
4260	2.98	0.058	0.70	1.38
4379	2.69	0.058	0.65	1.45
4970	2.36	0.065	0.83	1.14
5147	2.61	0.057	0.72	1.36
5591	2.67	0.054	0.72	1.30
6346	2.46	0.061	0.93	1.21
6657	2.50	0.055	0.79	1.38
6701	2.37	0.054	0.74	1.35
7455	2.25	0.052	0.72	1.33
7456	2.18	0.062	0.98	1.21
7588	2.23	0.052	0.72	1.34
7677	2.27	0.052	0.73	1.38
7722	2.29	0.052	0.72	1.36
7811	2.28	0.052	0.72	1.33
7899	2.20	0.053	0.73	1.34
8032	2.08	0.062	0.99	1.21
Averages:	2.48	0.056	0.75	1.27

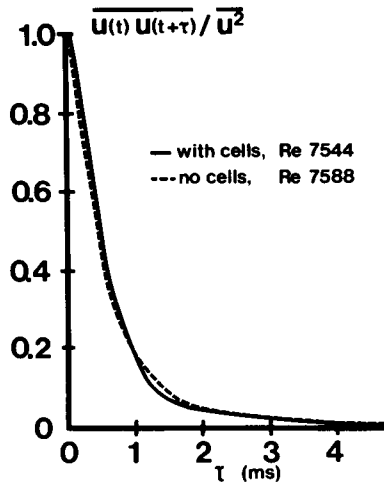


FIGURE 3 The autocorrelation coefficient is plotted versus the time increment,  $\tau$ , for the flows of a red cell suspension and a suspension of polystyrene spheres (no cells) with about the same Reynolds number.

length scales are slightly smaller than in the presence of red cells, consistent with the improved spatial resolution obtained with the smaller polystyrene spheres. The data indicate that the addition of the small number of red blood cells does not change the principal characteristics of the turbulent flow.

Fig. 3 shows a typical auto-correlation coefficient with and without red blood cells at approximately the same Reynolds number. The auto-correlation coefficient is a

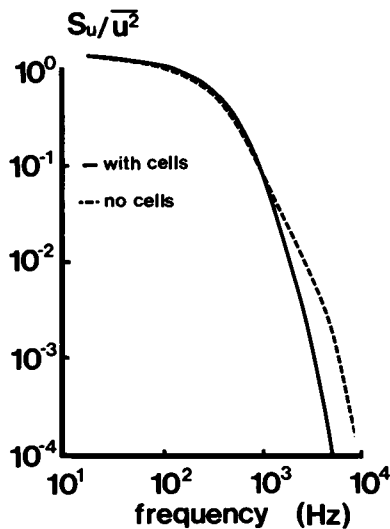


FIGURE 4 The spectrum Fourier transform of the auto-correlation coefficient is plotted for a flow of a dilute red blood cell suspension and for a dilute suspension of polystyrene spheres (no cells) with about the same Reynolds number (given in Fig. 3).

measure of the statistical character of the velocity fluctuations, and Fig. 3 shows that over time intervals ( $\tau$ ) greater than 4 ms, the two instantaneous mean velocities  $u(t)$  and  $u(t + \tau)$  are statistically independent. That the curve for the red cell suspension is almost identical with the curve in the absence of red cells suggests that the small volume fraction of red cells has only minimal influence on the turbulent flow properties of the isotonic NaCl solution.

Fig. 4 shows energy spectra (Fourier transforms of the auto-correlation coefficient) for the two cases of Fig. 3 at approximately the same Reynolds numbers with the area under each curve normalized to unity. In these plots the ordinates are proportional to the fraction of the total energy associated with the frequencies shown on the abscissa. The spectra are quite similar in the presence and absence of red blood cells.

