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The Cooperativity of Human Fetal and Adult Hemoglobins is Optimized: A Consideration Based on the Effectiveness of the Bohr Shift

Yan Zhang¹, Makoto Miki¹, Keisuke Sasagawa¹, Michisuke Kobayashi², Kiyohiro Imai³
and Michiyori Kobayashi^{4*}

¹Graduate school of Science and Technology, Niigata University

²Department of Physics, Faculty of Science, Niigata University

³Laboratory of Nanobiology, Graduate School of Frontier Biosciences, Osaka University,
Suita, Osaka, 565-0871 Japan

⁴Department of Biology, Faculty of Science, Niigata University, Niigata 950-2181 Japan

ABSTRACT—The physiological significance of the cooperativity of human hemoglobin (Hb) is considered from the viewpoint of the effectiveness of the Bohr shift at the sites of O₂ release and uptake across the placental membrane. The effects of the Bohr shift was examined by changing the O₂ saturation of Hb ($S_{(PO_2)}$) per unit change in P_{50} , $-dS_{(PO_2)}/dP_{50}$, where P_{50} is partial pressure of O₂ at half saturation. The Bohr shift at the sites of O₂ uptake and release was found to be highly effective in both fetal and maternal bloods at physiological degree of cooperativity (Hill's coefficient, $n=2.65$). From the results obtained in this paper, it is concluded that the positions of OECs of fetal and maternal Hbs are regulated to receive a maximal benefit from the Bohr shift, and that a relatively low n value of human tetrameric Hb is adequate for the O₂ and CO₂ exchange across the placental membrane.

Key words: hemoglobin, oxygen affinity, Bohr effect, cooperativity, oxygen equilibrium curve

INTRODUCTION

Hemoglobin (Hb) combines with O₂ reversibly. The oxygen equilibrium curve (OEC) of Hb, that is a plot of O₂ saturation (S) vs. partial pressure of O₂ (PO_2 or P), is characterized by its position and degree of sigmoidicity. The position or O₂-affinity of the OEC is represented by the oxygen pressure at half saturation (P_{50}), while the sigmoidicity or cooperativity can be expressed by Hill's coefficient (n_{max}) as the highest slope of the Hill plot of $\log(S/(1-S))$ vs. $\log P$ (Hill, 1910).

The O₂ affinity of Hb can be modified by carbon dioxide. An increase in the partial pressure of carbon dioxide (PCO_2) with a concomitant decrease in pH reduces the O₂ affinity of Hb. This modulation is known as the "classical Bohr effect" (Bohr *et al.*, 1904). Changes in pH without those in PCO_2 can also modulate O₂ affinity, and this effect is simply called the "Bohr effect". The magnitude of the Bohr effect is given by the change in $\log P_{50}$ per unit change in pH ($\Delta \log P_{50}/\Delta pH$) as Bohr coefficient. In the physiological pH range, the

Bohr coefficient for human Hb is -0.48 (Severinghaus, 1966). The rightward shift of the OEC upon the lowering of pH (the Bohr shift) facilitates the release of O₂ into fetal tissues without any change in the ambient O₂ pressure. The additional amount of O₂ released from Hb by the Bohr shift depends not only on the magnitude of the Bohr coefficient, but also on the position and the steepness of the OEC. In our previous study, the Bohr shift-dependent additional amount of O₂ released from human adult Hb in the venous blood (at $PO_2 = 40$ torr) was calculated in order to estimate the effectiveness of the Bohr shift (Itoh *et al.*, 2001). As a result, the position of the OEC of human adult Hb was found to be optimized so as to receive a maximal benefit from the Bohr effect.

