Pregnancy In Carriers of High-Affinity Hemoglobins

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Pregnancy in female carriers of abnormal hemoglobins with great avidity for oxygen provides a unique opportunity to assess the importance of the usual difference in oxygen affinity between fetal and maternal blood. Outcome of pregnancy was recorded for carriers of hemoglobins Bethesda, Osler, and Yakima, whose p50s (9.5, 9.1, and 12 mm Hg at pH 7.4) were far lower than that of a normal fetus (23 mm Hg at pH 7.3). Neither spontaneous abortions nor intrauterine growth retardation could be attributed to the presence of high oxygen affinity in the mothers. In vitro simulations suggested that neither maternal or fetal polycythemia alone was sufficient to adjust for perturbation of the normal situation, and increased uterine and/or fetal blood flow probably provided additional compensation.

In THEIR DESCRIPTION of a family in which erythrocytosis was associated with the presence of an abnormal hemoglobin with high oxygen affinity (Hb Yakima), Jones and his associates described an unusual incidence of spontaneous abortion in the proband’s sibship. It was suggested that the stillbirths might be a consequence of the hemoglobinopathy, because if the aborted fetuses had had normal hemoglobin, their oxygen affinity would have been lower than their mother’s, who were assumed to carry the abnormal hemoglobin. In 1967, Moore et al presented an opposing view in their report of the birth of a normal child to a carrier of Hb Zurich, which has somewhat increased affinity. Parer and his co-workers and Charache et al described normal children born to mothers with Hbs Rainier and Potomac, and in 1969 Battaglia et al presented experimental data concerning the same problem. They transfused sheep fetuses, replacing high-affinity fetal blood with low-affinity adult blood; no untoward consequences were observed, suggesting that a gradient of oxygen affinity across the placenta was not essential for oxygen transport. In 1971, Noye et al showed that normal adult red cells, transfused into infants in utero, retained the earlier observations that have been amply confirmed.

In a review of the literature, Charache and Murphy* found that equal numbers of normal children were born in families in which the mother was a carrier of a high-affinity hemoglobin and those in which the father was a carrier and the mother was normal (ie, increased maternal oxygen affinity did not appear to be detrimental to survival of normal fetuses). Increased affinity of blood of fetal carriers with normal mothers did not appear to be an advantage, at least in terms of survival during gestation.

Recently, contrasting views have been presented by Hebbel et al and Bauer et al, who pointed out that fetal death was too stringent a criterion, and suggested that intrauterine growth retardation might be a better reflection of fetal hypoxia. These investigators increased the oxygen affinity of pregnant rats by transfusion with carbamylated red cells, and found that fetal growth was, in fact, retarded.

We have recently studied the outcome of pregnancy in carriers of the very high-affinity hemoglobins Osler and Bethesda, as well as in members of the original family with Hb Yakima. In the first, one of two twins was also a carrier and the other was not. In the second, comparisons were made with a previous pregnancy, in which a carrier child exhibited intrauterine growth retardation. In the third, only normal children were born to two carrier mothers.

CASE REPORTS

Family A

(Figure 1A.) A 21-year-old pregnant woman was found to have polycythemia (hemoglobin 16.7 g/dL). She gave a history of "familial polycythemia," and her blood was shown to contain 51% of a fast-moving hemoglobin on agar electrophoresis (I. Steiman, C. Norwood, and S. Charache, in press). Oxygen affinity of her blood was increased (p50* = 12 and 15 mm Hg) on two determinations, but her blood contained 11% to 15% carboxy-hemoglobin (due to cigarette smoking). Oxygen affinity of her nonsmoking brother was also

*Oxygen pressure required for half saturation of blood at 37 °C, pH 7.4. Normal, 26 to 27 mm Hg.

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increased (p50 = 9.5 mm Hg; percentage of CO Hb = 3).

Her 1-year-old son had been delivered at 37.5 weeks, weighing 2,075 g with a ponderal* index of 2.0 [less than 10th percentile]; the placenta weighed 300 g. Apgar scores were 8 at one and five minutes. Hematocrits obtained during the neonatal period were 54% and 57%; the child was subsequently shown to be a carrier of Hb Bethesda. The patient herself had been a full-term infant, but weighed only 1,535 g at delivery; her mother also smoked heavily.

