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# Significance of Affinity and Cooperativity in Oxygen Binding to Hemoglobin of Horse Fetal and Maternal Blood

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**ABSTRACT**—The physiological significance of the position and shape of the oxygen equilibrium curve (OEC) of horse hemoglobin (Hb) is considered from the viewpoint of oxygen (O<sub>2</sub>) transport efficiency and the effectiveness of the Bohr effect. In horse fetal and maternal bloods, their physiological O<sub>2</sub> affinities are nearly optimized with respect to the effectiveness of the Bohr shift occurring at the O<sub>2</sub> release site, when it is measured by the change in O<sub>2</sub> saturation per unit change in  $P_{50}$ . With relatively low cooperativity ( $n=2.69$ ) of horse Hb under physiological conditions, the effectiveness of the Bohr shift for fetal blood at O<sub>2</sub> uptake site and maternal blood at O<sub>2</sub> release site is high. These facts imply that the position and the cooperativity of horse Hb OEC are optimized to receive maximal benefit from the double Bohr shift. Before exercise, the position of the OEC for adult mares is nearly optimized for the effectiveness of the Bohr shift occurring at the O<sub>2</sub> release site, whereas, at maximal exercise, the position of the OEC tends to become advantageous for O<sub>2</sub> transport efficiency.

**Key words:** cooperativity, effectiveness of the Bohr effect, hemoglobin, O<sub>2</sub>-Hb equilibrium curve, horse

## INTRODUCTION

An increase in partial pressure of CO<sub>2</sub> ( $PCO_2$ ) or a decrease in pH lowers the O<sub>2</sub> affinity of Hb. Thus, they shift the OEC to the right, causing release of additional O<sub>2</sub> from Hb to the tissues. This shift caused by concomitant  $PCO_2$  change and pH change is called the “classical Bohr effect” (Bohr *et al.*, 1904), while the shift of OEC only by pH change called the “Bohr effect”. In the case of fetal blood, the Bohr shift is considered to be of particular importance. The diffusion of CO<sub>2</sub> from fetal blood into maternal blood increases pH of fetal blood and shifts the fetal OEC to the left, the simultaneous decrease in pH of maternal blood shifts its OEC to the right (MaCarthy, 1943). This phenomenon is called the “double Bohr effect”. It has been considered that the enhanced difference in  $P_{50}$  between fetal and maternal bloods by the double Bohr effect promotes the mother-fetus transfer of O<sub>2</sub>.  $P_{50}$  is partial pressure of O<sub>2</sub> at half saturation.

The magnitude of the Bohr effect is quantitatively measured by the change in  $\log P_{50}$  per unit change in pH ( $d\log P_{50}/dpH$ ), which is called the “Bohr coefficient”. The

additional O<sub>2</sub> released from Hb by the Bohr shift without any change in venous  $PO_2$  is dependent on not only the magnitude of the Bohr coefficient but also the steepness of the OEC. In our previous study, we found that the position of the OEC of human adult Hb at rest is optimal for the effectiveness of the Bohr shift occurring at O<sub>2</sub> release sites (venous  $PO_2(PvO_2)=40$  torr) (Kobayashi *et al.*, 1996, Itoh *et al.*, 2001).

The cooperativity of O<sub>2</sub> binding is expressed in terms of the highest slope of the Hill plot ( $\log(S/(1-S))$  vs.  $\log P$  plot),  $n$  (Hill, 1910). The functional significance of the sigmoid shape of the OEC has been explained by efficient O<sub>2</sub> transport: the higher the cooperativity, the more O<sub>2</sub> is transported to the tissues. However, mammalian tetrameric Hb usually has relatively low cooperativity ( $n$  value of approximately 2.8). The physiological significance of this low cooperativity has not been explained so far. Recently, using the human OEC data, which was described by Adair's stepwise O<sub>2</sub> binding equation, it was reported that the relatively low cooperativity of the OEC of human tetrameric Hbs is designed to receive a maximal benefit from the double Bohr shift (Zhang *et al.*, 2003). In this study, the above-mentioned approach was applied to confirm the functional significance of the relatively low cooperativity of other mammalian tet-

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rameric Hbs.

In each many species of mammalian species, Hb of the fetal blood differs structurally from that found in the adult blood. The subunit structure of human adult Hb is  $\alpha_2\beta_2$ , and that of fetal Hb is  $\alpha_2\gamma_2$ . The amino acid sequences of the  $\beta$ -chain of adult Hb and the  $\gamma$ -chain of fetal Hb differ at 31 of 146 residues (Schroeder *et al.*, 1963). In contrast, the Hbs of the fetal and adult horse bloods have been shown to be structurally identical with each other (Stockell *et al.*, 1961; Comline and Silver, 1974). However, fetal blood has higher  $O_2$  affinity than maternal blood because of the low concentration of 2,3-diphosphoglycerate (2,3-DPG) in fetal red blood cells compared to that in maternal red blood cells (Bunn and Kitchen, 1973). There are literature values for not only the Bohr coefficient of horse blood ( $d\log P_{50}/d\text{pH} = -0.47$ , Bunn and Kitchen, 1973; Fenger *et al.*, 2000), but also  $PO_2$  for the arterial and venous blood and  $P_{50}$  for both fetal and maternal bloods (Comline and Silver, 1974; Comline and Silver, 1975). In addition, the OEC of horse adult blood, for which the four Adair constants (see below) were determined, is also available in the literature (Clerbaux *et al.*, 1993). To our knowledge, horse fetal blood OEC data, for which the four Adair constants have been determined, are not available in the literature. Then, we measured OECs of horse Hb at various 2,3-DPG concentrations and found that cooperativity is not sensitive to 2,3-DPG concentration. It is known that cooperativity is also insensitive to pH. These facts give the basis for generating an OEC for either adult or fetal Hb under given 2,3-DPG and pH conditions from the Adair constants for horse adult Hb. Using these calculated OECs, the physiological significance of the position and the cooperativity of horse Hb can theoretically be examined from the viewpoint of  $O_2$  transport efficiency and the effectiveness of the Bohr shift.