In the case of fetus, the PO_2 condition of the blood is restricted to a very low and narrow range (from 35 to 15 torr) compared to that of the maternal blood. Therefore, the role of the Bohr effect in fetal respiration seems to be especially important. The lowering of pH caused by the production of CO₂ in tissues induces a rightward shift of the OEC, which in turn facilitates the release of O₂ from the Hb. The diffusion of CO₂ from fetal blood into maternal blood across the placental membrane causes such a shift to the maternal blood OEC, promoting the release of O₂ from maternal blood. On

* Corresponding author: Tel. +81-25-262-6154;
FAX. +81-025-262-6154.
E-mail: komichi@bio.sc.niigata-u.ac.jp

the other hand, a reduction in fetal blood PCO_2 causes a leftward shift of the fetal blood OEC. The simultaneous rightward shift of the maternal blood OEC (with consequent decrease in O_2 saturation) and leftward shift of the fetal OEC (with consequent increase in O_2 saturation) facilitate the diffusion of O_2 from maternal blood to fetal blood. This phenomenon is known as the "double Bohr effect". It is thus important to examine the effectiveness of the Bohr shift at O_2 uptake site in fetal blood and compare it with that at O_2 release site in maternal blood.

Under physiological conditions, the O_2 affinity of Hb may be largely altered, but the cooperativity essentially remains constant. The physiological significance of the magnitude of cooperativity and its constancy in tetrameric Hb has not yet been fully explained. The aim of this paper is to present a theoretical treatment for the influence of cooperativity on the Bohr shift in O_2 uptake and release process in the placenta, and to understand the physiological significance of the rather low cooperativity of the tetrameric Hb.

MATERIALS AND METHODS

All the OEC data used in this study were taken from the previously published data for human fetal and adult Hb solutions measured under various experimental conditions (Imai, 1982; Imai and Yonetani, 1975; Imaizumi *et al.*, 1982; Tyuma *et al.*, 1973). These OEC data can be described by Adair's intermediate compound theory (Adair, 1925). According to the theory, the O_2 saturation of Hb is expressed as a function of p as follows:

$$S = (a_1 P + 2a_2 P^2 + 3a_3 P^3 + 4a_4 P^4) / (4(1 + a_1 P + a_2 P^2 + a_3 P^3 + a_4 P^4))$$

where $a_1 = 4k_1$, $a_2 = 6k_1 k_2$, $a_3 = 4k_1 k_2 k_3$ and $a_4 = k_1 k_2 k_3 k_4$ (1)

Here, k_i ($i=1$ to 4) differs from K_i ($i=1$ to 4) defined by Adair in the sense that the former is corrected for the statistical factor (intrinsic Adair constant).

Hypothetical OECs with arbitrary P_{50} values were constructed from the published set of four Adair constant values which were varied by multiplication with a common factor, *i.e.* k_i -constant. By doing this, the P_{50} value of the hypothetical OEC was varied without any change in shape.

The effect of changes in cooperativity (n) on the Bohr shift was investigated in a wide range of n value, using Hill's empirical equation:

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

where n is a conventional expression of cooperativity.

The effectiveness of the Bohr shift in maternal blood under physiological conditions was examined using the standard OEC for human whole blood with the P_{50} value of 26.7 torr and n value of 2.65. The Adair constant values for the standard OEC are: $k_1 = 0.0037 \text{ torr}^{-1}$, $k_2 = 0.047 \text{ torr}^{-1}$, $k_3 = 0.012 \text{ torr}^{-1}$, and $k_4 = 1.1 \text{ torr}^{-1}$ (Mohammed Mawjood and Imai, 1999; Imai, personal communication).

For fetal Hb solution, only a few accurate OEC data are available (Tyuma *et al.*, 1973). However, it is generally thought that there is no difference in shape between the fetal Hb OEC and the adult Hb OEC (Allen *et al.*, 1953). To construct the fetal Hb OECs, therefore, the OECs for adult Hb solution obtained under various experimental conditions were employed by changing their position but keeping their shape unchanged. The OEC for fetal blood was constructed from the standard OEC for adult blood, as well.

