ON THE VELOCITY OF OXYGEN DISSOCIATION OF HUMAN HEMOGLOBIN AND RED CELL

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In the preceding studies^{1,2)} it has been clarified that the velocity factor in the oxygenation of the red cell is to be subdivided into two categories, i. e. the factor related to gaseous diffusion and that related to chemical reaction between O_2 and Hb. Further, it has been realized that the reciprocal of the total velocity factor equals the sum of the reciprocals of the subdivided factors. This formula may be applied not only to the oxygenation, but also to the dissociation reaction, where the O_2 diffuses out of the red cell into the surrounding medium. The aim of the present study is to examine the applicability of the formula to the dissociation reaction on one hand and, on the other hand, to determine quantitatively the dissociation velocity of Hb in solution as well as in the red cell under physiological conditions.

In 1923, HARTRIDGE and ROUGHTON³⁾ measured the dissociation velocity of Hb solution and the red cell by using sodium hydrosulphite, $Na_2S_2O_4$, and found that the half-time of O_2 Hb dissociation in a laked Hb solution was 14 to 19 msec at room temperature and that in the red cell was 135 msec. In 1933, MILLIKAN⁴⁾ measured this velocity, following the same method, but his halftimes were twice as long as those shown by HARTRIDGE and ROUGHTON. Fifteen years later, LEGGE and ROUGHTON⁵⁾ studied the deoxygenation with ram blood; according to their report the half-times of O_2 Hb dissociation in solution were distributed around 30 msec, while those of the red cell were 170 to 210 msec at pH 7.1 and 140 to 160 msec at pH 6.8. Recently, LAWSON, HOLLAND and FORSTER⁶⁾ measured the deoxygenation velocity of the red cell by combining the dithionite method and a stopped flow method elaborated by SIRS and ROUGHTON.⁷⁾ The half-times they found were markedly shorter, being 34 msec at 37°C and 87 msec at 6.8°C. In 1955, GIBSON and others⁸⁾ calculated the dissociation velocity by measuring the displacement reaction of O_2 Hb + CO and obtained 28 sec⁻¹ on an average, which corresponded to a time constant of 36 msec. Thus, the half-time of the dissociation was roughly estimated as about 23 msec. Those data which appeared in literatures are

Received for publication March 1, 1966

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TABLE 1.
Half-times of the dissociation reaction measured by previous workers.

Species	Temp. (°C)	Half-time (msec)		Method	Author
		Solution	Red cell		
Sheep	15	35	135	adding $\text{Na}_2\text{S}_2\text{O}_4$	ROUGHTON (1926) ³⁾
"	22	14		"	"
Man	22	19		"	"
Sheep	22	28		"	MILLIKAN (1933) ⁴⁾
Man	22	38		"	"
Pig	22	47		"	"
Ram	15	30	140	"	LEGGE & ROUGHTON (1949) ⁵⁾
"	13.5	30-50	160-210	"	"
"	14	30	120-170	"	"
Man	18	23		adding CO in O_2Hb	GIBSON, et al. (1955) ⁸⁾
"	37		34	adding $\text{Na}_2\text{S}_2\text{O}_4$	LAWSON, et al. (1964) ⁶⁾
"	6.8		13	"	"
"	37		13	mixing with low PO_2 sol.	NAKAMURA & STAUB (1964) ¹⁰⁾

summarized in TABLE 1.

In the method using dithionite the O_2 tension in the medium was neglected, and, furthermore, according to GIBSON⁹⁾ there was a side reaction between the oxidation products of dithionite and Hb. Therefore, the experimental conditions in the dithionite method seems to be far different from the physiological conditions to be considered in the peripheral capillaries. In 1964, NAKAMURA and STAUB¹⁰⁾ measured the deoxygenation of the red cell by use of PO_2 measurement by a Pt electrode. The half-times observed were very short and were ranged from 16 to 10 msec. These values were 1/2 to 1/3 of the shortest values so far obtained.