## METHODS

Horse blood sample was purchased from Nippon biological material center. Hemoglobin was stripped of organic phosphates according to the method of Condo *et al.* (1992), and its OECs were measured with an automatic oxygenation apparatus developed by Imai and Yonetani (1977) and Imai (1981) at an Hb concentration of 60  $\mu\text{M}$  on a heme basis and 25°C. Oxygen saturation of Hb was calculated from the change in absorbance at 576 nm measured with a Shimadzu spectrophotometer (model UV 2000). The concentration of  $O_2$  in the sample cell was decreased by replacing air with pure  $N_2$  gas and its change was monitored with a Clark-type  $O_2$  electrode. The buffer solution used for OEC measurements was 0.05 M Tris-HCl (pH 7.4). Methemoglobin (Met-Hb) formed by auto-oxidation was reduced using an enzymatic reducing system as described by Hayashi *et al.* (1973). The Met-Hb concentration at the end of OEC measurement as determined as described by Evelyn *et al.* (1938), did not exceed 5% of total Hb. The 2,3-DPG concentrations were measured by the enzymatic procedure of Ericson and Verdier (1972). The experimentally obtained OEC data were analyzed by curve fitting method described by Imai (1981) to estimate the Adair constants. Using these Adair constants,  $P_{50}$  and  $n$  values were calculated.

The Adair equation (Adair, 1925) is given by

$$S = (A_1P + 2A_2P^2 + 3A_3P^3 + 4A_4P^4) / 4(1 + A_1P + A_2P^2 + A_3P^3 + A_4P^4) \quad (1)$$

where  $S$  is the fractional saturation of Hb with  $O_2$ ,  $A_i$  ( $i=1\sim 4$ ) is the Adair constants and  $p$  is the partial pressure of  $O_2$ .

The literature  $A_i$  values for horse adult whole blood at pH 7.4,  $PCO_2$  of 40 torr and 37°C are:  $A_1=3.103 \cdot 10^{-2} \text{ torr}^{-1}$ ,  $A_2=8.451 \cdot 10^{-4} \text{ torr}^{-2}$ ,  $A_3=1.447 \cdot 10^{-5} \text{ torr}^{-3}$  and  $A_4=3.961 \cdot 10^{-6} \text{ torr}^{-4}$  (Clerbaux *et al.*, 1993). The values of  $P_{50}$  and  $n$  are 23.8 torr and 2.69, respectively. Using Adair constants, theoretical OECs with various  $P_{50}$  values were generated by multiplying each  $PO_2$  value by a common factor. In this multiplication, the position of the OEC was shifted freely without changing the shape.

In order to investigate the effect of cooperativity on the effectiveness of the Bohr shift, Hill's empirical equation (Hill, 1910) and OEC data of horse Hb solutions measured under various experimental conditions (Imai, 1983) were used.

The  $O_2$  transport efficiency and the effectiveness of the Bohr shift of horse fetal and maternal bloods at rest were calculated using the following literature values of blood  $PO_2$  and  $P_{50}$  (Comline and Silver, 1974). Fetal umbilical venous  $PO_2$  (arterialized fetal blood), fetal umbilical arterial  $PO_2$  ("venous blood" coming from the fetus to the placenta), and physiological  $P_{50}$  under normal conditions were assumed to be 49, 33, and 27 torr, respectively. In the placental circulation, "arterial blood" and "venous blood" meant the blood flowing through the umbilical vein and the umbilical artery, respectively. Maternal arterial  $PO_2$ , uterine venous  $PO_2$  and physiological  $P_{50}$  values were assumed to be 95, 50 and 31 torr, respectively.

The  $O_2$  transport efficiency and the effectiveness of the Bohr shift during incremental exercise of horse adult were calculated using the  $PO_2$ ,  $P_{50}$  and  $n$  values reported by Fenger *et al.* (2000). The arterial  $PO_2$  ( $PaO_2$ ), venous  $PO_2$  ( $PvO_2$ ),  $P_{50}$  and  $n$  values before exercise were 106, 38, 22.9 torr and 2.649, respectively, and these at maximal exercise were 86, 20, 31.8 torr and 2.707, respectively.

All computations were performed on a personal computer (model PC-9821 AP2; Nippon Electric Co., Tokyo) using MS-FORTRAN.

## RESULTS AND DISCUSSION

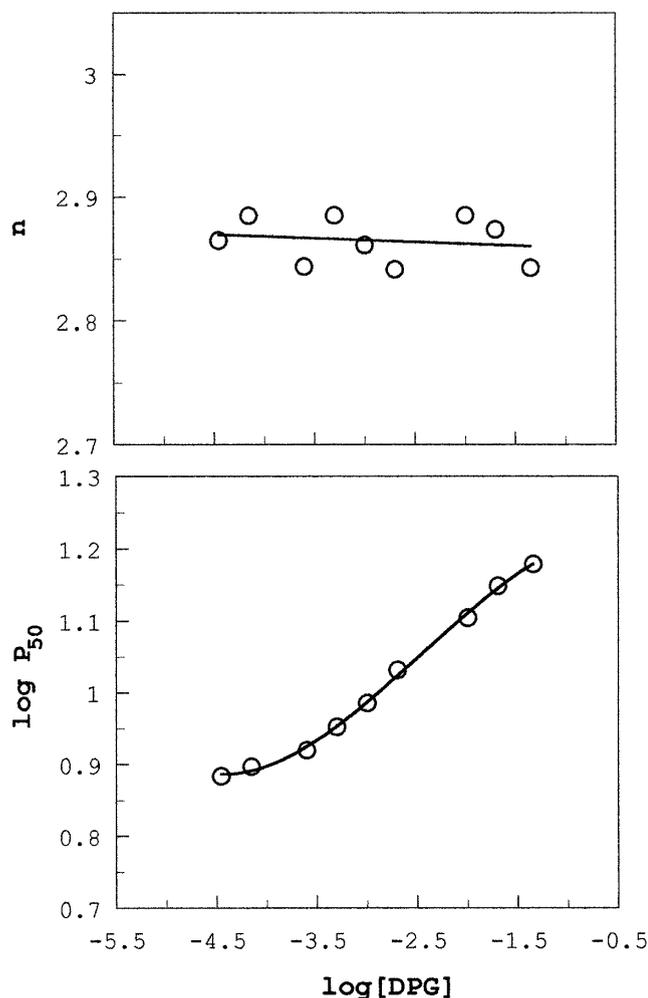
### Effect of 2,3-DPG on cooperativity of horse Hb

