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A New Look on the Position of the Oxygen Equilibrium Curve of Human Adult Hemoglobin at Rest and during Exercise with Special Reference to the Effectiveness of the Bohr Shift

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ABSTRACT—The position of oxygen equilibrium curve (OEC) for human adult Hb at rest is optimized with respect to the effectiveness of the Bohr shift which is measured by the change in O_2 saturation at a venous O_2 pressure of 40 torr per unit change in partial pressure of O_2 at half saturation ($dS_{(40)}/dP_{50}$). The effectiveness of the Bohr shift at the physiological P_{50} of 27 torr depends on the cooperativity of O_2 binding calculated by the Hill coefficient *n*, being maximized at $n = 4$.

The effectiveness of the Bohr shift during exercise, which was expressed as $dS_{(PvO2)}/dP_{50}$, was the highest at the PvO_2 (venous O_2 pressure) of 28 torr. The effectiveness of the Bohr shift at PvO_2 of 28 torr increased with increases *n* value (*n* value ranged from 2.65 to 3.27), while below PvO_2 of 15 torr the opposite was true.

As a whole, the position of the OEC of human adult Hb at rest is optimized with respect to the effectiveness of the Bohr shift while the efficiency of O_2 delivery is moderately maintained. On the other hand, during exercise the position of the OEC is adjusted to make the efficiency of O_2 delivery at high levels while the effectiveness of the Bohr shift is maintained at the same level as that at rest.

INTRODUCTION

The effect of carbon dioxide (CO_2) on the O_2 affinity of Hb was first described by Bohr *et al*. (1904). An increase in the partial pressure of CO_2 or a decrease in pH lowers the O_2 affinity of Hb, shifting the O_2 equilibrium curve (OEC) to the right. This effect caused by PCO_2 change and concomitant pH change is called the "classic Bohr effect" while the shift of OEC by pH change only is called the "Bohr effect". The magnitude of the Bohr effect is given by the change in log P_{50} (P_{50} is the O_2 pressure at which Hb is 50% saturated with O_2) per unit change in pH and is expressed as $\Delta\log P_{50}/\Delta pH$ (the Bohr coefficient). Under the physiological conditions, the Bohr coefficient for human adult Hb is -0.48. A moderate rightward shift of the OEC gives rise to additional O_2 unloading from Hb without any change in the ambient PO_2. The amount of O_2 released from Hb to the tissues upon a rightward shift of the OEC depends not only on the Bohr coefficient, but also on the position and the shape of the OEC (Kobayashi *et al*., 1996). In the previous paper, we calculated the O_2 saturation difference between PO_2 of 100 and 40 torr ($\Delta S_{(100-40)}$) and plotted it against P_{50} for human adult Hb, and its differentiation, which was considered to indicate the effectiveness of the Bohr shift, was maximized when P_{50} was 28 torr. This P_{50} is close to the physiological P_{50} of the Hb (27 torr). However, the $d\Delta S_{(100-40)}/dP_{50}$ vs. P_{50} plot intersected the abscissa at $P_{50} = 62$ torr. These phenomena can be explained by the large decreases in O_2 saturation of the arterial blood (PO_2 of 100 torr) caused by increase in P_{50}. This seems to imply that it is not adequate to use the slope of $\Delta S_{(100-40)}$ vs. P_{50} plot for accurate estimation of the effectiveness of the Bohr shift.

For this reason, in the present study the O_2 saturation of the venous blood ($S_{(PvO2)}$) was plotted against P_{50}, and additional O_2 released from Hb to the tissues caused by the Bohr shift was estimated from the slope of this plot ($dS_{(PvO2)}/dP_{50}$) at rest and during exercise. Based on the results obtained, we propose a new look on the physiological significances of the position and shape of the OEC for human adult Hb that had never been noticed by others. The present analysis which is

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concerned with the effect of pH change can be equally applied to the effect of PCO_2 change. Therefore the term "Bohr shift" used in this study includes both the effect of pH and that of PCO_2 which vary concomitantly in whole blood.