## DISCUSSION

### *Turbulence Properties of Dilute Red Blood Cell Suspensions*

We find that very dilute red cell suspensions (0.06% by volume) do not differ markedly from isotonic NaCl solutions in their turbulent flow properties. Flow is laminar below Reynolds number 2,000, transitional in the range of Reynolds numbers between 2,000 and 3,000, and fully turbulent above Reynolds number 3,000. Different results have been reported with more concentrated red cell suspensions and with whole blood. Coulter and Pappenheimer (10) studied bovine blood flowing in glass tubes (2.5–6.8 mm diameter). They reported that flow becomes turbulent at a Reynolds number (diameter-based) of  $1,940 \pm 60$  and that the critical Reynolds number is relatively independent of tube size, hematocrit, and temperature.

Munter and Stein (11) used a hot film anemometer to study turbulence in human blood forced through an orifice of 3.18 mm diameter. The turbulence intensity was found to depend markedly on the hematocrit (20–60%). Depending on the hematocrit and the Reynolds number, the turbulence intensity in the flowing blood could be greater than, less than, or equal to that in plasma. The microscale of the turbulence was found not to depend markedly on hematocrit. Stein and Sabbah (12), using similar techniques, found that human blood (10–40% hematocrit) has greater intensity of turbulent flow than isoviscous plasma over a range of Reynolds numbers. Turbulence intensity depended on hematocrit and maximum turbulence intensity occurred near 20% hematocrit.

### *Turbulent Flow during Oxygen Uptake in the Rapid Reaction Apparatus*

In the mixing chamber, convection due to turbulence is responsible for rapidly mixing deoxygenated red cells with oxygenated saline solution. As the mixture flows through the flow tube of the rapid reaction apparatus and the uptake of  $O_2$  proceeds, both diffusion and convection maintain the suspension well mixed with respect to the oxygen concentration. When the flow is turbulent, the mixing by convection is many



times faster than in laminar flow. One contribution of our work is to define the ranges of Reynolds numbers in which the flow of dilute suspensions of red cells is laminar, transitional, and turbulent. Some investigators have assumed, from the work of Coulter and Pappenheimer with rather concentrated suspensions of bovine red cells (10), that dilute red cell suspensions will show a transition to turbulent flow at Reynolds numbers just below 2,000. Our results indicate that this transition Reynolds number is grossly low for dilute red cell suspensions, which do not show a transition to unambiguously turbulent flow until the Reynolds number approaches 3,000. While most rapid reaction apparatus measurements of oxygen uptake have been made at Reynolds numbers that assure turbulence, this has not always been the case.

### *Convective Mixing in the Rapid Reaction Apparatus*

It is necessary to know the time scales of the turbulence as well as the length scales to consider the mixing by convection of the layers adjacent to the red cell with the rest of the suspending medium in a turbulent flow. Consider conditions in our experiments when the flow is demonstrably turbulent (e.g. at a Reynolds number around 4,400, with a mean flow velocity of 100 cm/s). Under these conditions the RMS velocity fluctuation (Table I) is about 2.25 cm/s. Using the relation that the time scale is equal to the length scale divided by the RMS fluctuation velocity (9), we obtain the time scales listed in Table III. Eddies in turbulent flow are transient structures. The time scale gives an indication of the life-span of the average eddy of a particular size. Over a time equivalent to several time scales, an eddy can be expected to have become completely mixed with other eddies. While the macroscale represents the largest eddy possible and the Kolmogoroff microscale represents the smallest eddy possible, the Taylor microscale cannot be associated with any particular group of eddies. It does, however, provide a useful estimate for the time scale associated with mixing of the eddies, in this case about 45 ms.

TABLE III  
APPROXIMATE VALUES FOR THE  
VARIOUS LENGTH SCALES ( $\lambda$ , macro-  
scale;  $\lambda$ , Taylor microscale;  $\eta$ , Kolmogoroff  
scale) AND THE TIME SCALE AP-  
PROPRIATE TO EACH LENGTH  
SCALE FOR REYNOLDS NUMBER  
ABOUT 4,400.

