Relative Affinity of Human Fetal Hemoglobin for Carbon Monoxide and Oxygen

By Rolf R. Engel, F. Lee Rodkey, John D. O'Neal and Harold A. Collison

The ratio of carbon monoxide (CO) to oxygen bound by hemoglobin A is 217 times greater than the ratio of these gas tensions in the equilibrium medium. This report shows that hemoglobin F has less preferential binding of CO over O₂ than hemoglobin A. Because of this difference between adult and fetal hemoglobin the diffusion gradient for CO across the placenta can not be equated with the difference in carboxyhemoglobin per cent saturation (COHb). This relationship is of interest when comparisons are made between maternal and fetal COHb levels to determine the direction of CO transfer. Such comparisons are pertinent when CO is used to evaluate placental function, and when CO is implicated as mediating an adverse effect on the fetus from maternal smoking.

Materials and Methods

Blood Samples

Placentas were obtained at Caesarean section and fetal blood was collected immediately in a heparinized vial by gravity flow through the dependent cord. At delivery, maternal blood samples were collected from an arm vein in a heparinized tube. Measurements were also made on venous blood samples from 4 children with thalassemia major, without recent blood transfusion. The whole blood samples were kept at 4°C for 1 to 10 days before the equilibration experiments.

Equilibration Procedure

An open or a closed system was used to equilibrate hemoglobin with a gas phase containing CO and O₂. Both of these alternate methods were identical to the procedures used in this laboratory for the determination of the relative affinity constant of adult blood for CO and O₂. The open system experiments required 4 to 6 hours for the blood to equilibrate with a gas stream of predetermined composition. For most experiments the closed system was used for only 2 hours because here equilibrium is established within 90 minutes between the confined gas and blood phases. Repeat experiments were conducted to evaluate the effect of variables such as pH, hemoglobin concentration, and the direction from which equilibrium is approached. Several comparisons were made with whole blood to check that the method of preparing buffered, undialyzed, hemoglobin

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solutions does not modify the results. All of the equilibrations were run in a 37 C. water bath.

Analytic Methods

The fraction of hemoglobin F was determined on all blood samples with Singer's alkali denaturation method. Hemoglobin solutions were prepared for this determination by treating washed cells with 4 volumes of distilled water and freezing twice. Before this method for hemolysis was adopted, a non-ionic detergent (Sterox) was used as a lysing agent. The fetal hemoglobin determinations obtained after hemolysis with Sterox were rejected because this agent introduced a spurious decrease in the assay for hemoglobin F. Total hemoglobin was assayed by the cyanmethemoglobin method. The CO and O₂ content of the equilibrated hemoglobin was measured gasometrically by the method of Sendroy and Liu on 2 ml. samples. For the equilibrated gas the O₂ concentration was measured by Haldane analysis and the CO concentration was determined by gas chromatography. A glass electrode (Instrumentation Laboratories) was used to measure the pH of the samples at 37 C.

Calculations

The above data on the CO and O₂ concentration of the blood and gas phases at equilibrium was used to calculate K as expressed by Douglas, Haldane, and Haldane in the relation:

$$K = \frac{[\text{COHb}]}{[\text{O}_2\text{Hb}]} \times \frac{[\text{O}_2]}{[\text{CO}]}$$

where K is the relative affinity constant of hemoglobin for CO and O₂, while [O₂] and [CO] are the concentrations in the gas phase, and COHb plus O₂Hb are the per cent saturation of hemoglobin by these gases.

To calculate the Kₐ for hemoglobin F the observed Kₒₕ was assumed to vary in direct proportion to the fraction of adult (a) and fetal (f) hemoglobin that was present in the medium. The Kₐ for hemoglobin A was taken to equal 217:

$$K_{o_b} = fK_F + aK_A$$

RESULTS

A total of 37 experiments were done on placental blood samples obtained from 15 pregnancies. In every case the ratio of COHb to O₂Hb was less than would be predicted for adult hemoglobin in equilibrium with the same gas tensions. The average Kₒᵢₕ for all 37 equilibrations was 178 with S.D. of ± 10. For the 14 experiments with a valid determination of the per cent fetal hemoglobin it was possible to calculate a mean Kᵢₕ of 172 with S.D. of ± 12 (Fig. 1).