Fig. 1 shows the effect of 2,3-DPG concentration on the  $O_2$  affinity and cooperativity of horse Hb. These data demonstrate a strong influence of 2,3-DPG on the  $O_2$  affinity, but little effect on cooperativity. Hill's coefficient ( $n$ ) value was nearly constant. Therefore, in this study, the OEC with an  $n$  value of 2.69 of adult blood measured under standard conditions pH 7.4,  $PCO_2$  40 mmHg, 37°C (Clerbaux *et al.*, 1993) was used to generate OECs for horse fetal and maternal bloods under various conditions.

### Calculation of $O_2$ transport by fetal and maternal blood

Fig. 2 illustrates an example calculation of the  $O_2$  transport by horse fetal and maternal bloods and the contribution of the Bohr shift occurring at the  $O_2$  uptake and release sites.

The additional  $O_2$  released from fetal blood due to the Bohr shift was estimated from the decrease in  $O_2$  saturation at  $PO_2$  of 33 torr,  $\Delta S_{(33A-33B)}$ . In the placenta, the additional  $O_2$  uptake by fetal blood due to the Bohr shift was estimated from the increase in  $O_2$  saturation at  $PO_2$  of 49 torr,



**Fig. 1.** Effect of 2,3-DPG on the  $O_2$  affinity and cooperativity of horse adult Hb.  $\log P_{50}$  and  $n$  values are plotted against 2,3-DPG concentration (mol/liter). These parameter values were obtained from the OECs measured at pH 7.4 and 25°C.

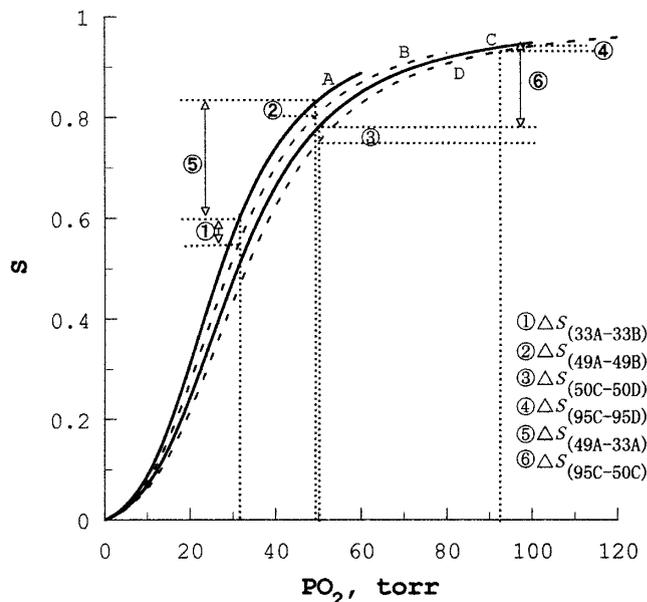
$\Delta S_{(49A-49B)}$ .

In maternal blood, the additional  $O_2$  released at  $PO_2$  of 50 torr due to the Bohr shift was represented by  $\Delta S_{(50C-50D)}$ , and the additional  $O_2$  uptake in the lungs caused by the Bohr shift at  $PO_2$  of 95 torr was represented by  $\Delta S_{(95C-95D)}$ .

The amount of  $O_2$  transported to the tissues was estimated from the arterio-venous difference in  $O_2$  saturation  $\Delta S_{(PaO_2-PvO_2)}$ .

#### **$O_2$ transport efficiency of fetal and maternal blood**

The slope of the OEC ( $dS/dP=S$ ) was quantified to represent the  $O_2$  transport efficiency of Hb (Kobayashi *et al.*, 1994), and this measure was used to compare the fetal and maternal bloods. The  $S'$  vs.  $P$  plot of fetal blood exhibited a steep slope under physiological  $O_2$  environment (at  $PO_2$  ranging from 33 to 49 torr) (Fig. 3A), indicating high  $O_2$  transport efficiency. In contrast, the slope of the OEC of maternal blood was rather flat under physiological  $O_2$  environment (at  $PO_2$  ranging from 50 to 95 torr), showing low  $O_2$  transport efficiency (Fig. 3B).

