STUDY ON THE OXYGENATION VELOCITY OF THE HUMAN RED CELL

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As described in the preceding paper¹⁾, HARTRIDGE and ROUGHTON²⁾ clarified in 1926 that the oxygenation velocity of the red cell was much slower than that of Hb solution. Then ROUGHTON and NICOLSON^{3,4,5)} studied theoretically the relation between the O₂ diffusion inside the red cell and the oxygenation. However, the data which were valuable to consider the relationship between the oxygenation velocity of the red cell and the pulmonary diffusing capacity were not obtained before 1955. In that year GIBSON and others⁶⁾ measured the oxygenation velocity of the red cell using the human red cell at 37°C. While SIRS and ROUGHTON⁷⁾ (1963) advanced this study further, using a stopped flow method instead of the rapid flow. Still, however, the oxygenation velocity in the range of S₀, from 70 to 100% had not been measured until quite recently.

In 1961 STAUB and others⁸⁾ measured the oxygenation of the human red cell by using a Pt electrode instead of the conventional photometric method. They measured the velocity all over the range from 0 to 95% of S₀, but the variation of the measured values was so large, that there remained an additional problem, whether the variation was inherent to the individual subject or was it only the error of the measurements.

Different from Hb, the red cell scatters the incident light. Therefore, it is fairly difficult to find a change in light absorption due to oxygenation in red cell suspension. When the suspension is made to flow through an observation tube, the amount of the scattered light depends on the flow velocity. Thus, a fluctuation appears on the photosignals, when the flow velocity is not constant. This is especially true, because the change in light absorption due to oxygenation is usually comparable to the noise due to the uneven velocity of flow; therefore, it is absolutely necessary to avoid the influence of light scattering. On the other hand, since the amount of the scattered light is indifferent to the wave length, the influence of it has so far been excluded by taking the difference of transmissions at two wave lengths^{7,9,10)}. In this

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FIG. 1. Schematic illustration of the whole arrangement.

as to be moved along the observation tube on a solid truck.

2) *Photometry.* Transmitted light intensities were measured at two wave lengths of 572 and 599 milli-micron, as shown in FIG. 2. The light going across the observation tube was divided into two parts by a neutral half-mirror, and then each part was made to pass through their respective interference filter to a photomultiplier. Both photo-signals from the photomultipliers were further amplified by a differential amplifier and then recorded on a pen recorder.

3) *Solutions and gases.* The red cell suspension was prepared by adding whole blood of 5 ml into 2 liters of a phosphate buffer solution. Before each measurement the blood sample was drawn freshly and heparinized. The buffer solution was composed of NaCl 7.88 g, KCl 0.26 g, KH_2PO_4 3 mg, Na_2HPO_4 0.85 g, NaHOC_3 2.4 g and distilled water 1.000 ml. pH of the solution was about 7.40 at P_{CO_2} 40 mmHg.

Three kinds of mixed gases were used for tonometry; their compositions were as follows: 1) P_{O_2} ranging from 180 to 220 mmHg and P_{CO_2} 40 to 60 mmHg, 2) P_{O_2} 4 to 40 mmHg and P_{CO_2} 40 to 60 mmHg and 3) pure O_2 . The pure O_2 was used to obtain the full oxygenated S_{O_2} level, that is, the full oxygenated suspension was made by mixing the red cell suspension of low P_{O_2} with the buffer solution bubbled with pure O_2 . Since the oxygenation proceeds very rapid at a hyperoxic level over 300 mmHg, the full saturated level can be attained easily through this method. When the reaction time is very short, the S_{O_2} is still lower than 100%. For getting a reliable level for 100% S_{O_2} however, this method was much better than the one in which the suspension of

FIG. 2. Block diagram of the photometric apparatus.

TABLE 1. Composition of mixed gases used for tonometry.