The experimental conditions and the results for all 37 equilibrations are summarized in Table 1. There is no evidence that any of the experimental variables encountered had an effect which exceeds the combined error of the 4 analytic procedures required for the determination of Kₒᵢₕ. Thus the observed scatter for different samples and for duplicate runs on the same sample of fetal blood can be attributed to an experimental error in the determination of Kₒᵢₕ of at least ± 5 per cent. Because Kᵢₕ is only 20 per cent less than Kₐ for hemoglobin A, only 25 per cent increments in the per cent fetal hemoglobin can be detected from Kₒᵢₕ. The mean Kₒᵢₕ for the 9 experiments with whole blood was 174 which is indistinguishable from the mean Kₒᵢₕ of 179 for the 28 experiments with hemoglobin solutions. Similarly the mean Kₒᵢₕ of 176 for 5 experiments with the open system was not signifi-
HUMAN FETAL HEMOGLOBIN

Fig. 1.—Effect of fetal hemoglobin on the relative affinity constant of blood. Thalassemia major and placental blood samples had lower K values than the blood from normal adult males or pregnant women.

Significantly different than the average $K_{obs}$ of 178 for the 32 experiments with the closed system. In 7 experiments equilibration was begun with a high COHb level and in 25 experiments O$_2$Hb was high initially. The mean $K_{obs}$ for both groups was 178 indicating that there was not a systematic failure to attain equilibrium. The mean $K_{obs}$ was the same for the 20 experiments with hemoglobin concentrations above 14 grams per cent and the 17 experiments with lower hemoglobin concentrations.

For 12 experiments the pH of the medium was between 6.4 and 7.2, 13 other experiments were performed in the pH range 7.2 to 7.5, and the remaining 12 equilibrations were conducted from pH 7.5 to 9.3. The means for the $K_{obs}$ of these three groups were 177, 175, and 182, respectively, suggesting that in this range pH does not influence the K of fetal hemoglobin.

A few additional equilibration experiments were done on other hemoglobin solutions to further exclude a possible effect from variables that are extraneous to the difference in globin structure (Fig. 1). Maternal venous blood samples obtained at the termination of pregnancies A and B had K values of 218 and 229. Thus pregnancy does not alter the K of adult blood.

Hemoglobin solutions from fetal blood samples H and N were diluted by 50 per cent with hemoglobin solutions prepared from normal adult blood. The $K_{obs}$ for the two determinations on these mixtures were 188 and 189 which is intermediate between the values observed on the separate fetal and adult hemoglobin solutions.
Table 1.—Determinations of K for Fetal Blood and for Hemoglobin F

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Equilibrium Values</th>
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<tr>
<td>Fetal Blood Sample</td>
<td>Equilibrium Values</td>
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<tr>
<td>Whole Blood O2Hb 15.7</td>
<td>pH 7.52</td>
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<tr>
<td>Hb solution O2Hb 17.5</td>
<td>COHb 0.583</td>
</tr>
<tr>
<td>Hb solution O2Hb 18.9</td>
<td>O2Hb 0.307</td>
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<tr>
<td>Whole blood O2Hb 18.9</td>
<td>Hb 164</td>
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<td>Whole blood O2Hb 19.8</td>
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* Only these experiments were performed in the open system and the initial ratio of COHb to O2Hb was slightly above or below the equilibrium value.

Hemoglobin solutions from 4 children with thalassemia major and 15 to 35 per cent fetal hemoglobin had K values of 185, 190, 192, and 196. All were below the normal range for hemoglobin A.