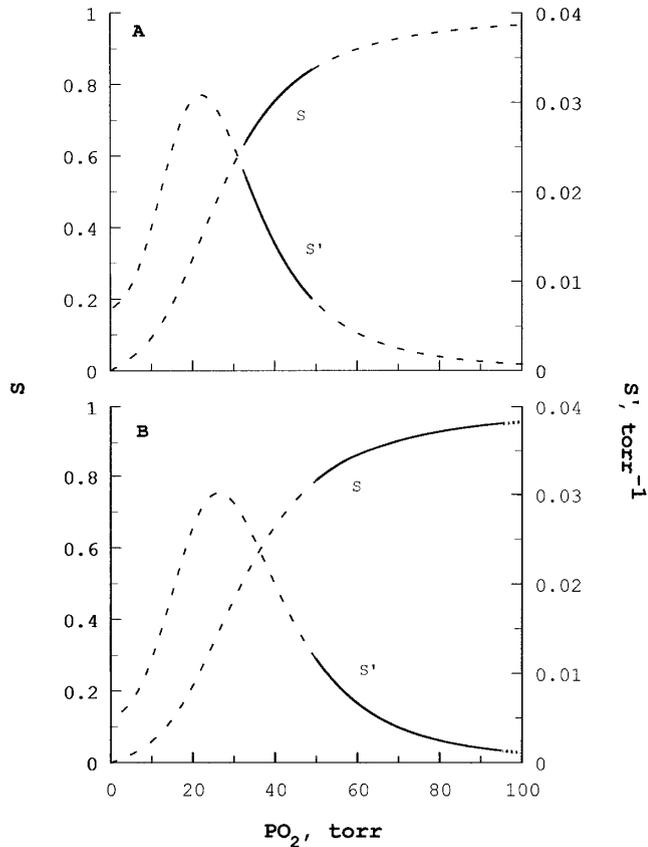


**Fig. 2.** Example calculation of the  $O_2$  transport and additional  $O_2$  release and uptake caused by Bohr shift of horse blood. Solid line A represents the OEC of fetal "arterial" blood, and broken line B represents that of fetal "venous" blood. Solid line C represents the OEC of maternal arterial blood, and broken line D represents that of maternal uterine venous blood.  $\Delta S_{(33A-33B)}$  (①) and  $\Delta S_{(50C-50D)}$  (③) represent the  $O_2$  released from fetal and maternal blood, respectively due to the Bohr shift.  $\Delta S_{(49A-49B)}$  (②) and  $\Delta S_{(95C-95D)}$  (④) represent the additional  $O_2$  uptake by fetal and maternal blood, respectively due to the Bohr shift.  $\Delta S_{(49A-33A)}$  (⑤) and  $\Delta S_{(95C-95C)}$  (⑥) represent the amount of  $O_2$  transported to the tissues by fetal and maternal blood without the Bohr shift, respectively. Hypothetical OECs with physiological  $P_{50}$  values were constructed using the Adair constants of OEC for horse blood under standard conditions (Comline and Silver, 1974).

#### **Relation to the optimal $P_{50}$ for $O_2$ transport efficiency**

In order to explain the significance of the position of the OEC of fetal blood, the arterio-venous difference in  $O_2$  saturation ( $\Delta S_{(PaO_2-PvO_2)}$ ) was calculated as a function of  $P_{50}$ . Fig. 4A shows the relationship between  $P_{50}$  and the fetal "venous blood"  $O_2$  saturation ( $S_{(33)}$ ), "arterial blood"  $O_2$  saturation ( $S_{(49)}$ ) and arterio-venous difference in  $O_2$  saturation ( $\Delta S_{(49-33)}$ ). The  $\Delta S_{(49-33)}$  vs.  $P_{50}$  plot had one maximum value. The  $P_{50}$  that gave the highest  $O_2$  transport efficiency was called the "optimal  $P_{50}$ " for  $O_2$  transport. There are slight difference in the physiological  $P_{50}$  of horse blood and the optimal  $P_{50}$  values. The amount of  $O_2$  transported at physiological  $P_{50}$  was slightly lower than that of the maximum value at optimal  $P_{50}$  value. In human fetal Hb, it is well known that the physiological  $P_{50}$  of human fetal Hb is close to the optimal  $P_{50}$  (Itoh *et al.*, 2001; Sold, 1982; Willford *et al.*, 1982). Therefore, the  $O_2$  transport efficiency of horse fetal blood is not so high as that of human fetal blood.

In maternal blood, the amount of  $O_2$  transported at physiological  $P_{50}$  (31 torr) was roughly one-half that of theoretically obtained maximum value (Fig. 4B). This indicates that maternal venous blood is a large  $O_2$  reservoir and a rightward shift of the OEC enhances  $O_2$  transport as



**Fig. 3.** OEC and  $S'$  vs.  $P$  plots of horse blood. **A:** Solid line indicate  $S$  and  $S'$  values calculated for the physiological  $PO_2$  range of fetal blood. **B:** Solid line indicate  $S$  and  $S'$  values calculated for the physiological  $PO_2$  range of maternal blood. These data were derived from hypothetical OECs with various  $P_{50}$  values that were constructed using the OECs shown in Fig. 2.

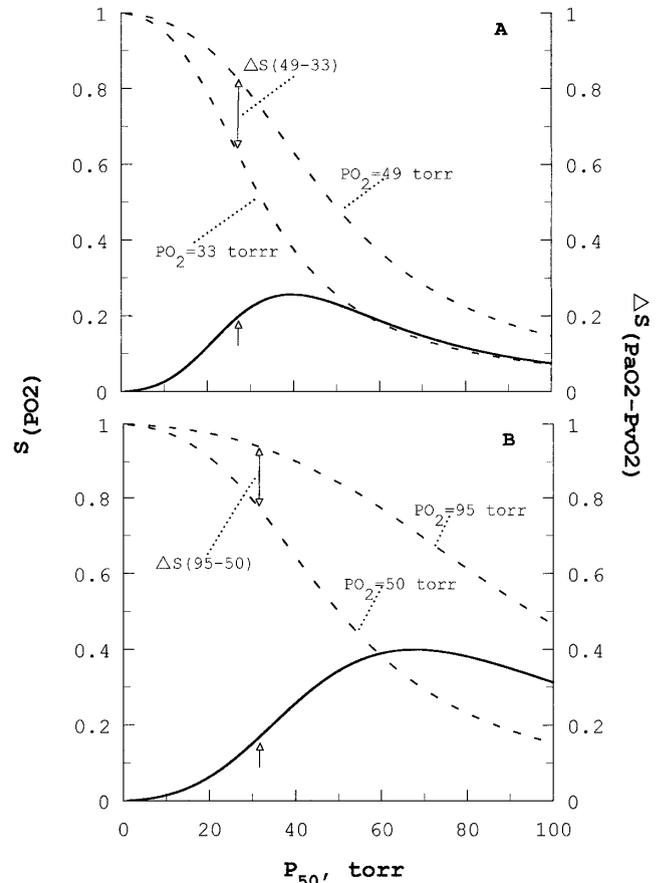
observed at hard exercise.