No.	Number of Subjects	Date	High O ₂ gas (mmHg)		Low O ₂ gas (mmHg)	
			PO ₂	P _{CO} ₂	PO ₂	P _{CO} ₂
1	2	May, 13-14	231	50	26	37
2	2	May, 15-17	200	29	29	37
3	3	May, 17-19	200	29	29	37
4	2	June, 21-23	185	47	9	55
5	1	June, 23	185	47	24	74
6	4	June, 24-25	205	40	9	55
7	4	June, 29-30	205	40	24	74
8	2	July, 5-6	205	40	29	56

100% SO₂ was additionally prepared as the standard suspension for the calibration, for the concentration of the red cell easily varied to a large extent in the red cell suspension.

The compositions of the high and low O₂ groups of the mixed gases used are tabulated in TABLE 1.

RESULTS

The oxygenation velocity was measured in 7 cases of different PO₂ and P_{CO}₂ with samples collected from normal 7 subjects, as shown in TABLE 1. In the experiments of No. 2 and 3, the day by day variation was examined with the same subjects under the same experimental conditions. The recorded curves shown in FIG. 3 obtained in the experiment No. 8. As shown in this figure duplicate measurements were carried out at three levels of SO₂, i.e. initial SO₂, the full oxygenated SO₂ and the changing SO₂ between the two. The initial SO₂ of the experiment No. 8 was 57.5%; this figure was determined by referring to the initial PO₂ and the O₂ dissociation curve of blood. The error of the duplicate measurements was within 1% in SO₂.

In FIG. 4 are shown the changes of SO₂ along the reaction time which were measured in the experiment No. 6. The plotted points are the mean values of 4 different subjects and the arrows illustrated at the points represent standard deviations around the mean. The standard deviations were about 2.5% in SO₂ in the case of the experiment No. 6 and 1.3% in the case of No. 7. As seen in TABLE 1, PO₂ and P_{CO}₂ of the original red cell suspension was 9 and 55 mmHg and those of the buffer solution were 205 and 40 mmHg. Thus, when they were mixed with the equal amount to each other, the initial PO₂ of the solution surrounding the red cell should be 107 mmHg and the P_{CO}₂, 47 mmHg. It follows that the oxygen molecule should have diffused into the red cell and CO₂ diffused out of the red cell. In the case of No. 7 the same type of diffusion should have occurred, for P_{CO}₂ of the buffer solution was

higher than that of the initial red cell suspension. Consequently, the BOHR effect may be included in the oxygenation reactions which are represented by the curves in FIG. 4.

As described already, in the experiments No. 2 and 3 the S_{O_2} -time curves were measured duplicately on the same subjects under the same conditions of P_{O_2} and P_{CO_2} , but on different days. The variations of the measured S_{O_2} were similar to those observed in the experiments No. 6 and 7. Therefore, the variations expressed by standard deviations seems to be due mainly to the measuring procedure, and not to the individual variation of the oxygenation velocity.

During the 4th experiment the oxygenation of Hb solution was also measured as shown in FIG. 5, for the purpose to determine the oxygenation velocity at S_{O_2} range of more than 90% on one hand, and to check the mixing effect of the mixing chamber used on the other hand. The electrolyte composition of Hb solution was similar to the one described in the previous paper; except that 1.05 g $NaHCO_3$, instead of 2.40 g, was added to 1l of the buffer solution.

FIG. 3. Actual records of the light intensities at 6 different reaction times obtained in the No. 8 experiment. In each record the transmissions were measured at three levels of S_{O_2} , i.e. 57.5%, 100% and the varying level of the mixed suspension.

The pH of the solution was 7.26 at 40 mmHg P_{CO_2} . The P_{O_2} and P_{CO_2} of Hb solution were 9 and 55 mmHg respectively. As the P_{O_2} after mixing was comparatively high, the oxygenation occurred very fast, and S_{O_2} reached 90% within 10 msec after mixing. However, the oxygenation velocity from 90% to 100% were obviously slowed down; this tendency was observed also in the oxygenation process of the red cell.