During her second pregnancy, the patient was hospitalized much of the time and was given supplemental oxygen. This child was delivered at 38½ weeks, and weighed 2,445 g. Placental weight was greater than that in the first pregnancy, 450 g. Apgar scores were 9 at one and five minutes (and the ponderal index was 2.15). On the day after delivery, the hematocrit was 64%; 5½ months later, the child’s hemoglobin was 16.8 g/dL. He also was shown to be a carrier of Hb Bethesda.

The patient’s mother and an aunt were also carriers of Hb Bethesda. Neither woman had had any spontaneous abortions. The mother had one normal child, whose birth weight was 2,070 g. The aunt had three children, one of which was normal and had weighed 2,892 g at birth.

Family B

(Figure 1B.) A 31-year-old woman, known to be a carrier of Hb Osler (one of the “daughters” in a previous report13), developed hypertension, proteinuria, and diabetes during her fourth pregnancy. Her oxygen affinity was not measured, but the p50 of her mother’s blood was 9.1 mm Hg (3.5% CO). Prior pregnancies had been unremarkable, two of the three children being carriers of the abnormal hemoglobin; the normal child had weighed 2,665 g at birth. Her hemoglobin concentration was 16.1 g/dL before delivery. Because of gestational diabetes and hypertension, elective Caesarean section was carried out at 36 weeks. One twin weighed 2,268 g (ponderal index 2.18, 10th percentile) and the other 2,110 g (ponderal index 2.61, >50th percentile).11 The diamnionic dichorionic placenta weighed 1,400 g. Apgar scores were 8 at one minute and 8 and 9 at five minutes, and their neonatal courses were unremarkable. Hemoglobin concentrations were recorded as 22.1 for the smaller twin and 25.4 and 17.6‡ for her sister; hemoglobin concentrations were 13.9 and 12.0 when the two children were studied 2½ months later. The smaller twin was shown to be a carrier of Hb Osler; both children also had sickle cell trait.

The patient’s sister had been pregnant six times, and delivered six children. The two normal children weighed 2,140 and 3,955 g at birth, while the four carrier children weighed 2,920 to 3,090 g. One of the carrier daughters had been pregnant twice; the normal child weighed 2,665 g and the carrier weighed 3,444 g at birth.

Family C

(Figure 1C.) Patient III-8 in a previous report,1 a carrier of Hb Yakima, had two carrier daughters whose p50s were about 12 mm Hg.1 Each of these women had two normal children. Birth weights of the children ranged from 2,807 to 3,742 g, and ponderal indices from 2.11 to 2.37. Neither woman had had a spontaneous abortion. All four children have only Hb A, and hemoglobin values at 3 to 4 months of age ranged from 11.2 to 12.0 g/dL.

MATERIALS AND METHODS

Hemoglobin electrophoresis at pH 8.6 and 6.0 was performed on cellulose acetate and agar plates. Fetal hemoglobin was purified from cord blood using Bio Rex 70 and developer 6.13 Hemoglobin Bethesda was purified by chromatography on Bio Rex 70 (Bio-Rad Laboratories, Richmond, Calif) using developers 6 and 9. Globin chains were separated with phosphate buffers containing 8 mol/l urea13 and then lyophilized.

Portions of the α and β chains prepared from Hb Bethesda were aminoethylated and then hydrolyzed with trypsin as previously described.16 The tryptic peptides from about 3 mg of hydrolysate of modified and unmodified globin chains were separated by high-performance liquid chromatography (HPLC) using an ammonium acetate-acetonitrile developer system18 and a 10 x 250-mm Altex Ultrasphere ODS C18 column.19 Peaks from the preparative chromatogram, suspected of containing two or more peptide fragments, were rechromatographed by HPLC using the same column but with a potassium phosphate-acetonitrile developer system.20 Hydrolysis of isolated peptides was done with 6 N HCl in evacuated, sealed

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*Weight in g × 100/(length in cm)^3, an index of intrauterine growth.11

‡There was no record of whether one of these samples was from a heel stick.
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glass tubes by heating for 22 hours at 110 °C. The amino acid compositions were determined by automatic amino acid analysis.