METHOD

A similar method described in the previous paper²⁾ was used in this experiment, except that compositions of the gases used for tonometry were different and the oxygenated Hb solution as well as the oxygenated red cell suspension were mixed with O_2 free buffer solution. The PO_2 and PCO_2 of the mixed gases are tabulated in TABLE 2. PO_2 's of higher oxygen group were 23 and 32 mmHg in case of Hb solution and were 88, 68 and 61 mmHg in case of the red cell experiment. PO_2 's of lower oxygen group were 1.6 and 3.2 mmHg. PCO_2 was kept at about 40 mmHg throughout this experiment. pH of Hb solution was 7.26 and that of the red cell suspension was 7.40.

TABLE 2.
Compositions of mixed gases used for tonometry.

	No.	Number of Subjects	Date	High P_{O_2} gas (mmHg)		Low P_{O_2} gas (mmHg)	
				P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}
Hb	1	3	Nov. 18-22	32	40	1.6	39
	2	4	Nov. 27-28	23	40	3.2	40
			Dec. 8				
Red cell	1	3	Oct. 7-8	88	42	1.6	46
	2	4	Oct. 10-13	68	35	3.2	40
	3	3	Oct. 11-14	61	39	1.6	46

FIG. 1. Changes of S_{O_2} due to the dissociation of O_2Hb in laked Hb solution. The numbers on the curve show the experiment number of TABLE 2.

RESULTS

1) *Hb solution.* The deoxygenation was measured with samples collected from three or four normal subjects and in the two cases of No. 1 and 2 of different S_{O_2} range as shown in TABLE 1. The S_{O_2} changes plotted along the reaction time are shown in FIG. 1. The plots are the means of 6 or 8 respective measurements and the arrows show the standard deviations around the means. The deviations were 1.0 to 2.3% in S_{O_2} .

The velocities shown in this figure were very fast in comparison with the data previously measured. Two thirds of the changes of S_{O_2} were finished within 5 msec after mixing. The S_{O_2} decreased at first exponentially with a lapse of time. As the reaction proceeded, however, it deviated from the exponential curve. Actually, the changing rate, dS/dt , was proportional to the 1.6th power of the difference between the real S_{O_2} of Hb, S , and the supposed S_{O_2} corresponding to the P_{O_2} of the surrounding medium, S_m , as shown in FIG. 2. The S_m was obtained from the P_{O_2} in the medium by referring to the O_2 dissociation curve of Hb solution shown in FIG. 5 of the previous paper¹⁾. The

FIG. 2. Time rate of S_{O_2} changes, dS/dt , plotted against the difference between the real S_{O_2} of Hb, S , and the supposed S_{O_2} corresponding to P_{O_2} of the surrounding medium, S_m , which was obtained by referring to the O_2 dissociation curve published on the previous paper.¹⁾

relation shown in FIG. 2 is expressed numerically by

$$dS/dt = 63.4 (S - S_m)^{1.6}. \quad (1)$$

As is clear from Eq(1), the deoxygenation velocity had no distinct relation to the P_{O_2} difference between the medium and Hb, that is, to the difference between the real P_{O_2} and the back pressure. The latter P_{O_2} , P_{Hb} , was obtained from the O_2 dissociation curve of Hb solution by referring to the S_{O_2} . In other words, the velocity factor of deoxygenation in Hb solution, F_s' , which was obtained according to the following formula (refer to Eq(1) in the previous paper²⁾) was no longer independent of the P_{O_2} difference, $P_{Hb} - P$, where P represents the real P_{O_2} of solution.

$$F_s' = - \frac{0.4 \times 10^{-2} \cdot dS/dt}{P_{Hb} - P}. \quad (2)$$

FIG. 3. Deoxygenation velocity factor of Hb, F_s' , plotted against the reaction time. The velocity factor was obtained through

$$F_s' = - \frac{0.4 \times 10^{-2} \cdot dS/dt}{P_{Hb} - P},$$

where P_{Hb} was derived from the O_2 dissociation curve of pH 7.26 by referring to S_{O_2} of Hb.