MATERIALS AND METHODS

The experimental data used for the present analysis were taken from the published standard OEC for human adult whole blood (Hohammed Mawjood and Imai, 1999), as well as the OECs for human adult Hb solutions that were measured under various experimental conditions (Imai, 1982; Imai and Yonetani, 1975; Imaizumi *et al*., 1982; Tyuma *et al*., 1973). All these OECs were described by the Adair equation (Adair, 1925). According to the equation, the O_2 saturation (S) is expressed as a function of the partial pressure of O_2 (P) as follows:

$$S = (k_1P+3k_1k_2P^2+3k_1k_2k_3P^3+k_1k_2k_3k_4P^4) / (1+4k_1P+6k_1k_2P^2+4k_1k_2k_3P^3+k_1k_2k_3k_4P^4) \quad (1)$$

where, ki (i = 1 to 4) is the intrinsic association constant at the ith oxygenation step (the stepwise Adair constant). The Adair constant values for the standard OEC are k_1 = 0.0037 torr^{-1}, k_2 = 0.047 torr^{-1}, k_3 = 0.012 torr^{-1}, and k_4 = 1.1 torr^{-1} (P_{50} = 26.7 torr, n = 2.65 at 37°C, pH 7.4 and PCO_2 of 40 torr; Hohammed Mawjood and Imai, 1999; Imai personal communication). The hypothetical OECs with different P_{50} values were constructed from the published set of four Adair constants, which were varied by multiplication with a common factor, *i.e*., ki · constant (i = 1 to 4). On this multiplication, the position of the hypothetical OEC, that was expressed by S vs. log P plot, was shifted along the abscissa without any change in shape.

The effect of change in cooperativity (n) on the effectiveness of the Bohr shift were investigated in a wide range of n value, using Hill's empirical equation (Hill, 1910),

$$S = P^n/(P^n + P_{50}{}^n) \quad (2)$$

where n is a quantitative expression of the cooperativity of O_2 binding (the degree of sigmoidicity of OEC).

RESULTS AND DISCUSSION

Calculation of O_2 transported by Hb and the effectiveness of the Bohr shift

The total amount of O_2 available for the tissues is determined by the quantity of O_2 delivered by unit volume of the blood and the rate of blood flow. In this paper, the rate of blood flow was not considered, because it is a separate subject concerning the regulatory system of circulation. Fig. 1 illustrates the standard OEC for the arterial whole blood (37°C, pH 7.4 and PCO_2 of 40 torr) of human adult. The quantity of O_2 transported by Hb is defined by the O_2 saturation difference between at PO_2 of 100 torr and of 40 torr ($\Delta S_{(100-40)}$). The rightward shifted OEC (broken line) stands for the venous blood. The amount of additional O_2 released from Hb caused by the Bohr shift is defined by the O_2 saturation difference between arterial and venous blood at PO_2 of 40 torr, and designated as ($S_{(40)a}$–$S_{(40)v}$). Total quantity of O_2 delivered by Hb is given by $\Delta S_{(100-40)}$ plus ($S_{(40)a}$–$S_{(40)v}$).

Fig. 2 shows the relation between $\Delta S_{(100-40)}$ and P_{50}, and between $S_{(40)}$ and P_{50}. As shown by the broken line, the O_2

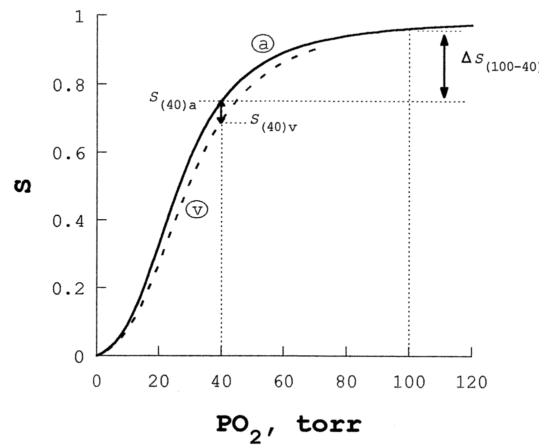

Fig. 1. The O_2 equilibrium curves of human adult Hb, showing the relationship of blood O_2 transport. The full curve (solid line, ⓐ) applies to arterial blood, while the broken line applies to venous blood (ⓥ). $S_{(40)a}$ and $S_{(40)v}$ represent the O_2 saturation of arterial and venous blood at PO_2 = 40 torr, respectively. $\Delta S_{(100-40)}$ represents the O_2 transported by blood without the Bohr shift. ($S_{(40)a}$–$S_{(40)v}$) is the additional O_2 released from Hb caused by the Bohr shift. $\Delta S_{(100-40)}$ plus ($S_{(40)a}$–$S_{(40)v}$) are the total quantity O_2 delivered by blood from the lungs to the tissues. The standard OEC data set of human adult whole blood, which was measured under physiological conditions, 37°C, pH 7.4 and a PCO_2 of 40 torr, taken from Hohammed Mawjood and Imai (1999), was used for arterial blood while the curve for venous blood was calculated from the Adair constant values of the standard OEC.