Length scale	Corresponding time scale
<i>mm</i>	<i>ms</i>
$\lambda = 1.33$	59
$\lambda = 1.00$	44
$\eta = 0.069$	3

### *Kinetics of O<sub>2</sub> Uptake in the Rapid Reaction Apparatus*

Assume that in the flow-type rapid reaction apparatus the incoming streams are completely mixed in the mixing chamber. As the mixture flows down the flow tube at a Reynolds number of 4,400, to ensure turbulent flow, the red cells take up oxygen and the solution immediately surrounding each red cell becomes somewhat depleted of oxygen. Diffusion and convection will tend to restore the oxygen concentration in the layers immediately surrounding the red cells toward the mean value of the O<sub>2</sub> concentration in the whole suspension. To the extent that the replenishment of oxygen to these layers lags behind the uptake by the cells, these boundary layers will remain somewhat oxygen-depleted and the kinetics of oxygen uptake by the cells will behave as if there were an "unstirred layer" surrounding each red cell.

Most of the measurements of oxygen uptake by red cells have been made under conditions such that the uptake process requires 10–100 ms. Our data suggest that the replenishment by convective mixing of oxygen in the layer immediately around the red cell requires on the order of 45 ms and thus partly limits the rate of O<sub>2</sub> uptake observed under these conditions. Koyama and Mochizuki (13) found that red cells in a flow-type rapid reaction apparatus take up oxygen some 16% slower at  $Re = 3,000$  than at  $Re = 6,000$ . This is qualitatively consistent with the data we obtained on dilute red cell suspensions (Table I). The data of Table I show that a typical red cell at  $Re = 3,000$  is contained in an eddy about 0.8 mm (Taylor microscale) across, while the red cell at  $Re = 6,000$  is in a somewhat larger eddy (about 1.0 mm across). Calculation of the time scales, however, shows that at  $Re = 3,000$  the time scale for mixing of the eddies is about 50 ms, while at  $Re = 6,000$  mixing occurs faster (time scale of 33 ms). On going from Reynolds number 3,000 to 6,000, the decrease in the time scale of mixing is proportionally greater than the increase in the size of the eddies, so that if mixing by convection were limiting, the rate of uptake observed should increase on increasing the Reynolds number.

### *Effect of Unstirred Layers on O<sub>2</sub> Uptake*

Sha'afi et al. (7) used the delay in the onset of red cell shrinking when a red cell suspension was rapidly mixed with a hypertonic solution to estimate the thickness of the unstirred layer around the cell. From a mixing chamber the suspension flows down an observation tube with  $Re$  from 8,720 to 10,900. Flow was rapidly stopped and a record of cell volume (from light scattering measurements) vs. time was obtained. The delay in onset of cell shrinking was consistent with the presence of an unstirred layer about 5.5  $\mu\text{m}$  thick around the red cell. Sha'afi et al. were able to show that this unstirred layer had a negligible effect on the red cell water permeability coefficients measured previously by Sidel and Solomon (14).

If the resistance to oxygen uptake previously postulated to reside in the red cell membrane were really due to an unstirred layer, how thick would the unstirred layer need to be? As discussed in the Introduction, a number of experimental results are consistent with the assumption that the ratio of the oxygen permeability of the red

cell membrane to that of the red cell interior is 1.5. That is,  $\lambda_{\text{rc}} = (D_m/t_m)/(D_c/t_c) = 1.5$ , where  $D_m$  and  $D_c$  are the diffusion coefficients for oxygen in the membrane and cell interior, respectively, and  $t_m$  and  $t_c$  are the thickness of the membrane and half-thickness of the cell, respectively. If we consider that the membrane has no effect on oxygen uptake, but that an unstirred layer around the red cell is responsible for the unexpectedly slow uptake of  $\text{O}_2$ , the above equation will apply, but with  $D_m$  and  $t_m$  referring to the unstirred layer. Take the half-thickness of the red cell to be  $0.8 \mu\text{m}$ ,  $D_m = 2 \times 10^{-5} \text{ cm}^2/\text{s}$  and  $D_c = 0.75 \times 10^{-5} \text{ cm}^2/\text{s}$  (16). Solving the above equation for  $t_m$  and substituting the numerical values just given, we obtain a calculated unstirred layer thickness of  $1.4 \mu\text{m}$ . That is to say that an unstirred layer roughly one quarter as thick as that observed by Sha'afi et al. would suffice to explain the resistance to oxygen uptake hitherto postulated to be due to the red cell membrane.