Oxygen affinity of whole blood was measured at 37 °C by tonometry with gases of known pO₂ and a pCO₂ of approximately 40 mm Hg, using an IL 282 Cooximeter (Instrumentation Laboratory, Lexington, Mass), and a Radiometer America (Westlake, Ohio) capillary pH electrode, with subsequent correction to a whole blood pH of 7.4. Oxygen affinity of dilute hemolysates and of mixtures of pure fetal hemoglobin with dilute hemolysates were measured by previously described methods.12

RESULTS

Members of Family A are newly recognized carriers Hb Bethesda.21,22 The diagnosis was established by identifying the presence of a histidyl residue in place of the normal tyrosine at the 145th position of the β chain. The amino acid composition of the abnormal β chain revealed one more histidine than present in the normal. The tryptic peptide pattern of the abnormal β chain showed a shift from the normal position of the peptide corresponding to βT-15. Amino acid analysis of the abnormal βT-15 peptide before and after acid hydrolysis indicated that it contained only histidine as a dipeptide. No other abnormalities were detected in either the α or β chain of this abnormal hemoglobin.

Oxygen affinity of blood from the three mothers was clearly increased. Oxygen affinity of cord blood from the children could not be measured, but affinity of dilute (1.5 × 10⁻⁵ mol/L hemoglobin) mixtures of 30% of a carrier's hemolysate with 70% purified fetal hemoglobin was measured in bis-TRIS buffers containing excess 2,3-diphosphoglycerate (DPG) (1.4 × 10⁻⁴ mol/L), and compared with those of a normal adult and those of carriers, measured under the same conditions. To permit comparisons between curves and estimates of oxygen delivery, a graphic maneuver was employed. "Fetal" and maternal curves were plotted separately. The curve for the normal adult hemolysate was then transposed to the right, so that its p50 (originally 10.7 mm Hg) became equal to that of normal adult whole blood at pH 7.4 (26.6 mm Hg). Each point on the carrier curves was then multiplied by the same factor (26.6/10.7), shifting the curves to new p50s but maintaining their shapes and relative positions. A similar maneuver was carried out for the "fetal" samples, moving a normal fetal hemolysate curve to the p50 of normal fetal blood at pH 7.3,23 and then multiplying the points on the "fetal carrier" curves by the same factor (Fig 2).

Data for the two maternal samples and a sample from a normal woman were replotted in terms of oxygen content in Fig 3A; the curve for the second patient was corrected for 13% carboxyhemoglobin by decreasing the oxygen capacity by that fraction. When oxygen was delivered to the placenta at a pO₂ of 40 mm Hg,24 the two polycythemic women delivered 0.8 and 1.3 mL O₂ per deciliter of blood, respectively, compared with 2.2 mL from a normal woman.
simulated fetal samples are shown in Fig 3B. Between umbilical vein and artery pO\textsubscript{s} of 27 and 15 mm Hg, the two fetal samples could bind 5.7 and 8.3 mL O\textsubscript{2} per deciliter of blood, compared with 7.5 mL for a normal fetus.

**DISCUSSION**

Although the presence of abnormal hemoglobin with high oxygen affinity imposes some stress on the oxygen transport system, hematopoietic and cardiovascular adjustments permit normal function in affected individuals.\textsuperscript{25,26} The observations reported here concern a more complex system, in which oxygen pressures may be as low as those in exercising muscle, but two sets of compensatory mechanisms are available, in the mother and in the fetus.\textsuperscript{27}

If increased maternal oxygen affinity causes either an increased incidence of fetal loss, or intrauterine growth retardation, one would expect to find an above-average number of spontaneous abortions or small babies in the three families described here. Other than in the father's generation of Family C (in which the carrier parent was unknown), abortions were not observed. Both children born to the carrier of Hb Bethesda (Family A) were small, as were she and her sister, but the heavy cigarette smoking in all adult members of her immediate family makes it difficult to attribute decreased birth weight to the presence of the abnormal hemoglobin. The mean birth weight of all babies born to carrier women in the three families was 2,912 g (SD 757), not an abnormal value.