The normal blood PO_2 values used for calculations in the present study are as follows: maternal arterial PO_2 at rest, 100 torr; maternal uterine venous PO_2 , 40 torr; fetal umbilical venous PO_2 (arterialized fetal blood), 35 torr; fetal umbilical arterial PO_2 ("venous blood" coming from the fetus to the placenta), 15 torr. The P_{50} values for fetal and maternal blood are 20 and 26 torr (Battaglia and Meschia 1986; Dejours 1975), respectively. In the placental circulation, "arterial blood" and "venous blood" mean the blood flowing through the umbilical vein and the umbilical artery, respectively.

All computations were performed on a personal computer (Model PC-VC500, Nippon Electric Co., Tokyo, Japan) using MS-FORTRAN.

RESULTS AND DISCUSSION

Contribution of the Bohr effect to oxygen transport by human blood

Fig. 1 illustrates the contribution of the Bohr shift to the release and uptake of O_2 in human blood. The OEC of fetal Hb ($P_{50}=20$ torr, A) lies to the left of that of maternal Hb ($P_{50}=26$ torr, C). B and D represent the OECs right-shifted by the Bohr effect. The additional amount of O_2 delivered to tissues by fetal blood as a result of the Bohr shift was estimated from a decrease in O_2 saturation at PO_2 of 15 torr ($\Delta S_{(15A-15B)}$ (①)). Hence, the total amount of O_2 transported to fetal tissues by fetal blood was $\Delta S_{(35A-15B)}$. At the pla-

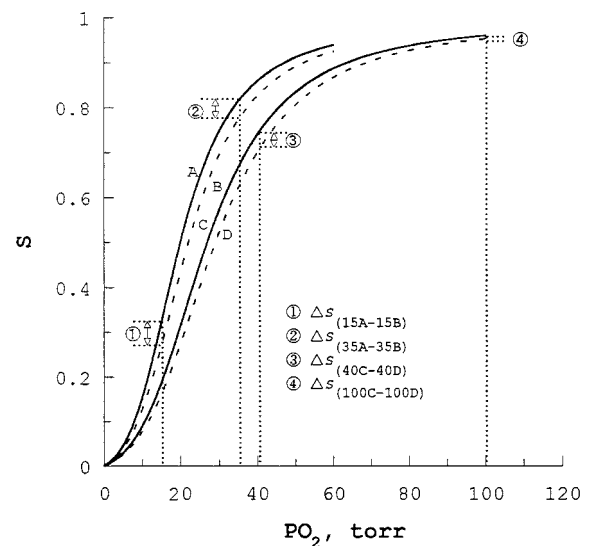


Fig. 1. Graphical expression of additional amounts of O_2 unloading and loading caused by the Bohr shift in human fetal and maternal blood, respectively. Solid line A stands for fetal arterial blood, and broken line B for fetal venous blood. Solid line C stands for maternal arterial blood, and broken line D for maternal uterine venous blood. $\Delta S_{(15A-15B)}$ (①) and $\Delta S_{(40C-40D)}$ (③) represent the additional amount of O_2 released from fetal blood ($P_{50}=20$ torr) and maternal blood ($P_{50}=26$ torr), respectively, as a result of the Bohr shift. $\Delta S_{(35A-35B)}$ (②) and $\Delta S_{(100C-100D)}$ (④) represent the Bohr shift-dependent additional amount of O_2 loaded to fetal and maternal blood, respectively. $\Delta S_{(35A-15B)}$ represents the total O_2 delivered by fetal blood to fetal tissues in the presence of the Bohr shift. The human adult standard OEC measured under physiological conditions by Mohammed Mawjood and Imai (1999) was used for generating the necessary OECs.

centa, the additional amount of O₂ loading to fetal Hb by the Bohr shift was $\Delta S_{(35A-35B)}$ (②). In maternal blood, the additional amount of O₂ released from maternal Hb by the Bohr shift was $\Delta S_{(40C-40D)}$ (③), and the additional amount of O₂ loading to the Hb caused by the Bohr shift was $\Delta S_{(100C-100D)}$ (④).