#### Effectiveness of Bohr shift in fetal and maternal blood

The effectiveness of the Bohr shift at various  $P_{50}$  was estimated from the change in  $O_2$  saturation per unit change in  $P_{50}$ , i.e. the slope of the  $S_{(PO_2)}$  vs.  $P_{50}$  plot. The slope ( $dS_{(PO_2)}/dP_{50}$ ) was usually a negative value because  $S_{(PO_2)}$  was decreased with an increase in  $P_{50}$ . Therefore, in this study, the magnitude of the effectiveness of the Bohr shift was expressed as  $-dS_{(PO_2)}/dP_{50}$ .

Fig. 5A shows the effectiveness of the Bohr shift at the  $O_2$  uptake and release sites (at  $PO_2$ s of 49 and 33 torr, respectively) for fetal blood. The theoretical highest effectiveness of the Bohr shift at  $O_2$  release site was observed at  $P_{50}$  of 23 torr. Clearly the physiological  $P_{50}$  value is close to the optimal  $P_{50}$  value for the effectiveness of the Bohr shift. At  $O_2$  uptake site, there are a slight difference in the physiological  $P_{50}$  and optimal  $P_{50}$  values. This implies that the position of the OEC of fetal blood is nearly optimal with respect to the effectiveness of the Bohr shift at  $O_2$  release site.

In fetal blood, the highest value at the  $O_2$  release site ( $P_{VO_2}=50$  torr) was observed at  $P_{50}$  of 38 torr (Fig. 5B). This

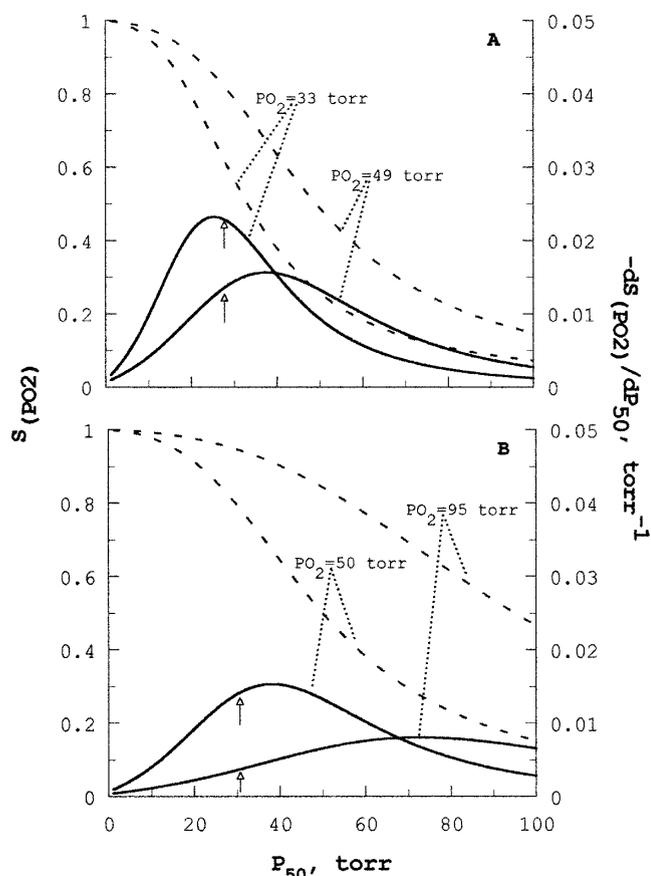


**Fig. 4.**  $O_2$  saturation at arterial and venous  $PO_2$  values and arterio-venous difference in  $O_2$  saturation ( $\Delta S_{(PaO_2-PvO_2)}$ ) of horse fetal and maternal bloods as a function of  $P_{50}$ . Hypothetical OECs with various  $P_{50}$  values were constructed using the OEC shown in Fig. 2. Open arrows are positioned at the physiological  $P_{50}$  for each blood. **A:** Broken lines represent the  $O_2$  saturation of fetal "arterial"  $PO_2$  of 49 torr and "venous" ( $PO_2$  of 33 torr) blood. The solid line represents the fetal arterio-venous difference in  $O_2$  saturation ( $\Delta S_{(49-33)}$ ) of fetal blood. **B:** Broken lines represent the  $O_2$  saturation of maternal arterial ( $PO_2$  of 95 torr) and uterine venous ( $PO_2$  of 50 torr) blood. Solid line represents the maternal arterio-venous difference in  $O_2$  saturation ( $\Delta S_{(95-50)}$ ) of maternal blood.

optimal  $P_{50}$  value is somewhat different from the physiological  $P_{50}$  value (31 torr). In contrast, at  $O_2$  uptake site there was a large difference between the theoretical optimal  $P_{50}$  value (72 torr) and the physiological  $P_{50}$  value. Therefore, the position of the OEC of maternal blood is nearly optimal to maximize the effectiveness of the Bohr shift in venous blood. The effectiveness of the Bohr shift at  $O_2$  uptake site of fetal blood is almost equal to that of maternal blood at  $O_2$  release site with physiological  $P_{50}$ . This phenomenon is similar to that observed in human bloods (Zhang et al., 2003)

#### Influence of cooperativity on effectiveness of the Bohr shift in fetal and maternal blood

Using physiological  $PaO_2$ ,  $PvO_2$  and  $P_{50}$  values, the influence of cooperativity on the effectiveness of the Bohr shift was investigated for a wide range of  $n$  values.