In the experiment No. 4, the oxygenation of the red cell was observed at

FIG. 4. Changes of O_2 saturation, S_{O_2} , plotted against the reaction time obtained in the experiments of Nos. 6 and 7.

two levels of pH's of 7.40 and 6.85. In the latter case NaHCO_3 was added to the solution in the concentration of $1.05 \text{ g} \cdot \text{l}^{-1}$ instead of $2.40 \text{ g} \cdot \text{l}^{-1}$, and thus its pH became 6.85, while the P_{CO_2} was the same in both cases. The closed circles in FIG. 5 represent the S_{O_2} in the suspension of the 6.85 pH. As the affinity of the red cell for O_2 is lowered in an acid solution, the terminal S_{O_2} level was markedly low in the suspension of the low pH than that in the suspension of pH 7.46. The oxygenation velocity from 10 to 60% S_{O_2} , however,

FIG. 5. Changes of O_2 saturation, S_{O_2} , plotted against the reaction time obtained in red cell suspension of pH 7.40 and 6.85 and in Hb solution of pH 7.26.

was of the same magnitude between the two kinds of suspension.

The plotted point at 18 msec deviated largely upward from the smooth curve. This may be ascribed to the fact that the full oxygenated level could not be attained in such a short period as 18 msec even at a hyperoxic level of more than 300 mmHg. In other words, the procedure to mix the buffer solution saturated with pure O₂ and the red cell suspension of low P_{O₂}, seemed not to be satisfactory to obtain the full saturated suspension in such a short period.

As in the preceding paper¹⁾, the oxygenation velocity was compared by values of the velocity factor, F_c . The values were calculated by dividing the O₂ quantity taken up by 1 ml red cells in a second by the P_{O₂} difference between the red cell and the surrounding medium. Let the P_{O₂}'s of the medium and the red cell be P and P_c. The practical calculation was performed by the following formula, P_c being estimated from the O₂ dissociation curve of blood at pH 7.40 by referring to the S_{O₂} measured.

$$F_c = \frac{0.4 \times 10^{-2} ds/dt}{P - P_c}, \quad (1)$$

where 0.4×10^{-2} represents the O₂ combining capacity of 1 ml of red cells versus 1% increase of S_{O₂}, and ds/dt is the increasing rate of S_{O₂} of the red cell.

The velocity factors, F_c , obtained during every 10 msec along eight S_{O₂}-time curves are shown in FIG. 6. The numbers written on the curves of

FIG. 6. Velocity factor of oxygenation of the red cell, F_c , plotted against the O₂ saturation. The numbers noted on the curves are the experimental numbers shown in TABLE 1.

FIG. 6 correspond to the experiment number shown in TABLE 1. Because θ derived by FORSTER and ROUGHTON¹¹⁾ is the velocity factor expressed per one minute for the blood of 1 ml, it is about 30 times as great as F_c . As expected from the theoretical study the factor converged to 0 as the saturation proceeded. However, the measured values were about 2/3 of the theoretical values obtained by the author¹²⁾ and about 1/3 of the measured values reported by STAUB and others⁸⁾.

P_{CO_2} of the red cell suspension and the buffer solution were ranged from 37 to 74 mmHg. However, P_c was estimated always by use of the O_2 dissociation curve of pH 7.40. Therefore, the error due to the variation of the

FIG. 7. Relationship between the velocity factors of the red cell, F_c , and the Hb solution, F_s , and the O_2 undersaturation $(100 - S_{O_2})$.

dissociation curve is certainly implied in the calculation of the velocity factor.