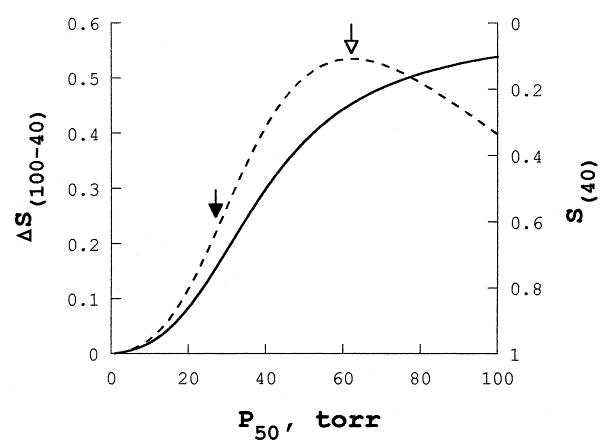

Fig. 2. The effect of P_{50} on the $\Delta S_{(100-40)}$ and the $S_{(40)}$. The broken line represents the dependence of the O_2 saturation difference between at PO_2 = 100 and PO_2 = 40 torr ($\Delta S_{(100-40)}$) on P_{50}. The solid line represents the relationship between $S_{(40)}$ and P_{50}. The open arrow indicates the maximal $\Delta S_{(100-40)}$ value, and the closed arrow indicates the $\Delta S_{(100-40)}$ at physiological P_{50} of 27 torr. All the calculated lines were obtained using the four Adair constants of the OEC in fig. 1.

transported by Hb increased gradually with increases in P_{50}, reaching its maximal value at P_{50} of 62 torr. The P_{50} value that gives the maximal O_2 transport may be called the "optimal P_{50} for O_2 transport" (Sold, 1982; Willford *et al*., 1982). At the physiological P_{50} value for adult whole blood (P_{50} = 27 torr), the O_2 transported by Hb was relatively low ($\Delta S_{(100-40)}$ = 0.22). This value was about two-fifths that of the theoretical maximal value ($\Delta S_{(100-40)}$ = 0.54). As shown by the solid line, the $S_{(40)}$

value decreased with increases in P_{50}, and the $S_{(40)}$ vs. P_{50} plot was sigmoid.

Effectiveness of the Bohr shift at rest

As the venous PO_2 of 40 torr, the change in $S_{(40)}$ per unit change in P_{50} ($dS_{(40)}/dP_{50}$), was calculated from the slope of the $S_{(40)}$ vs. P_{50} plot to estimate the effectiveness of the Bohr shift (Fig. 3). The $dS_{(40)}/dP_{50}$ value depended on P_{50}, and the minimal value (the highest effectiveness of the Bohr shift) occurred at P_{50} of 30 torr (the solid line, Fig. 3). This indicates that when the P_{50} value is adjusted to 30 torr, the quantity of additional O_2 released from Hb to the tissues caused by the Bohr shift is maximized. When the P_{50} value is either above or below 30 torr, the $dS_{(40)}/dP_{50}$ value becomes smaller. Therefore, the P_{50} value that gives the minimal $dS_{(40)}/dP_{50}$ value may be called the "optimal P_{50} for the effectiveness of the Bohr shift". It should be noted that there is only a slight difference in the $dS_{(40)}/dP_{50}$ value between at the optimal P_{50} and at the physiological P_{50}. It is, therefore, concluded that the position of the OEC at rest is optimized to achieve the maximal action of the Bohr effect.

The $dS_{(40)}/dP_{50}$ vs. P_{50} plot was compared with the previously reported $d\Delta S_{(100-40)}/dP_{50}$ vs. P_{50} plot (the broken line, Fig. 3). The two optimal P_{50} values for the effectiveness of the Bohr effect in both of the plots are almost equal. These plots deviated from each other more at high P_{50} values, and the $d\Delta S_{(100-40)}/dP_{50}$ vs. P_{50} plot intersected the abscissa at P_{50} = 62 torr. The reason for the zero and negative values of $d\Delta S_{(100-40)}/dP_{50}$ above P_{50} = 62 torr can be ascribed to the substantial decreases in the O_2 saturation at 100 torr with increases in P_{50}. A zero value of additional O_2 released from Hb indicates that the Bohr shift has no effect. On the other hand, $dS_{(40)}/dP_{50}$ value tends to zero but never intercepts the abscissa even at high P_{50} values. From these points of view, the $dS_{(40)}/dP_{50}$ is more rational for accurate estimation of the effectiveness of the Bohr shift.