If K is constant for hemoglobin F along the entire dissociation curve then the ratio of CO to O2 bound by hemoglobin F should be a linear function of the ratio of CO to O2 in the gas phase. In Figure 2 lines are plotted for hemoglobin A and F with slopes corresponding to the K values of 217 and 172 respectively. The results for the fetal blood samples have been plotted on this graph without a correction for the specific fetal hemoglobin content.
Fig. 2.—Equilibrium ratios of CO to O₂ in gas and blood phases. The values for maternal hemoglobin (x) fall along the line corresponding to hemoglobin A, while the determinations on placental blood (●) cluster around the line calculated for hemoglobin F.

The ratio of CO to O₂ in the gas covers a threefold range while the corresponding values for COHb range between 31 per cent and 58 per cent. Over this restricted portion of the dissociation curve the experimental data maintain a reasonably constant relationship to the predicted line for hemoglobin F. If it can be shown that K_F is a constant along the entire dissociation curve then the CO dissociation curve for hemoglobin F can be predicted from the O₂ dissociation curve of hemoglobin F by simply dividing the oxygen tensions by K_F.

The predicted, in vitro, equilibrium COHb levels for hemoglobins F and A as a function of the ambient CO tension is presented in Figure 3. In the range of physiologic concentrations a newborn and adult sharing the same environment can be expected to have approximately a 20 per cent difference in the equilibrium COHb.

**DISCUSSION**

These data are presented in response to previous statements that the relative CO affinity of fetal hemoglobin is unknown. Although the structures of adult and fetal hemoglobin are well defined the preferential binding of CO over O₂ remains to be explained. Therefore the present finding of a lower relative affinity between CO and fetal hemoglobin could not have been predicted from the fact that the two hemoglobins differ simply in the substitution...
Fig. 3.—Carboxyhemoglobin per cent saturation versus carbon monoxide tension. At physiologic concentrations of CO the predicted, in vitro, equilibrium COHb of hemoglobin F is approximately 20 per cent less than the COHb of adult blood.

The following considerations are cited in support of the thesis that the lower K of fetal hemoglobin results from the disparate globin structures and not from some other difference between adult and newborn blood.

1. The mean $K_{obs}$ for 9 equilibration experiments with whole blood was 174 which is not significantly different than the mean $K_{obs}$ of 181 for the 9 hemoglobin solutions prepared from the same placental blood samples. If either a plasma factor or an aspect of red cell structure were critical then the results on the hemoglobin solutions should differ from the values obtained on whole blood.

2. In 2 experiments solutions of hemoglobin from adult and placental blood were mixed. The K values obtained for these mixtures varied in direct proportion to the amount of fetal and adult hemoglobin. This indicates that the different hemoglobin molecules bind CO independently and that the medium did not modify the K of either adult or fetal hemoglobin.

3. Two hemoglobin solutions, prepared from maternal blood, obtained at the time of delivery, had normal adult K values indicating that pregnancy is
not associated with some obscure factor which lowers the relative CO affinity of hemoglobin on both sides of the placenta.

4. Blood from four children with thalassemia major and 15 to 36 per cent fetal hemoglobin had an average K of 191 which is intermediate between the range observed on normal adults and on placental blood samples with higher fetal hemoglobin levels. This shows that hemoglobin F has a lower K than hemoglobin A regardless of whether it is formed in utero or at a later age. Whether the two situations produce hemoglobin F with an identical K remains unresolved since only 4 patients were studied and at low concentrations of hemoglobin F the calculated K_F is less reliable.

The lower relative CO affinity of fetal hemoglobin appears to be inconsequential in maintaining normal intra-uterine homeostasis. Even in the rare case of CO poisoning during pregnancy the fetus will usually succumb or incur permanent brain damage before the mother. However, a comprehensive description of the diffusion gradient for CO must consider not only the CO content of the maternal and fetal blood but also the hemoglobin concentration, oxygen tension, pH and K on either side of the placenta. When adjacent compartments containing adult and fetal human blood are equilibrated about a 20 per cent difference in the COHb is to be expected because the K of hemoglobin F is that much lower (Fig. 3).