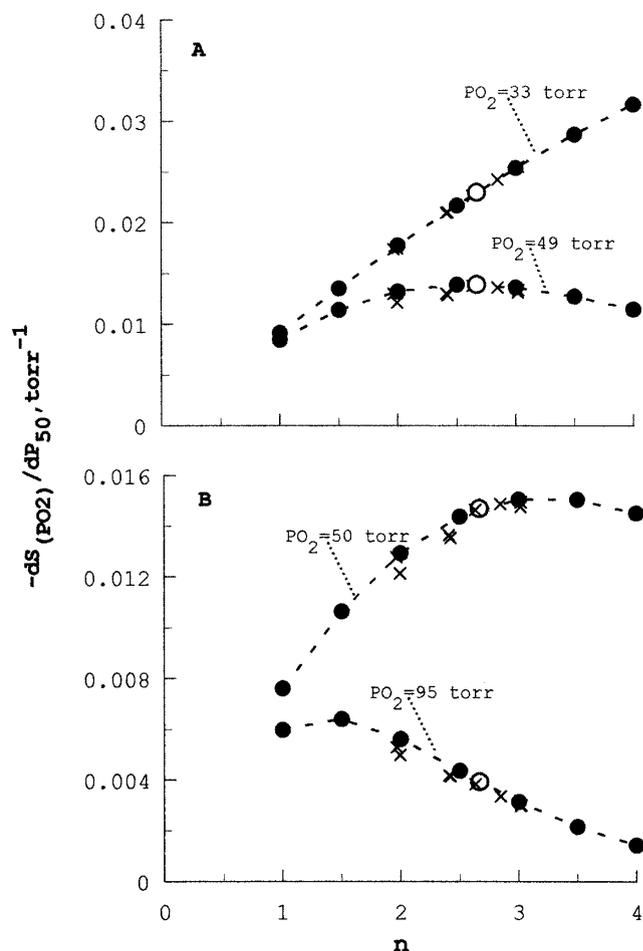
**Fig. 5.** Effectiveness of Bohr shift of arterial and venous horse blood as a function of  $P_{50}$ . Arrows indicate the physiological  $P_{50}$ . Hypothetical OECs with various  $P_{50}$  values were constructed using the OEC shown in Fig. 2. **A:** Dashed lines represent  $O_2$  saturation of Hb ( $S_{(PO_2)}$ ) in fetal "arterial" ( $PO_2$  of 49 torr) and fetal "venous" ( $PO_2$  of 33 torr) blood. Solid lines represent the effectiveness of the Bohr shift at the  $O_2$  uptake site in fetal "arterial" blood,  $-dS_{(49)}/dP_{50}$ , and that at  $O_2$  release site in umbilical "venous" blood,  $-dS_{(33)}/dP_{50}$ . **B:** Dashed lines represent  $O_2$  saturation of Hb ( $S_{(PO_2)}$ ) in maternal venous and arterial blood with  $PO_2$  of 50 and 95 torr. Solid lines represent the effectiveness of the Bohr shift at the  $O_2$  uptake site in arterial blood,  $-dS_{(95)}/dP_{50}$ , and that at the  $O_2$  release site in uterine venous blood,  $-dS_{(50)}/dP_{50}$ .

In fetal blood, the effectiveness of the Bohr shift at the  $O_2$  release site ( $dS_{(33)}/dP_{50}$ ) was increased with an increase in  $n$  value (Fig. 6A), and reached the highest value at approximately  $n=7$  (this point is out of range in Fig. 6A). Conversely, at the  $O_2$  uptake site of fetal "arterial" blood, the highest effectiveness of the Bohr shift ( $dS_{(49)}/dP_{50}$ ) was observed at  $n=2.6$  (Fig. 6A).

In maternal blood, the highest value at the  $O_2$  release site ( $dS_{(50)}/dP_{50}$ ) was observed at  $n=3.3$ , and that at the  $O_2$  uptake site ( $dS_{(95)}/dP_{50}$ ) was observed at  $n=1.5$  (Fig. 6B).

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

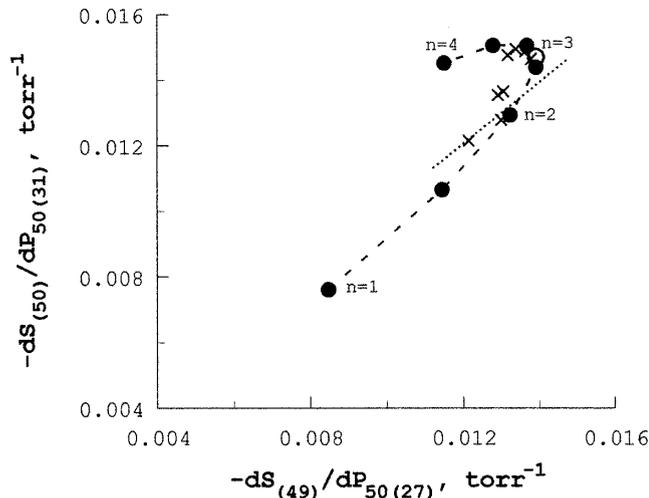
The effectiveness of the Bohr shift at physiological  $P_{50}$  was compared between at the  $O_2$  uptake site (fetal "arterial"



**Fig. 6.** Influence of cooperativity on effectiveness of the Bohr shift in horse blood. The effectiveness of the Bohr shift as a function of  $n$  value were calculated using OEC data of horse whole blood measured under standard condition ( $\circ$ ) (Comline and Silver, 1974), OEC data of horse Hb solutions measured under various experimental conditions ( $\times$ ) (Imai, 1983) and Hill's empirical equation ( $\bullet$ ). **A:** Effectiveness of the Bohr shift in fetal "arterial" blood,  $-dS_{(49)}/dP_{50}$ , and that in "venous" blood,  $-dS_{(33)}/dP_{50}$ , are plotted against  $n$ . **B:** Effectiveness of the Bohr shift in maternal arterial blood,  $-dS_{(95)}/dP_{50}$ , and that in uterine venous blood,  $-dS_{(50)}/dP_{50}$ , are plotted against  $n$ .

blood) and that at  $O_2$  release site (maternal uterine venous blood) to consider the gas exchange across the placental membrane. As shown in Fig. 7, the two  $dS_{(PO_2)}/dP_{50}$  values at  $PO_2$  values of 49 and 50 torr were nearly equal at  $n$  values below 3.0. The highest values were observed at an  $n$  values ranged from 2.5 to 3.0. The  $n$  value of horse Hb under standard conditions was reported to be 2.69 (Clerbaux *et al.*, 1993). If there was large difference in effectiveness of the Bohr shift of both bloods, the increase or decrease in  $CO_2$  and  $H^+$  concentration in the fetal blood could not be prevented. The results obtained in this study seem to show that relatively low cooperativity is adequate for gas exchange across the placental membrane.