Influence of the position of the OEC on the effectiveness of the Bohr shift in fetal umbilical venous and arterial blood

Fig. 2 shows the theoretically derived effects of P_{50} on O₂ saturation of Hb ($S_{(PO_2)}$, dashed lines) and the effectiveness of the Bohr shift ($dS_{(PO_2)}/dP_{50}$, solid lines) in the fetal placental circulation. The degree of the effectiveness of the Bohr shift in the “venous blood” and “arterial blood” was estimated from the slope of the $S_{(15)}$ vs. P_{50} plot and $S_{(35)}$ vs. P_{50} plot, respectively. The slope ($dS_{(PO_2)}/dP_{50}$) is usually negative, because $S_{(PO_2)}$ decreases with increases in P_{50} . The $-dS_{(PO_2)}/dP_{50}$ vs. P_{50} curves are bell-shaped having a single maximum. The highest effectiveness of the Bohr shift occurred at P_{50} of 12 torr in “venous blood” and at P_{50} of 28 torr in “arterial blood”. At these P_{50} values, the Bohr shift can achieve maximal action. Hence, the P_{50} values of 12 and 28 torr are the optimal P_{50} for the effectiveness of the Bohr shift for fetal umbilical “venous blood” and “arterial blood”, respectively.

As shown by the double-headed arrow in Fig. 2, the effectiveness of the Bohr shift in fetal blood with physiological P_{50} value at the site of O₂ release ($-dS_{(15)}/dP_{50}$ (20)) is slightly higher than that at the site of O₂ uptake ($-dS_{(35)}/dP_{50}$ (20)), but it is of interest that there is only a small difference between $-dS_{(35)}/dP_{50}$ (20) and that of the maximal $-dS_{(35)}/$

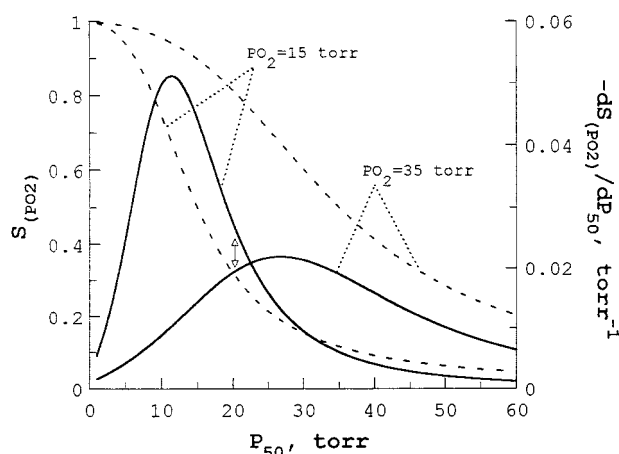


Fig. 2. Oxygen saturation of Hb as a function of P_{50} and the effectiveness of the Bohr shift of fetal umbilical blood in the placental circulation. The dashed lines represent the O₂ saturation of Hb ($S_{(PO_2)}$) in fetal blood with PO_2 of 15 and 35 torr. The solid lines represent the effectiveness of the Bohr shift in fetal umbilical “venous blood”, $-dS_{(15)}/dP_{50}$, and that of fetal umbilical “arterial blood”, $-dS_{(35)}/dP_{50}$. Double-headed arrow indicates the physiological P_{50} of fetal blood. Calculation was carried out using the human adult standard OEC.

dP_{50} value. Here, the number in parentheses following “ P_{50} ” expresses the P_{50} value at which its differential is taken. The difference in the effectiveness of the Bohr shift at the site of O₂ release and O₂ uptake was also pointed out in the human adult venous and arterial blood in our previous paper (Itoh *et al.*, 2001). The effectiveness of the Bohr shift in adult venous blood ($-dS_{(40)}/dP_{50}$ (27)) was about seven times more efficient than that of the arterial blood with physiological P_{50} of adult Hb ($-dS_{(100)}/dP_{50}$ (27)), implying that the effectiveness of the Bohr shift at the site of O₂ loading is less important. In the lungs, the O₂ uptake seems to be ensured by stable high alveolar O₂ pressure. In contrast to adult blood, the effectiveness of the Bohr shift of fetal blood at the site of O₂ loading is as important as that at the site of O₂ release, because the PO_2 and PCO_2 environment of the fetal blood are extremely different from that of alveolar gas.

