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Erythropoiesis oxygen affinity
fetus placenta

The Effect of Hemoglobin F-Chesapeake ($\alpha_2^{92\ \text{Arg.} \rightarrow \text{Leu}}\gamma_2$) on Fetal Oxygen Affinity and Erythropoiesis

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Summary

A carrier of hemoglobin Chesapeake, born of a normal mother, had a cord-blood hematocrit of 60%. The oxygen affinity of his blood was increased. Hemoglobin F-Chesapeake (α_2Chesγ_2), partially purified from the infant's blood, had oxygen affinity greater than that of hemoglobin A, but less than that of the adult form of the abnormal hemoglobin (α_2Chesβ_2). These findings suggest that the conformation of that part of the γ chain which contacts the site of amino acid substitution in Hb F-Chesapeake is similar to the analogous region of hemoglobpn F. They also support the hypothesis that regulation of erythropoiesis in late fetal life is similar to that of the adult, and is under fetal control.

Speculation

The conformation of that part of the γ chain which contacts the site of amino acid substitution in hemoglobin F-Chesapeake is similar to the conformation of the analogous region of the γ chain of hemoglobin F. Erythropoiesis in late fetal life is determined by the oxygen affinity of fetal blood (as well as by placental blood flow and structure, fetal cardiac output, and tissue oxygen utilization), and probably is regulated by erythropoietin produced by the fetus.

Hemoglobin components of umbilical cord blood include F ($\alpha_2\gamma_2$), F_1 ($\alpha_2\gamma_2^{\text{acetyl}}$), and A ($\alpha_2\beta_2$) (16). Carriers of an α-chain mutant (x) have two other components, the fetal and adult forms of the abnormal hemoglobin, $\alpha_2^x\gamma_2$ and $\alpha_2^x\beta_2$ ... and perhaps the acetylated form of the abnormal fetal hemoglobin $\alpha_2^x\gamma_2^{\text{acetyl}}$. If the adult form of the abnormal hemoglobin has abnormal physiologic properties, the fetal form should also have them, if structural interactions between the α^x and γ chains are similar to those between α^x and β in the adult.

Frier and Perutz (7) have compared the structures of deoxyhemoglobins A and F. One of the 39 differences in amino acid sequence between the two hemoglobins is replacement of glutamic acid βCD2 (Similarities between structures of similar chains are most evident when positions within helices are compared, and residues in the same position within a helix usually play similar roles in molecular function. βCD2 = the second amino acid in the region between the C and D helices of the β chain. γC5 = the fifth amino acid in the C helix of the γ chain.) by aspartic acid in the γ chain. In deoxyhemoglobin, this difference causes loss of a hydrogen bond between βCD2 and arginine αFG4. The guanidinium group of αFG4 moves slightly, and a new bond is formed between the arginine and γC5.

In hemoglobin Chesapeake, replacement of arginine αFG4 by leucine causes loss of the βCD2 → αFG4 bond. This loss is probably responsible for the increased oxygen affinity of the adult form of the abnormal hemoglobin (5, 7). Substitution of leucine for arginine should also prevent formation of an αFG4 → γC5 bond, and if the general conformation of the molecule is not altered, the fetal analogue of hemoglobin Chesapeake (Hb F-Chesapeake) ($\alpha_2^{\text{Ches}}\gamma_2$) should have increased oxygen affinity. Availability of a cord blood sample from a newborn carrier of hemoglobin Chesapeake permitted us to study this question, and permitted speculation on the regulation of erythropoiesis in late fetal life.

CASE STUDY

The patient is the son of a normal woman and of a carrier of hemoglobin Chesapeake (III-30 in reference 4). The mother's hemoglobin has normal electrophoretic mobility, and she was not anemic during the pregnancy. The child's birth weight was 3,453 g and he appeared normal on physical examination. The placenta was intact and no abnormalities were seen. At birth, the child's cord blood hematocrit was 60% (normal 52.3; SD = 5.3) (8); at the age of 6 months, it was 37.5% (normal 37; SD = 2.0), (14). Postpartum growth and development have been normal; he was in the 50th percentile for weight and the 60th for height at the age of 16 months.

MATERIALS AND METHODS

Cord blood was collected in EDTA, and studied by techniques previously described from this laboratory (4). Hemoglobin fractions were measured by elution from cellulose acetate strips. Chromatography of carboxyhemoglobin was carried out on columns (2.5 × 25 cm) containing Whatman DE-52, using CO-saturated or KCN-containing glycine-NaCl buffers at 4° C or at room temperature, with a flow rate of 96 ml/hr (1). After equilibration with buffer containing 0.005M NaCl, fractions were eluted with a 2-step gradient, from 0.005–0.03M NaCl, and from 0.03–0.06M NaCl. Samples were concentrated by ultrafiltration; carbon monoxide was removed just before analysis of oxygen affinity by exposure to a bright light under a stream of oxygen.