The velocity factor of Hb, F_s , was also calculated through the same procedure as Eq(1). In FIG. 7, F_s are plotted together with the mean values of F_c versus the undersaturation on a logarithmic graph. F_s values at the lower S_{O_2} range were rewritten from the preceding paper¹¹. The logarithm of F_s was linear against that of the undersaturation as expressed experimentally by

$$F_s = 0.00106(100 - S_{O_2})^{1.6}. \quad (2)$$

Eq(2) shows that F_s had a non-linear relation to the undersaturation. Using the values of F_s given by this equation, the F_c values of FIG. 7 which were derived by a curve fitting method were expressed as follows:

$$F_c = \frac{0.025 \times F_s^{1.26}}{0.025 \times F_s^{1.26}}. \quad (3)$$

The broken line illustrated in FIG. 6 shows the curve given by Eq(3).

DISCUSSION

The non-linear relation of F_s to the undersaturation was confirmed in this study as well as in the preceding paper. As the velocity factor means the velocity of the transfer or the conductivity of O_2 from solution to Hb in the red cell, the reciprocal of the factor should indicate the resistance to the oxygenation. The reciprocal of F_c is expressed from Eq(3) as

$$1/F_c = 1/0.025 + 1/F_s^{1.26}. \quad (4)$$

The total residence to the oxygenation of Hb in the red cell may, thus, be considered to be given by the sum of both the resistances to the diffusion and the chemical reaction. The resistance to the diffusion should be independent of the S_{O_2} , therefore, the first term of Eq(4) seems to indicate the resistance to the diffusion and the second term, that to the combination reaction of O_2 and Hb in the red cell. As shown in FIG. 7, F_s is smaller than the unity, hence, $F_s^{1.26}$ is smaller than F_s itself. This leads to the conclusion that the oxygenation velocity of Hb is slower in the red cell than in the laked Hb solution.

THEWS^{12,13} stated that the oxygenation velocity of the red cell was limited only by the diffusion, because the chemical reaction of Hb was very fast. Certainly the oxygenation of Hb occurs very fast in the S_{O_2} range of 0 to 50%, but in the upper S_{O_2} range, above 70%, the velocity turns slow. Hence his treatment will not hold for the oxygenation of the range over 70%.

The relation of F_c to S_{O_2} is very important when considering the relationship of F_c to the red cell component of the pulmonary diffusing capacity, D_B . BOHR¹⁵ introduced an integral method to calculate the average P_{O_2} of the capillary blood of the lung. In his theory, he took account of only the diffusion

rate through the alveolar membrane and he assumed the diffusion rate always constant or indifferent to the back pressure, P_c . However, the oxygenation velocity is neither so rapid as to be disregarded nor it is independent of P_c . In FIG. 8 is illustrated the dependency of F_c upon the back pressure on a semi-logarithmic graph. The line in this figure was obtained from the F_c

FIG. 8. Velocity factor of the red cell, F_c , plotted against the back pressure, P_c , which was obtained from the O_2 saturation by referring to the O_2 dissociation curve.

curve in FIG. 7 by converting the undersaturation into the back pressure, P_c . As shown in FIG. 8, the logarithm of F_c was obviously linear against P_c over the P_{O_2} range of 40 to 100 mmHg. Therefore, there are two problems concerning BOHR's integral method: one is, what the average P_{O_2} obtained by his method means, and the other is whether or not the red cell component of the diffusing capacity, D_B , can be estimated by use of the relation of F_c and P_c shown in FIG. 8.

Suppose that 1 ml of red cells are travelling through the alveolar gas phase with O_2 tension, P_A . When the contact time, T_c , is divided into n time intervals, there are derived the following relations in the individual intervals similarly to Eq(1):

$$\begin{aligned}\frac{\Delta C_1}{P_A - P_1} &= F_1 \Delta t, \\ \frac{\Delta C_2}{P_A - P_2} &= F_2 \Delta t, \\ &\dots\dots\dots, \\ &\dots\dots\dots, \\ \frac{\Delta C_n}{P_A - P_n} &= F_n \Delta t,\end{aligned}\tag{5}$$

where ΔC_i , P_i and F_i are the change of O_2 content in 1 ml red cells, the back pressure and the velocity factor of the red cell.