Together with the previous conclusion that the position of the OEC for fetal Hb is optimized for O₂ delivery (Sold, 1982; Willford *et al.*, 1982; Kobayashi *et al.*, 1996), the present result indicates that the position of the fetal Hb seems to be well adapted to maintaining the effectiveness of the Bohr shift at high levels at both O₂ loading and release sites. The higher effectiveness of the Bohr shift at the site of O₂ release relative to that at the O₂ uptake may be adequate for preventing the accumulation of proton and CO₂ in the fetal tissues. Further, as will be described later, the equal or higher effectiveness of the Bohr shift in fetal blood than in maternal blood at the placenta (Fig. 4) will be also adequate for O₂ and CO₂ exchange across the placental membrane. Because the Bohr coefficient of fetal Hb has been reported to be almost equal value for adult Hb (Bohr coefficient = -0.51 for fetal Hb and -0.48 for adult Hb, Mann and Romney, 1968; Severinghaus, 1966), the equal or higher effectiveness of the Bohr shift may be permitted to receive the maximum benefit from the double Bohr effect.

Influence of cooperativity on the effectiveness of the Bohr shift in fetal blood at the PO_2 of umbilical venous and arterial blood

The influence of cooperativity on the effectiveness of the Bohr shift at PO_2 values of 15 and 35 torr was theoretically derived from a Hill's equation using the physiological P_{50} (20 torr) that covered a wide range of n values from 1 to 4 and from experimentally obtained OECs of human adult Hb solution (Fig. 3). Dashed lines represent the effectiveness of the Bohr shift calculated from the Hill equation. The effectiveness of the Bohr shift of “venous blood” $-dS_{(15)}/dP_{50}$ (20), increased with an increase in n value and reached its highest value at $n=5.0$. Further increases in n value reduced the effectiveness of the Bohr shift. Similar trends are observed in fetal umbilical “arterial blood” ($-dS_{(35)}/dP_{50}$ (20)). It is interesting that the highest effectiveness of the Bohr shift was observed at a relatively low n value (2.9). The crosses and closed circles represent the effectiveness of the Bohr shift calculated from the experimentally obtained OECs of adult Hb solutions and the human adult standard OEC.

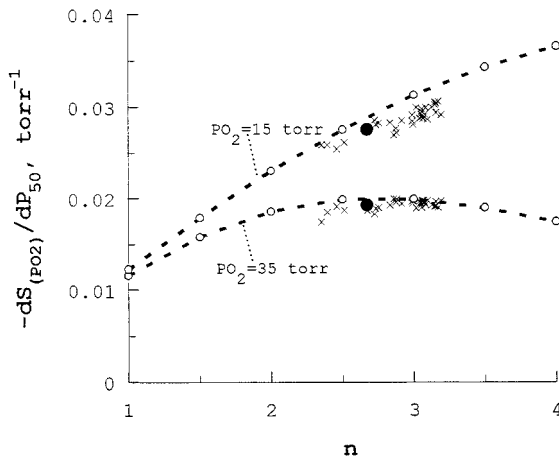


Fig. 3. Influence of cooperativity (n) on the effectiveness of the Bohr shift in fetal umbilical blood. The effectiveness of the Bohr shift in the umbilical “venous blood”, $-dS_{(15)}/dP_{50(20)}$, and that in the umbilical “arterial blood”, $-dS_{(35)}/dP_{50(20)}$, were plotted against n . The quantity, $-dS_{(P_{O_2})}/dP_{50}$, represented by dashed lines were calculated using a Hill’s equation. Crosses represent the $-dS_{(P_{O_2})}/dP_{50(20)}$ values obtained from the OECs of human adult Hb solution taken from Imai (1982), Imai and Yonetani (1975), Imaizumi *et al.* (1982) and Tyuma *et al.* (1973). Closed circles represent the $-dS_{(P_{O_2})}/dP_{50(20)}$ values calculated from the human adult standard OEC.