The reported values for maternal COHb do not exceed fetal levels by 20 per cent, presumably because the blood in the umbilical and uterine veins does not attain equilibrium in the placenta for oxygen tension, pH or CO tension. Significant variations in ambient CO and a slow half time for transferring CO between the maternal and fetal circulation can explain the observed discordance between maternal and fetal CO levels. Although there is a positive correlation between maternal and fetal COHb levels some investigators report higher levels for the mother while others find higher COHb in placental blood. The dynamic events attending delivery probably add to the variable COHb results so that a rise in COHb from increased endogenous CO production by the human fetus has not been discerned.

Longo, Power and Forster have made the only other report of K_F in a mammalian species. They found values of 218 and 216 for the K of maternal and fetal sheep blood at 19 C. Whether an equivalent K_F applies to fetal sheep blood at normal body temperature remains to be determined, since the K of adult sheep blood decreases to 162 at 37 C. The K of adult human blood is also inversely related to temperature.

Although pH is known to influence the oxygen affinity of fetal hemoglobin the experimental results for K between pH 6.4 and 9.3 were indistinguishable. This would indicate that in the case of fetal hemoglobin the Bohr effect is proportionately the same for CO and O_2. One might expect that K_F and K_A would show similar changes with pH since Antonini found that fetal and adult hemoglobin have the same Bohr effect for oxygen. However, in contrast to our experiments which show no pH effect on K_F or on K_A, Allen and Root found that the K of whole blood from adult men, dogs and rats is pH dependent with a peak at the pH of normal plasma. The experiments of
Allen and Root are not strictly comparable to our data since they changed pH by altering the CO₂ tension, while no CO₂ was added to the gas phase in the present experiments.

The observed difference between the K of adult and fetal whole blood samples or hemoglobin solutions may reflect a phenomenon such as the action of organic polyphosphates described by Benesch, Benesch, and Yu. Thus in the presence of millimolar concentrations of 2,3 diphosphoglycerate, adult hemoglobin will have a decrease in oxygen affinity. If the interaction between these organic phosphates and fetal hemoglobin is less on a qualitative or quantitative basis then there may be an explanation for the observation that the difference in O₂ affinity between hemoglobin A and F is not demonstrable when purified hemoglobin preparations are compared. Determinations of the K for adult and fetal hemoglobin solutions, stripped of organic phosphates, would help resolve the question of whether these trace compounds selectively depress the O₂ affinity of adult hemoglobin without producing a proportionate decrease in the CO affinity. Without this information it can not be concluded that adult and fetal hemoglobin have the same absolute affinity for CO and that the 20 percent decrease in the relative affinity constant (Kₐ) of fetal blood can be accounted for by the reduced O₂ affinity of adult blood.

**SUMMARY**

Human placental blood has a lower relative affinity for CO than adult blood. By correcting for the fraction of hemoglobin A in placental blood it was calculated that hemoglobin F has a relative affinity constant of 172 ± 12, which is approximately 20 per cent less than the K of hemoglobin A.

**SUMMARIO IN INTERLINGUA**

In humanos, sanguine placental ha un plus basse affinitate relative pro CO que sanguine ab adultos. Post introducer un correction pro le fraction de hemoglobina A presente in sanguine placental, il esseva possibile calcular que hemoglobina F ha un constantt tie affinitate relative de 172 ± 12, lo que es approximativemente 20 pro cento minus que le valor correspondent de hemoglobina A.

**ACKNOWLEDGMENTS**

We wish to thank Drs. Paul Berk, Marion E. Erlandson, and S. L. Leikin for samples of thalassemia blood. Placental blood samples were obtained through the co-operation of the Obstetric Department at the National Naval Medical Center, Bethesda, Maryland. Miss Minna Feld confirmed the fetal hemoglobin determinations independently.

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