The F_s' decreased with decrease of the P_{O_2} difference, that is, it was high at the beginning of the reaction and decreases gradually as shown in FIG. 3. The F_s' values in FIG. 3 were calculated at every 2.5 msec intervals along two curves of FIG. 1.

On the other hand, it was a function of S_{O_2} ; it increased as S_{O_2} decreased. However, it did not become smaller than $0.5 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ even at the S_{O_2} range over 90%. In other words, the velocity factor in the deoxygenation of $O_2\text{Hb}$ in solution was always greater 20 times that of the velocity factor for O_2 diffusion inside the red cell, $0.025 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ which was observed in the preceding paper²⁾.

2) *Red cell suspension.* The deoxygenation velocity of the red cell was measured with five normal subjects under three conditions as shown in TABLE

FIG. 4. Changes of S_{O_2} of the red cell due to the dissociation reaction. The two curves bordering the shaded areas were obtained by numerical integration of

$$dS/dt = F_c'(P_c - P)/0.4 \times 10^{-2},$$

where F_c' 's were constantly taken as $0.02 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ for the upper curves and $0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ for the lower curves.

2. The changes of S_{O_2} are plotted in FIG. 4 against the reaction time. The standard deviations around the means around the means plotted were about 1.2% in S_{O_2} . Each of the three series of plotted points was almost involved inside the respective shaded area between the two border curves. These curves were obtained by numerical integration of the following equation,

$$dS/dt = F_c'(P_c - P)/0.4 \times 10^{-2}, \quad (3)$$

where P_c is P_{O_2} in the red cell, P that of the medium and F_c' is the velocity factor of deoxygenation of the red cell. In calculation of the upper curve in FIG. 4, F_c' was constantly taken as $0.02 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ and in that of the lower curves it was $0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$. In the No. 1 and 2 experiments the plotted points lined up almost along the curve of $F_c' = 0.02 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$, but in the third experiment, the points were distributed between the upper and lower curves. Obviously the deoxygenation in the last case was faster than in other cases. On the other hand, the deoxygenation velocity in each case seemed to be greater at the beginning of the reaction, for the plotted S_{O_2} points at the early time were always closer to the curve of $F_c' = 0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$ than at the later time of reaction. This fact is supposed to reflect the characteristic of F_s' shown in FIG. 3.

In general, the deoxygenation took longer time as S_{O_2} decreased. Hence, the half-time grew longer with the decrease of S_{O_2} . The half-times obtained from the three curves from top to bottom in FIG. 4. were 35, 50 and 65 msec respectively. These values were similar to those shown by LAWSON and others. However, they are not so long as those of ROUGHTON and his coworkers.

DISCUSSION

The deoxygenation velocity has widely been measured by ROUGHTON and his group, but it was always measured in a solution with dithionite. Hence, it may be reasonable that the data presented here are largely different from theirs. The main differences between ours and theirs may be focussed on two points, i.e. 1) the rapidity of the reaction, and 2) the dependency of the velocity upon S_{O_2} , as far as the dissociation reaction of $O_2\text{Hb}$ in laked solution was concerned.

The dissociation velocity of $O_2\text{Hb}$ in laked solution was very fast and the reaction was completed within 40 msec. The half-time of dissociation was less than 5 msec. On the other hand, the rate of S_{O_2} change, dS/dt , was not linearly proportional to S_{O_2} , but to the 1.6th power of the S_{O_2} difference between the surrounding medium and Hb, $(S - S_m)$. In other words, the deoxygenation of $O_2\text{Hb}$ has also a non-linear relation against the S_{O_2} . As described in the foregoing papers^{1,2)}, the velocity factor of the oxygenation of Hb in solution was given experimentally by the following equation,

$$F_s = 1.06 \times 10^{-3} \cdot (100 - S_{O_2})^{1.6}.$$

Therefore, the number of the power, 1.6 just agreed in both the reactions of oxy- and deoxygenation.