The effectiveness of the Bohr shift calculated from the OECs of human adult Hb solution are slightly lower than those calculated using the Hill equation.

Comparison of the influence of P_{50} on the effectiveness of the Bohr shift in fetal umbilical “arterial blood” with that in maternal uterine venous blood

The importance of the Bohr shift in gas exchange at the placental membrane was evaluated by comparing the effectiveness of the Bohr shift in fetal blood, $-dS_{(35)}/dP_{50}$, with that in maternal uterine venous blood, $-dS_{(40)}/dP_{50}$.

Fig. 4 shows theoretically derived effects of P_{50} on the effectiveness of the Bohr shift in maternal uterine venous blood, $-dS_{(40)}/dP_{50}$, at physiological n value (2.65). As already described, the P_{50} value of 30 torr, that gives the highest $-dS_{(40)}/dP_{50}$ value, is relatively close to the physiological P_{50} value of maternal blood (26 torr). It is also important to note that the effectiveness of the Bohr shift in fetal umbilical “arterial blood”, $-dS_{(35)}/dP_{50(20)}$, and that in maternal uterine venous blood, $-dS_{(40)}/dP_{50(26)}$, are nearly equal.

Influence of cooperativity on the effectiveness of the Bohr shift in maternal blood

Fig. 5 illustrates the theoretically derived effect of cooperativity (n) on the effectiveness of the Bohr shift in maternal uterine venous blood, $-dS_{(40)}/dP_{50(26)}$, calculated in the range of n values from 1 to 4. The $-dS_{(40)}/dP_{50(26)}$ value increases with increases in n value, reaches its highest value at n of 4.0., and decreases on further increases in n . The effectiveness of the Bohr shift of maternal uterine venous blood, $-dS_{(40)}/dP_{50(26)}$ values, calculated from the

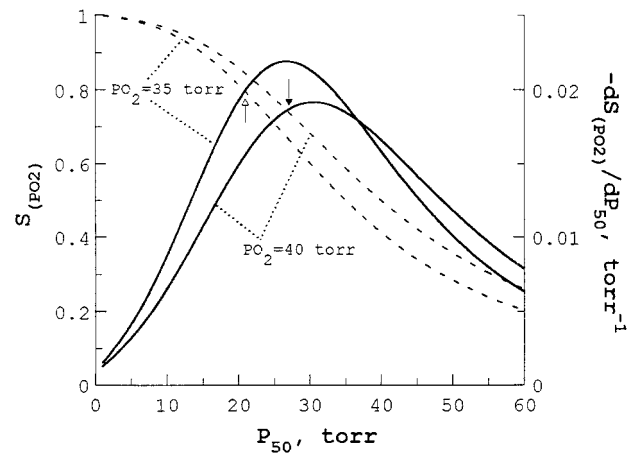


Fig. 4. P_{50} -dependences of oxygen saturation of Hb and the effectiveness of the Bohr shift in fetal and maternal blood. Dashed lines express P_{50} -dependences of oxygen saturation of maternal uterine venous blood at PO_2 of 40 torr and fetal “arterial blood” in the umbilical vein at PO_2 of 35 torr. Solid lines express P_{50} -dependences of $-dS_{(35)}/dP_{50(20)}$ (fetal blood) and $-dS_{(40)}/dP_{50(26)}$ (maternal blood). Open and closed arrows indicate the physiological P_{50} values for fetal and maternal blood, respectively.