Oxygen affinity of dilute partially purified hemoglobins (10^{-5}M) was measured in 0.05M bis-Tris 0.10M NaCl at 25° C, according to the method of Imai (10); oxygenation of the sample was assumed to be complete at a $pO_2 > 500$ mm Hg. Deoxygenation was accomplished by equilibration with oxygen-free nitrogen; a few crystals of sodium dithionite were added to achieve complete deoxygenation when the pO_2 was < 1.0 mm Hg. A methemoglobin-reducing system (9) was added to all samples and absorption spectra were devoid of a peak at 630 nm at the end of deoxygenation (before addition of dithionite).

RESULTS

Electrophoresis of cord blood and fractions from chromatography showed that the child was a carrier of a fast hemoglobin, there

being 24% of a component assumed to be F-Chesapeake ($\alpha_2^{Ches}\gamma_2$) and a trace of a component thought to be the adult hemoglobin ($\alpha_2^{Ches}\beta_2$). Globin electrophoresis by Dr. Rose Schneider confirmed the presence of α^A, α^{Ches}, γ, and β chains, as well as the acetylated γ chains of Hb F_1 (15, 16). The p50 of the cord blood was 17 mm Hg, rather than our normal value for cord blood of approximately 20. Normal values from other laboratories range from 19.4 ± 1.8 (13) to 23 ± 1.8 mm Hg (11).

When a hemolysate of the infant's blood was chromatographed on DEAE cellulose at room temperature using KCN-containing buffer, four peaks were obtained instead of the usual three obtained with normal cord blood (Fig. 1). The last peak was assumed to be Hb F-Ches, because of the latter's electrophoretic mobility, while adult Hb Chesapeake was assumed to be included in the Hb F peak. Another portion of the hemolysate was chromatographed at 4°C, using CO-saturated rather than KCN-containing buffers. This modification of the technique was used to minimize formation of methemoglobin. Resolution of the components was not as good as in the original method, but the final peak was used for measurement of the O_2 affinity of Hb F-Ches. Subsequent electrophoresis on cellulose acetate strips showed that the sample contained about 40% of Hb F_1.

Oxygen affinity of the partially contaminated sample of Hb F-Ches was higher than that of Hb A or F, but not as high as that of Hb Chesapeake (Fig. 2). Oxygen affinity of pure Hb F-Chesapeake may be higher than that of the adult form of the abnormal hemoglobin, for F-Chesapeake is probably represented by the steep initial portion of the curve, and Hb F_1, which was the major contaminant of the sample, has a relatively high p50 (2). These data suggest that the high oxygen affinity of the infant's cord blood was the result of the presence of Hb F-Chesapeake.

DISCUSSION

Oxygen affinity of the infant's cord blood and the affinity of partially purified Hb F-Chesapeake were both increased which suggests that loss of the αFG4 → γC5 bond in Hb F-Chesapeake has the same effect as loss of the αFG4 → βCD2 bond in the adult form of the abnormal hemoglobin, making the deoxy-conformation less stable and increasing the proportion of molecules in the high affinity oxy-conformation. Unfortunately, an insufficient amount of blood was available to give more than a semi-quantitative estimate of the degree to which O_2 affinity of the abnormal fetal hemoglobin was increased.

Oxygen affinity of the child's blood was somewhat increased, and his hematocrit was at the upper limit of normal. Adult carriers of Hb Chesapeake are somewhat more abnormal in both regards: they have p50's about 20 mm Hg (normal 25–26), and males have hematocrits of 50–58% (normal 40–54) (4). Findings in the child

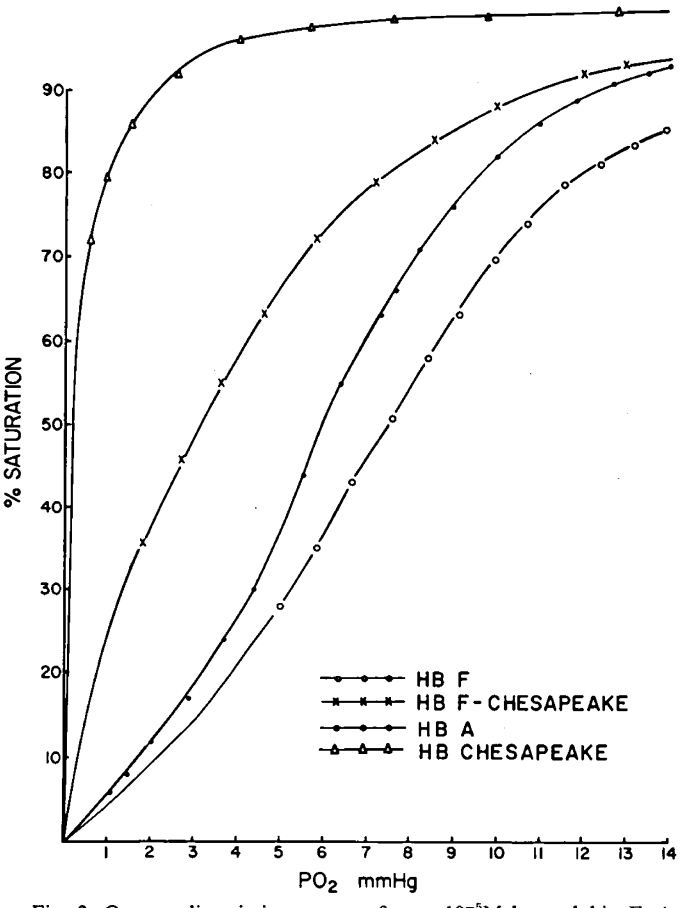

Fig. 2. Oxygen dissociation curves of pure 10^{-5}M hemoglobin F, A, and Chesapeake, and partially purified hemoglobin F-Chesapeake (see text) (25°C, 0.05 M bis-Tris 0.1 M NaCl, pH 7.4).