### Conclusions

The length and times scales reported in this article for the turbulent flow of dilute suspensions of red blood cells are consistent with the hypothesis that under the conditions employed in previous measurements of the rate of oxygen uptake by red cells, some limitation was present due to incomplete mixing by convection (an effective unstirred layer) during the time span of the measurement. Further research is needed to define more precisely the roles of the red cell membrane and the unstirred layer in determining the rate of oxygen uptake by the red cell in vitro and in the pulmonary capillary.

We are grateful to Virginia H. Huxley for help in performing the analyses of hemolysis and to Dr. Don Danielson for initiating our collaboration. This research was supported an Institutional Subgrant from the University of Virginia, National Science Foundation Grant ENG 7522488, and National Institutes of Health Grants HL 17967 and KO4-HL-00014 (Research Career Development Award to H.K.).

*Received for publication 3 November 1976.*

### REFERENCES

1. HARTRIDGE, H., and F. J. W. ROUGHTON. 1923. A method of measuring the velocity of very rapid chemical reactions. *Proc. R. Soc. Lond. A.* **104**:376.
2. ROUGHTON, F. J. W. 1959. Diffusion and simultaneous chemical reaction velocity in haemoglobin solutions and red cell suspensions. *Progr. Biophys. Biophys. Chem.* **9**:55.
3. NICOLSON, P., and F. J. W. ROUGHTON. 1951. A theoretical study of the influence of diffusion and chemical reaction velocity on the rate of exchange of carbon monoxide and oxygen between the red blood corpuscle and the surrounding fluid. *Proc. R. Soc. Lond. B. Biol. Sci.* **138**:241.
4. CARLSEN, E., and J. H. COMROE, JR. 1958. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. *J. Gen. Physiol.* **42**:83.
5. KREUZER, F., and W. Z. YAHR. 1960. Influence of red cell membrane on diffusion of oxygen. *J. Appl. Physiol.* **15**:1117.
6. KUTCHAI, H., and N. C. STAUB. 1969. Steady-state, hemoglobin-facilitated  $\text{O}_2$  transport in human erythrocytes. *J. Gen. Physiol.* **53**:576.
7. SHA'AFI, R. I., G. T. RICH, V. W. SIDEL, W. BOSSERT, and A. K. SOLOMON. 1967. The effect of the unstirred layer on human red cell water permeability. *J. Gen. Physiol.* **50**:1377.

8. MORTON, J. B., and W. H. CLARK. 1971. Measurements of two-point velocity correlations in a pipe flow using laser anemometers. *J. Physics E. Sci. Instrum.* 4:809.
9. BATCHELOR, C. K. 1960. *The Theory of Homogenous Turbulence*. Cambridge University Press, Cambridge.
10. COULTER, N. A., and J. R. PAPPENHEIMER. 1949. Development of turbulence in flowing blood. *Am. J. Physiol.* 159:401.
11. MUNTER, W. A., and P. D. STEIN. 1974. Turbulent blood flow and the effects of erythrocytes. *Cardio-vasc. Res.* 8:338.
12. STEIN, P. D., H. N. SABBAGH, and E. F. BLICK. 1975. Contribution of erythrocytes to turbulent blood flow. *Biorheology*. 12:293.
13. KOYAMA, T., and M. MOCHIZUKI. 1969. A study on the relationship between the oxygenation velocity of the red blood cell and the flow velocity in a rapid flow method. *Jpn. J. Physiol.* 19:534.
14. SIDEL, V. W., and A. K. SOLOMON. 1957. Entrance of water into human red cells under an osmotic pressure gradient. *J. Gen. Physiol.* 41:243.
15. FORSTER, R. E. 1964. Rate of gas uptake by red cells. *Handb. Physiol.* Sec. 3, Respiration. 1:827.
16. KREUZER, F. 1970. Facilitated diffusion of oxygen and its possible significance; a review. *Respir. Physiol.* 9:1.