OECs of human adult Hb were slightly lower than that obtained from the Hill equation. For comparison, the effectiveness of the Bohr shift in fetal umbilical “arterial blood”, $-dS_{(35)}/dP_{50(20)}$ is also drawn in the figure. At n value of 3.0, both the maternal and fetal values are nearly equal and relatively high.

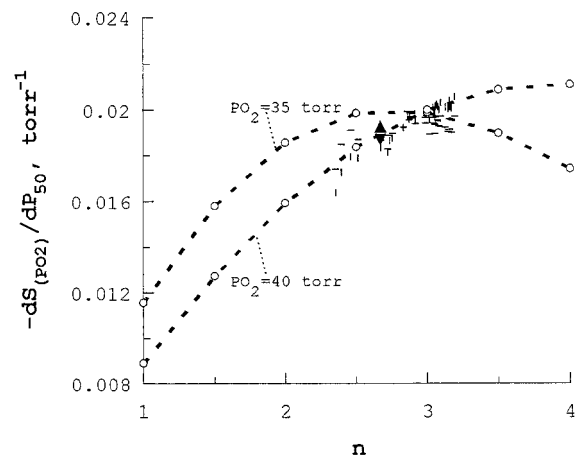


Fig. 5. Influence of cooperativity (n) on the effectiveness of the Bohr shift in fetal and maternal blood. The effectiveness of the Bohr shift in fetal umbilical “arterial blood”, $-dS_{(35)}/dP_{50(20)}$, and that in maternal uterine venous blood, $-dS_{(40)}/dP_{50(26)}$ are plotted against n . The dashed lines represent the $-dS_{(P_{O_2})}/dP_{50}$ values calculated from the Hill equation. The $-dS_{(35)}/dP_{50(20)}$ (—) and $-dS_{(40)}/dP_{50(26)}$ (|) values were calculated from the same OECs for human Hb solutions as those used in Fig. 3. The $-dS_{(35)}/dP_{50(20)}$ (▲) and $-dS_{(40)}/dP_{50(26)}$ (▼) values were calculated from the human adult standard OEC.

Correlation between the effectiveness of the Bohr shift in fetal umbilical "arterial blood" and that in maternal uterine venous blood

The correlation between the effectiveness of the Bohr shift in fetal umbilical "arterial blood", $-dS_{(35)}/dP_{50(20)}$, and that in maternal uterine venous blood, $-dS_{(40)}/dP_{50(26)}$, is shown in Fig. 6. The bold dotted line is the result obtained from the Hill equation. The plus signs and closed circles represent the effectiveness of the Bohr shift calculated using the human Hb OECs. Both quantities increased with increases in n , reaching nearly equal highest values at a relatively low n value (about 3.0). Further increases in n value resulted in marked decreases in the $-dS_{(35)}/dP_{50(20)}$ value. It is interesting that high effectiveness of the Bohr shift is observed at a relatively low n value (from 2.5 to 3.5).