As to the deoxygenation of the red cell, it must be emphasized that the S_{O_2} points plotted in FIG. 4 were distributed between two S_{O_2} -time curves calculated with $F_c' = 0.02$ and $0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$. FIG. 6 in the preceding paper²⁾ showed that the velocity factor of its oxygenation was in the range from 0.02 and $0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$, when the combination reaction of O_2 and Hb was very fast. Analogously, since the velocity factor for the diffusion should be independent of the direction of diffusion, the velocity factor of deoxygenation was expected to approach some value between 0.02 and $0.03 \text{ sec}^{-1} \cdot \text{mmHg}^{-1}$, when the dissociation of $O_2\text{Hb}$ would occur very fast. In reality, from FIG. 4 it is conceivable that the dissociation velocity was very fast, and, thus, the velocity factor agreed well with the diffusion factor.

As seen in TABLE 1, the half-times of deoxygenation of the red cell obtained by NAKAMURA and STAUB were 1/3 to 1/5 of those obtained in this study. This difference seems to be due to the difference in the method used. In the method using the P_{O_2} measurement, P_{O_2} in solution must change with the lapse of the reaction time, in other words, the concentration of red cells in suspension should be increased high enough as P_{O_2} change could be detected. Thus, the P_{O_2} increase caused by the S_{O_2} decrease might result in an early stop of the reaction, namely, a shorter half-time.

Apart from the kinetics of the deoxygenation, the velocity factor of deoxygenation is very important in considering the velocity of O_2 supply in the peripheral capillary. In 1922, KROGH¹¹⁾ studied the O_2 diffusion in tissues using a model of a standard cylinder. In his study, the deoxygenation of the red cell was assumed to be so rapid as to be disregarded. Actually, however, the P_{O_2} in blood plasma must be, more or less, lower than that of the red cell. In general, it is very difficult to calculate the P_{O_2} distribution in the standard cylinder, as stated by THEWS¹²⁾. However, a rough estimation of the average P_{O_2} difference between the red cell and the blood plasma may be attained from Eq(3) as follows.

Let the volume of red cells flowing per min through the tissue of 1 ml be Q , the contact time, T_c and the average P_{O_2} difference between the red cell and the plasma, $\overline{P_c - P_p}$, then the O_2 consumption of the tissue per min, $\dot{V}_{O_2}$, may be given by

$$Q \cdot T_c \cdot F_c' (\overline{P_c - P_p}) = \dot{V}_{O_2}. \quad (4)$$

Therefore, if $F_c' = 0.025 \text{ sec}^{-1} \text{ mmHg}^{-1}$, $Q = 0.05 \text{ ml} \cdot \text{min}^{-1}$, $T_c = 0.5 \text{ sec}$, O_2 consumption = 0.5 Vol % of the tissue, then the average, P_{O_2} difference $\overline{P_c - P_p}$, will be calculated as 6.4 mmHg. According to KROGH the P_{O_2} difference between the capillary surface and the points at the greatest distance from the

capillary was about 40 mmHg. Hence, the difference of 6.4 mmHg is considered not to be too small.

SUMMARY

The deoxygenation velocities of Hb solution and the red cell suspension have been measured at 37°C by mixing the oxygenated Hb and red cells with an O₂ free solution.

The dissociation of O₂Hb in hemolysed solution occurred very fast and the half-time was within 5 msec. Moreover, it was clarified that the rate of change in S₀, of Hb solution obeyed the following formula,

$$dS/dt = 63.4(S - S_m)^{1.6},$$

where S_m is the S₀, corresponding to P₀, of the surrounding medium.

In the red cell suspension, the reaction proceeded comparatively slowly and the half-times were ranged from 35 to 65 msec. The rate of S₀, change was expressed by use of the velocity factor, F_c', as follows:

$$dS/dt = F_c'(P_c - P_m)/0.4 \times 10^{-2},$$

where P_c and P_m are the P₀, in the red cell and the medium respectively. F_c' seemed to be fairly constant and it did not deviate out of the range of 0.02 to 0.03 sec⁻¹ · mmHg⁻¹.

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