2,3-DPG has little effect on the Bohr coefficient of horse blood (Pellegrini *et al.*, 1996) and this seems to suggest that

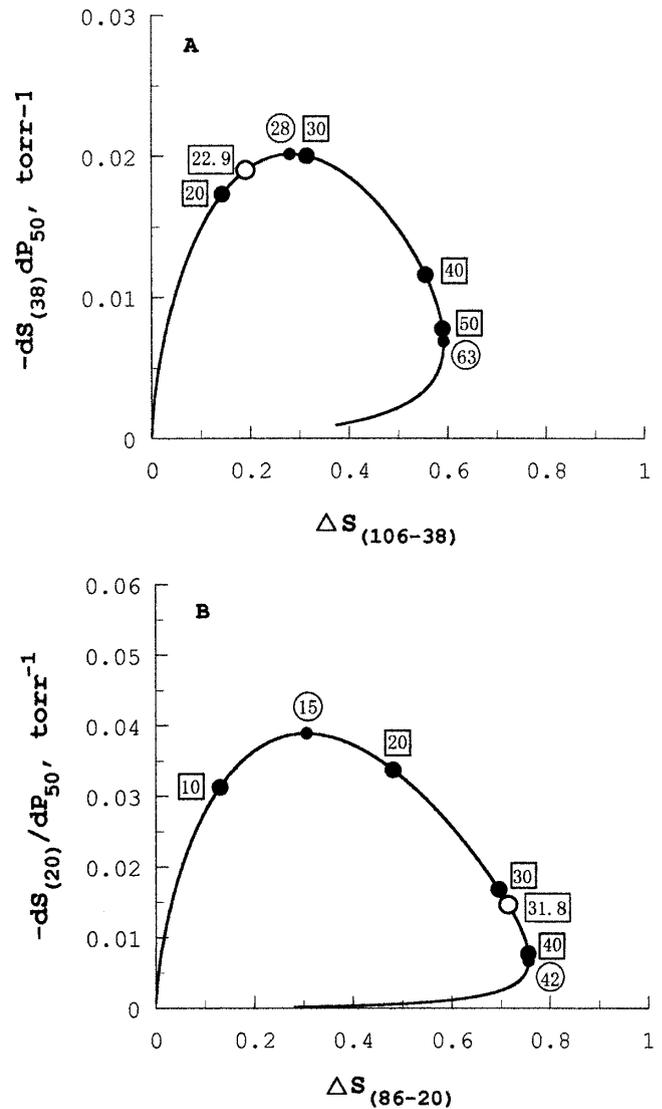


**Fig. 7.** Correlation between effectiveness of Bohr shift in horse fetal blood and that in horse maternal blood at various  $n$  values. The effectiveness of the Bohr shift at the  $O_2$  uptake site in fetal “venous” blood ( $-dS_{(49)}/dP_{50(27)}$ ) is plotted against that at the  $O_2$  release site in maternal uterine venous blood ( $-dS_{(50)}/dP_{50(31)}$ ). The straight dotted line represents the relation:  $-dS_{(49)}/dP_{50} = -dS_{(50)}/dP_{50}$

fetal blood have the same Bohr coefficient as that of maternal blood. From these results, it is concluded that the physiological  $P_{50}$  values of horse fetal and maternal bloods are appropriate for the effectiveness of the Bohr shift occurring at the  $O_2$  uptake and release sites. The relatively low cooperativity of fetal and maternal bloods is well suited for the efficient coupling of  $O_2$  and  $H^+$  transport through the placental membrane. These theoretically obtained results confirm the results on human Hbs reported in our previous paper (Zhang *et al.*, 2003)

### **$O_2$ transport efficiency and effectiveness of the Bohr shift before exercise and at maximal exercise**

Using literature values of *in vivo*  $O_2$  environment and  $P_{50}$  during incremental exercise in horse (Fenger *et al.*, 2000), we calculated  $O_2$  transport efficiency at  $O_2$  release site and the effectiveness of the Bohr shift before exercise and at maximal exercise. The fetal umbilical arterial  $PO_2$  and physiological  $P_{50}$  are different to the literature values used in Fig. 2 (Comline and Silver, 1974). The  $O_2$  consumption rate at maximal exercise was approximately 20 times higher than that before exercise. Fig. 8 shows the relationship between the  $O_2$  transport efficiency and the effectiveness of the Bohr shift at various  $P_{50}$  values with  $n$  fixed at physiological values. Before exercise, the physiological  $P_{50}$  (22.9 torr) was nearly optimal for the effectiveness of the Bohr shift. The  $O_2$  transport efficiency is about 2/5 that of the maximal value (Fig. 8A). In contrast, at maximal exercise, the physiological  $P_{50}$  (31.8 torr) tended to be advantageous for  $O_2$  transport efficiency (Fig. 8B). The effectiveness of the Bohr shift was low, approximately 3/4 that before exercise. The decrease in  $O_2$  affinity and the effectiveness of the Bohr shift at maximal exercise indicate both a decrease in



**Fig. 8.** Relationship between effectiveness of Bohr shift at venous  $PO_2$  (the ordinate) and  $O_2$  transport efficiency (the abscissa) of horse blood before exercise (A) and at maximal exercise (B) at physiological  $n$  and various  $P_{50}$  values. Open circles represent the  $-dS_{(PvO_2)}/dP_{50}$  and  $\Delta S_{(PaO_2-PvO_2)}$  values obtained at physiological  $P_{50}$  values. The number in the squares attached to the line represent  $P_{50}$ . The numbers in the circles represent the  $P_{50}$  which gives the maximal effectiveness of the Bohr shift or  $O_2$  transport efficiency.

pH and an increase in  $PCO_2$  must occur. In fact,  $PvCO_2$  and pH values changed from 49.9 torr and 7.43 before exercise to 82.9 torr and 7.15 at maximal exercise (Fenger *et al.*, 2000).