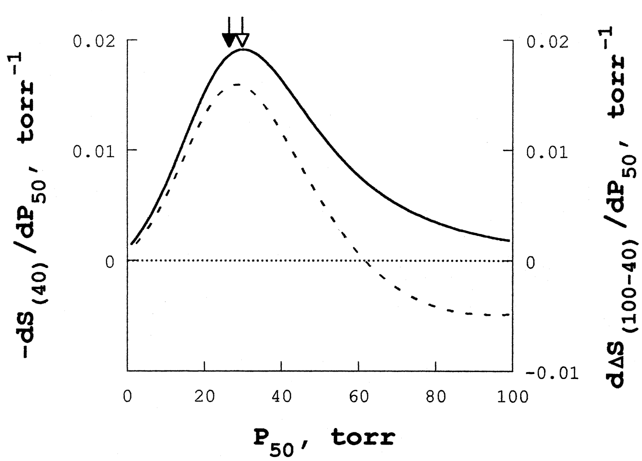

Fig. 3. Change in $S_{(40)}$ per unit change in P_{50} ($dS_{(40)}/dP_{50}$) as plotted against P_{50} (solid line). The open arrow indicates the minimal $dS_{(40)}/dP_{50}$value, and the closed arrow indicates the $dS_{(40)}/dP_{50}$ value at the physiological P_{50} of 27 torr. Note that the $dS_{(40)}/dP_{50}$ value is negative. The $d\Delta S_{(100-40)}/dP_{50}$ vs. P_{50} plot (broken line) was shown for comparison.

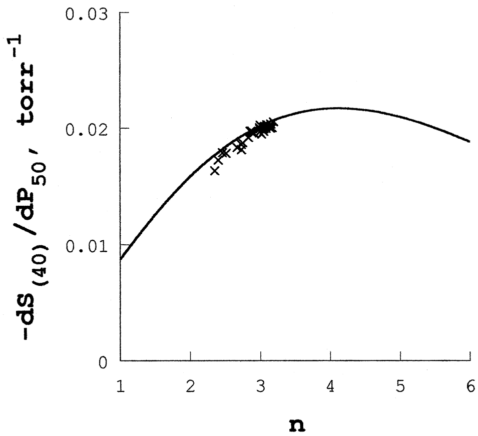

Fig. 4. The effect of cooperativity (n) on the $dS_{(40)}/dP_{50}$ at the physiological P_{50} of 27 torr. The $dS_{(40)}/dP_{50}$ values (×) were calculated using Adair equation from the human Hb OEC data sets taken from Imai (1982), Imai and Yonetani (1975), Imaizumi *et al.* (1982) and Tyuma *et al.* (1973). Data represented by the solid lines was calculated using Hill's equation.

The effect of cooperativity (n) on $dS_{(40)}/dP_{50}$ was investigated using the Adair equation and the Hill equation over a wide range of n value, from 1 to 6 (Fig. 4). The $dS_{(40)}/dP_{50}$ value calculated with physiological P_{50} of 27 torr decreased with increases in n, reaching the minimal value at about $n = 4$ (note that the $dS_{(40)}/dP_{50}$ value is negative). This phenomenon is interesting because the molecule of human adult Hb has a tetrameric structure.

In the lungs, pH increases with lowering of CO_2 pressure, the OEC moves to the left and enhances the O_2 loading. To estimate the effectiveness of the Bohr shift in arterial blood, the same calculation was applied to the arterial blood (PO_2 = 100 torr) to obtain $dS_{(100)}/dP_{50}$ values. The degree of the effectiveness of the Bohr shift at physiological P_{50} of 27 torr was very low, about one-seventh that of the venous blood. This implies that the Bohr shift seems to be much less important for O_2 loading at the lungs.

The effectiveness of the Bohr shift during exercise

As the venous O_2 pressure (PvO_2) falls to 20 torr during exercise, the effectiveness of the Bohr shift ($dS_{(PvO2)}/dP_{50}$) was calculated at physiological P_{50} (27 torr) in the range of PvO_2 from 10 to 55 torr (Fig.5). The $dS_{(PvO2)}/dP_{50}$ value was a convex function of PvO_2, and the minimal value was observed at an intermediate PvO_2 of 28 torr: at this PvO_2 the Bohr shift is most effective. The $dS_{(PvO2)}/dP_{50}$ value at PvO_2 of 20 torr is approximately equal to that at rest where PvO_2 is 40 torr. When PvO_2 is about 35 torr, the theoretical minimal $dS_{(PvO2)}/dP_{50}$ shown by broken line is equal to that of at physiological P_{50} of 27 torr.

The effectiveness of the Bohr shift at the intermediate PvO_2 (28 torr) increased with increases in n value. The reverse effect of n was observed below PvO_2 of 15 torr. The strong dependence of $dS_{(PvO2)}/dP_{50}$ on PvO_2 at low PvO_2 values seem to explain the lower critical point of PvO_2 during hard exercise.