According to BOHR the sum of the n left hand terms, $\sum_0^n C_i / (P_A - P_i)$, can be obtained by planimetry of the area, in which the abscissa is C and the ordinate is $1/(P_A - P)$. Therefore, the algebraic or time average of the n terms is given by dividing the area by n . Let P^* be the average back pressure obtained by the planimetry, then the above average is expressed by $(C_n - C_0) / n(P_A - P^*)$. On the other hand, let F_m be the time average of the velocity factor during the contact time, there holds the following relation between both the averages of the left and right hand terms of Eqs(5).

$$\frac{C_n - C_0}{P_A - P^*} = F_m \cdot T_c,\tag{6}$$

or

$$\frac{C_n - C_0}{T_c} = F_m (P_A - P^*).\tag{7}$$

Eq(7) states that the product of F_m and $(P_A - P^*)$ shows the average concentration increase during the contact time, T_c .

In FIG. 9 are shown three curves of O_2 uptakes by 1 ml red cells in a second, dC/dt , against the back pressure, when $P_A = 100$ mmHg. The first curve shows $dC/dt = F_c(100 - P_c)$, the second curve shows $dC/dt = F_m(100 - P_c)$, where F_m is the time average of F_c . The third curve is the time average of

the O_2 uptake, $F_m(100-P^*)$. Strictly speaking, the P^* must be different from the back pressure, P_m , which has such a certain relation to F_m as shown by the line of FIG. 8. However, P^* and P_m agreed practically well with each other as indicated by the intersection of these three curves in FIG. 9.

Originally BOHR's method was introduced for obtaining such a constant velocity factor as the diffusion constant. In that case, the P^* derived by his method is evidently the time average of the back pressure. When the velocity factor is not constant, the P^* calculated is not the time average. However, the F_m obtained by using the P^* through Eq(6) is really the time average of the velocity factor. And between the P^* and F_m there may hold the relation as illustrated by the line in FIG. 8.

FIG. 9. O_2 uptake of 1 ml of red cells in a second, dC/dt , plotted against the back pressure, where the alveolar O_2 tension, P_A , was assumed to be 100 mmHg and the diffusion through the alveolar membrane was disregarded. As F_c decreases with increase of P_c , the real O_2 uptake, $F_c(P_A - P_c)$, decreases exponentially as P_c increases. In Bohr's integral, the velocity factor, F_c , was treated, in the P_c range measured, as a constant averaged value, F_m . Therefore, the O_2 uptake was considered to decrease linearly as P_c increased, as shown by $F_m(P_A - P_c)$. In both cases, the average O_2 uptake, $F_m(P_A - P^*)$ agreed well with each other as shown by the intersection of those three curves.

According to GIBSON and others the total diffusing capacity, D_L , is expressed by the following,

$$1/D_L = 1/D_B + 1/D_M, \quad (8)$$

Where D_M is the membrane component of the diffusing capacity. On the other hand, according to MOCHIZUKI and FUKUOKA D_B is given by $D_B = Q \cdot F_c \cdot T_c$, where Q is the red cell volume flowing through the total lung capillary in one minute. Thus, if D_L 's are measured at two levels of F_c , the two components of D_L may be evaluated by use of F_c values shown in FIG. 8.

SUMMARY

The oxygenation velocities of the red cell and Hb were studied by means of the rapid flow method with 7 normal subjects. The SO_2 was measured by taking the difference in transmitted light intensities at two wave lengths, so that the influence of the uneven scattering could be excluded fairly well.

The oxygenation velocities were mainly measured over the SO_2 range from 70 to 100%, and they were expressed by the velocity factor. As to the velocity factor of Hb, F_s , the same result as shown in the preceding paper was confirmed in this study. That is, F_s was proportional to $(100 - SO_2)^{1.26}$. On the other hand, the velocity factor of the red cell, F_c , was clarified to be given by

$$F_c = \frac{0.025 \cdot F_s^{1.26}}{0.025 + F_s^{1.26}}.$$

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