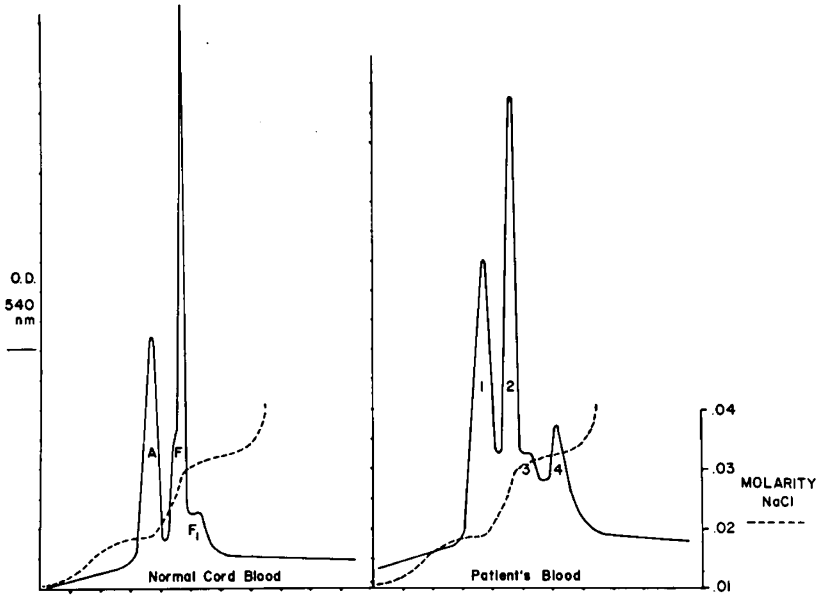

Fig. 1. Chromatogram of the infant's cord blood (on DEAE cellulose), compared to a chromatogram of a normal cord blood (*left*). Letters on the left refer to hemoglobin components. On the *right*, peak 1 was assumed to be hemoglobin A ($\alpha_2\beta_2$); 2, hemoglobin F ($\alpha_2\gamma_2$); 3, hemoglobin F_1 ($\alpha_2\gamma_2^{acetyl}$) plus hemoglobin Chesapeake ($\alpha_2^{Ches}\beta_2$), and 4, hemoglobin F-Chesapeake ($\alpha_2^{Ches}\gamma_2$).

support the hypothesis that erythropoiesis in late fetal life is regulated by fetal production of erythropoietin (6, 18), but demonstration of significant polycythemia in a newborn with a hemoglobin of very high oxygen affinity would be a better test of the proposition.

At the present time, 16 fetal hemoglobins with abnormal γ chains have been described (17). Oxygen affinity has not been reported for any of them. Of the nearly 90 known α-chain variants, at least 10 have increased affinity. Carriers must have had an abnormal fetal hemoglobin at birth, but neonatal polycythemia has not been reported in either these patients or the infants with γ-chain variants. That could be 1) because interaction between the α and γ chains was different from α-β interaction; 2) because many other kinds of stress can cause neonatal polycythemia (12) and the significance of an abnormal hemoglobin was not appreciated; or 3) because the difference between the oxygen affinity of normal fetal blood and that containing the abnormal hemoglobin was not high enough to cause a significant increase in hemoglobin level.

The authors favor the latter suggestion, for hematologic changes in adult carriers of high affinity α-chain variants are not striking. If modulation of erythropoiesis in late fetal life is similar to that in adults, newborn carriers of high affinity α- or γ-chain variants may have hemoglobin concentrations which are not definitely increased, but are only at the upper end of the normal range. The hypothesis may therefore not be provable by this type of evidence, but if carriers have *low* normal hemoglobin levels in the absence of a cause of "anemia", it must be viewed with skepticism.

We obviously think that pregnancies such as that which permitted this study are of great interest, from the points of view of both structure-function relationships in hemoglobin and the regulation of red cell production. We urge that the results of such pregnancies be reported, for they are valuable observations in fetal physiology.

CONCLUSION

At birth, the cord blood of a carrier of Hb Chesapeake contained 24% of Hb F-Chesapeake ($\alpha_2^{Ches}\gamma_2$); the p50 was 17 mm Hg (normal = 20 mm Hg), and the hematocrit was 60%. Oxygen affinity of partially purified Hb F-Ches was higher than that of Hb A, F, and F_1, but not as high as that of the adult form of Hb Chesapeake ($\alpha_2^{Ches}\beta_2$).

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