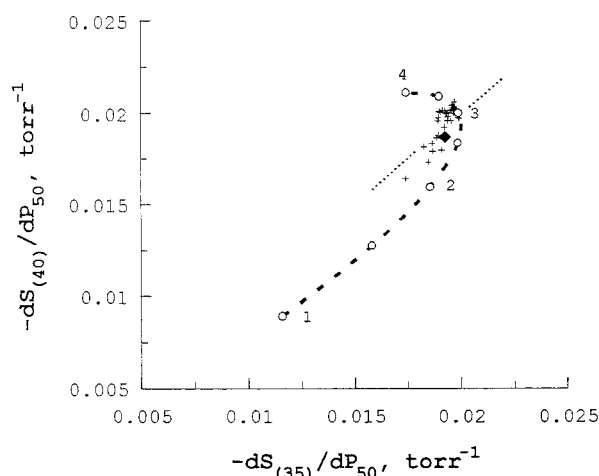


Fig. 6. Correlation between the effectiveness of the Bohr shift in fetal and maternal blood at various n values. The effectiveness of the Bohr shift in fetal umbilical "arterial blood" ($-dS_{(35)}/dP_{50(20)}$) was plotted against that in maternal uterine venous blood ($-dS_{(40)}/dP_{50(26)}$). Bold dotted line was constructed from the two lines in Fig. 5, and numbers attached to this line represent the value of n . Symbols (+) indicate the $-dS_{(PO_2)}/dP_{50}$ values obtained from OEC data sets of human adult Hb solutions. Symbols (● and ▲) are the same as those in Fig. 5. Straight thin dotted line represents the relation: $-dS_{(35)}/dP_{50(20)} = -dS_{(40)}/dP_{50(26)}$.

The effect of cooperativity on the O₂ transport efficiency of fetal blood

Fig. 7 shows the amount of O₂ transported by fetal blood ($\Delta S_{(35A-15B)}$) calculated at various n values. This amount gradually increased with increases in n , indicating that there is no optimal cooperativity for O₂ transport efficiency.

The amino acid substitution of Ser-143 of fetus Hb γ chain for His-143 of adult Hb β chain causes a reduction of the acid Bohr effect for fetal Hb (Perutz *et al.*, 1980). However, this functional difference occurs at a low pH range which is far from the physiological condition.

It is well known that the high oxygen affinity of fetal blood relative to that of adult blood is ascribed to the weakened interaction of fetal Hb with 2,3-diphosphoglycerate

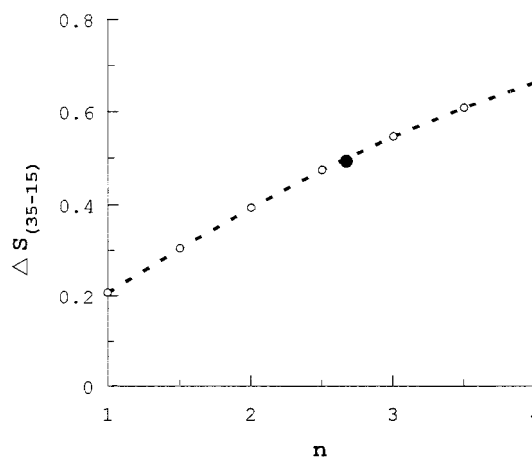


Fig. 7. Influence of cooperativity on the efficiency of O₂ transport by fetal blood at physiological P_{50} . Dotted line represents $\Delta S_{(35A-15B)}/(20)$ value calculated from the Hill equation. The closed circle represents the $\Delta S_{(35A-15B)}/(20)$ value calculated from the human adult standard OEC.

(DPG). The amino acid substitution described above causes a weakening of the interaction of DPG with fetal Hb since His-143 makes up part of the DPG binding site of adult Hb. Although fetal Hb has a lower oxygen affinity than adult Hb in the absence of DPG, the former shows a higher affinity than the latter in the red cell where DPG is present (Tyuma and Shimizu, 1970). The difference in allosteric properties between fetal Hb and adult Hb does not affect our present results of analysis since the effectiveness of the Bohr shift was measured by the factor, $dS_{(PO_2)}/dP_{50}$, that was calculated from whole blood OECs. The role of DPG was to produce the difference in oxygen affinity between the fetal blood and the adult blood.

Conclusions

Although the principal role of fetal Hb is in the transport of O₂ from the placenta to peripheral tissues, the Bohr shift is also important to enhance the O₂ and CO₂ exchange between fetal blood and maternal one across the placental membrane. From the results obtained in the present study, it is concluded that the positions of the OECs of fetal and maternal Hbs are mutually adjusted to receive a maximal benefit from the double Bohr effect, and that the relatively low cooperativity of tetrameric human Hb ($n=2.65$) is adequate for nearly maximizing the effectiveness of the Bohr shift in O₂ and CO₂ exchange processes across the placental membrane.

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