The  $P_{50}$  of the OEC before exercise, which is lower than  $PvO_2$ , is nearly optimized with respect to the effectiveness of the Bohr shift. On the other hand, at maximal exercise, the physiological  $P_{50}$ , which is higher than  $PvO_2$  but lower than  $PaO_2$ , tends to be advantageous for  $O_2$  transport efficiency. These trends are basically similar to those of human Hb (Itoh *et al.*, 2001)

## REFERENCES

- Adair GS (1925) The O<sub>2</sub> equilibrium curve of hemoglobin. *J Biol Chem* 63: 529–545
- Bohr C, Hasselbalch KA, Krogh A (1904) Ubereinen in biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung ubt. *Skand Arch Physiol* 16: 402–412
- Bunn HF, Kitchen H (1973) Hemoglobin function in the horse: The role of 2,3-diphosphoglycerate in modifying the oxygen affinity of maternal and fetal blood. *Blood* 42: 471–479
- Clerbaux TH, Gusti P, Detry B, Cao ML, Frans A (1993) Comparative study of the oxy-haemoglobin dissociation curve of four mammals: Man, Dog, Horse and Cattle. *Comp Biochem Physiol* 106A: 687–694
- Comline RS, Silver M (1974) A comparative study of blood gas tensions, oxygen affinity and red cell 2,3-DPG concentrations in fetal and maternal blood in the mare, cow and sow. *J Physiol* 242: 805–826
- Comline RS, Silver M (1975) Placental transfer of blood gases. *Br Med Bull* 31: 25–31
- Condo SG, Coletta M, Cicchetti R, Argentin G, Guerrieri P, Marini S, El-Sherbin and Giardina B (1992) The “natural” hybrid haemoglobin from mule. Inter relationships with its parent haemoglobins from horse and donkey. *Biochem J* 282: 595–599
- Ericson A, Verdier CH (1972) A modified method for the determination of 2,3-diphosphoglycerate in erythrocytes. *Scand J Clin Lab Inv* 29: 85–90
- Evelyn KA, Malloy HT (1938) Micro determination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *J Biol Chem* 126: 655–662
- Fenger CK, McKeever KH, Hinchcliff KW, Kohn CW (2000) Determinants of oxygen delivery and hemoglobin saturation during incremental exercise in horses. *Am J Vet Res* 61: 1325–1332
- Hayashi A, Suzuki T, Shin M (1973) An enzymatic reduction system for met-myoglobin, and its application to functional studies of oxygen carrier. *Biochim Biophys Acta* 310: 309–316
- Hill AV (1910) The possible effects of aggregation of the molecule on its dissociation curve. *J Physiol* 18: 4–7
- Imai K, Yonetani T (1977) The hemoglobin-oxygen equilibrium associated with subunit dissociation. 1. An approach with the Hill scheme. *Biochim Biophys Acta* 490: 164–170
- Imai K (1981) Measurement of accurate oxygen equilibrium curves by an automatic oxygenation apparatus. *Meth Enzymol* 76: 438–449
- Imai K (1984) Correlations between the Monod Wyman Changeux model parameters and their implications in oxygenation of mammalian haemoglobins. In *Hemoglobin* (AG Schnek & C Paul, eds.), Brussels Free University, Brussels, pp 83–102
- Itoh R, Sasagawa K, Kimura S, Ishigaki K, Imai K, Kobayashi M (2001) 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. *Zool Sci* 18: 905–908
- Kobayashi M, Ishigaki K, Kobayashi M, Imai K (1994) Shape of the haemoglobin-oxygen equilibrium curve and oxygen transport efficiency. *Respir Physiol* 95: 321–328
- Kobayashi M, Kimura S, Ishigaki K, Makino N, Imai K (1996) Significance of O<sub>2</sub> affinity of fetal and adult human hemoglobins. *Zool Sci* 13: 661–664
- McCarthy EF (1943) The oxygen affinity of human maternal and fetal hemoglobin. *J Physiol* 102: 55–61
- Pellegrini MG, Corda EM, Manga L, Olianias A, Sanna MT, Fais A, Masala B (1996) The hemoglobin polymorphism of the Sardinian wild draft horse and the oxygen binding properties of the four different horse hemoglobins. *Ital J Biochem* 46: 7–14
- Schroeder WA, Shelton JR, Shelton JB, Cormick J and Jones RT (1963) The amino acid sequence of the  $\gamma$ -chain of human fetal hemoglobin. *Biochemistry* 2: 992–1008
- Sold MJ (1982) Is there an optimal  $P_{50}$  of hemoglobin? *Anesthesia* 37: 640–645
- Stockell A, Perutz MF, Muirhead H, Glauser SC (1961) A comparison of adult and foetal horse haemoglobin. *J Mol Biol* 3: 112–116
- Willford DC, Hill EP and Moores WY (1982) Theoretical analysis of optimal  $P_{50}$ . *Respir Environ Physiol* 52: 1043–1048
- Zhang Y, Miki M, Sasagawa K, Kobayashi M, Imai K, Kobayashi M (2003) The cooperativity of human fetal and adult hemoglobins is optimized: a consideration based on the effectiveness of the Bohr shift. *Zool Sci* 20: 23–29

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