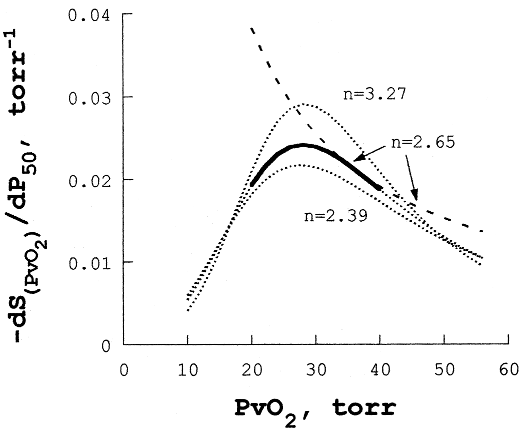

Fig. 5. The dependence of $dS_{(PvO2)}/dP_{50}$ value on PvO_2 value as varied from 10 to 55 torr at P_{50} of 27 torr. Bold solid line overlaid on the dotted line of $n = 2.65$ represents the $dS_{(PvO2)}/dP_{50}$ value under physiological O_2 condition (PvO_2 ranges from 40 to 20 torr). Dotted line represents the $dS_{(PvO2)}/dP_{50}$ value at $n = 2.39$ and 3.27. The broken line represents the minimal $dS_{(PvO2)}/dP_{50}$ value at the optimal P_{50} for the effectiveness of the Bohr shift and physiological n value of 2.65. The OEC data set of $n = 2.65$ was the same as that of fig. 1, and the OEC data set of n of 2.39 and 3.27 were taken from Imai (1982).

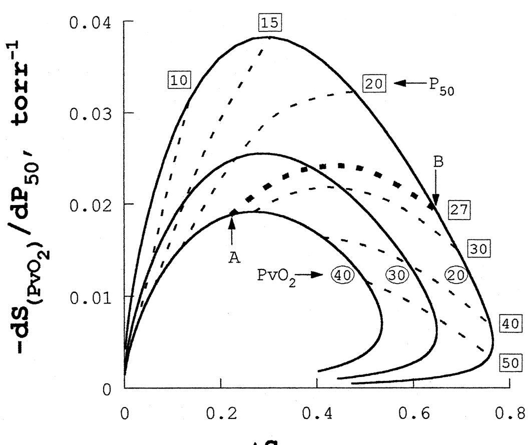

Fig. 6. The relationships between $dS_{(PvO2)}/dP_{50}$ and $\Delta S_{(100-PvO2)}$ at various PvO_2 and P_{50} values. The numbers in the circles represent the PvO_2 values; the numbers in the squares represent the p_{50} values. The OEC data set used for the calculation was the same as that of fig. 1 ($n = 2.65$). The solid lines represents the relationships when P_{50} is varied with PvO_2 fixed at either 20, 30 or 40 torr. The thin broken lines represent the relationships when PvO_2 is varied with P_{50} fixed at the value indicated. The bold broken line represents the relationship at physiological P_{50} of 27 torr. Point A represents the PvO_2 at rest, and point B represents the PvO_2 at hard exercise.

Fig. 6 illustrates the relationship between the effectiveness of the Bohr shift ($dS_{(PvO2)}/dP_{50}$) and the ability of O_2 transport ($\Delta S_{(100-PvO2)}$) at various P_{50} values at three different PvO_2 levels of 40, 30 and 20 torr (PaO_2 was fixed at 100 torr). As seen in the figure, relatively higher O_2 affinity is advantageous for the effectiveness of the Bohr shift, while lower O_2 affinity tends to be advantageous for O_2 transport. The trace of the data point as PvO_2 is varied with physiological P_{50} of 27 torr is shown by the bold broken line. The position of the OEC at rest ($P_{50}<PvO_2<PaO_2$) is optimized with respect to the effectiveness of the Bohr shift while the efficiency of O_2 transport is moderately maintained (point A). On the other hand, during exercise ($PvO_2<P_{50}<PaO_2$), the physiological P_{50} tends to be advantageous for the ability of O_2 transport while the effectiveness of the Bohr shift is remained at the same level as that at rest (point B).

In summary, the quantitative analysis performed in our present study gave several new aspects of physiological importance. The OEC is adjusted to such the position that the Bohr shift is optimized at rest while the O_2 transport is made highly efficient during exercise remaining the degree of the effectiveness of the Bohr shift at the same level as that at rest. These situations seem to be the results from the molecular adaptation, involving fine tuning of the allosteric properties, of the tetrameric human Hb to the *in vivo* O_2 environments and the circulation regulatory system.

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