Since \textbeta-globin chain synthesis begins in the first trimester of pregnancy, the experiments of Bauer et al\textsuperscript{10} suggest that a difference in birth weight might be evident between normal and carrier children born to carrier women (ie, high affinity in the fetus might offset high affinity in the mother). When mean birth weight of all normal children born to carrier women (2,972; SD 624) was compared to that of all carrier children born to carrier women (2,865; SD 868), no difference was evident, nor was there a difference from carrier children born to normal women in Family B (mean birth weight 3,222; SD 605). Further, birth weights of the twins born in Family B did not differ from each other or from normal, although one was a carrier and the other was not. Similar findings were present in the aunt of the proband in Family A: she had a twin brother and both weighed 3,636 g at birth, but it is not known whether their mother or father was the carrier.

Oxygen transport to the fetus not only depends on relative oxygen affinity of maternal and fetal blood, but blood flow across both sides of the placenta, as well as the oxygen binding capacity (ie, hemoglobin concentration) and perhaps, pO\textsubscript{2} of maternal and fetal blood.\textsuperscript{24,27,29,30} Maternal polycythemia compensates for some, but not all, of the disability imposed by low p50 (Fig 3A); although there are inconsistencies in the clinical data, it appears that an increased hemoglobin concentration in the fetus may provide further compensation (Fig 3B). Babies born to mothers with cyanotic heart disease,\textsuperscript{31} and lambs born to ewes maintained at high altitudes,\textsuperscript{32} show a similar response, indicating that at least in late pregnancy, fetal erythropoietin production can respond to environmental stress.\textsuperscript{33,34} Similar conclusions are suggested by data from studies of intrauterine transfusions\textsuperscript{6} and a newborn carrier of Hb Chesapeake.\textsuperscript{35}

It is unfortunate that samples of cord blood were not available from any of the children, and that a comparison cannot be made between the hemoglobin concentrations of the children born to the carrier of Hb Osler. The curves shown for simulated fetal blood (Fig 2) are, at best, rough approximations and should be considered as no more than hypothetical illustrations. The p50s of carriers' blood estimated from these curves were 11.3 (Osler) and 10.7 (Bethesda) mm Hg, in reasonable correspondence with the actual values (9.1 and 9.5 mm Hg), suggesting that exaggeration of the difference between normal and abnormal hemoglobins produced by oxygen affinity measurements in dilute solution did not create a larger error, and that the effects of such allosteric effectors as H\textsuperscript{+}, CO\textsubscript{2}, DPG, and temperature were not grossly exaggerated. Under the conditions of the experiments, dilute hemolysates of normal cord blood were indistinguishable from adult hemolysates—probably because the samples were sampled in the presence of excess DPG. Similar results were obtained if no DPG was added; no attempt was made to "strip" samples of endogenous DPG, since it was not possible to obtain additional blood samples from the two mothers.

These rough approximations suggest that perhaps transplacental oxygen transport could not be maintained by fetal and maternal polycythemia alone (Fig 2), and that adjustments in uterine and/or fetal blood flow were needed to preserve oxygenation. As noted above, cardiovascular adjustments also appeared necessary in an earlier study of exercise capacity in carriers of similar hemoglobins.\textsuperscript{32} Studies in animals\textsuperscript{16} and in men\textsuperscript{37} exposed to high altitudes suggest that possession of a high affinity hemoglobin might be an advantage to a fetus within the uterus of a woman who was also a carrier. Comparison of the twins born to the carrier of Hb Osler suggests that that may be the case, because the twin with Hb Osler had a higher ponderal index (greater than 50th v 10th percentile).

It is difficult to reconcile our observations in humans
with results of experiments in rats. The studies of Bauer et al. were performed shortly before delivery, and there was not sufficient time for placental hyper- 

trophy or maternal (or fetal) erythrocytosis to develop in response to the induced change in oxygen affinity. In the experiments of Hebbel et al., placental hyper- 

trophy was observed, but maternal erythrocytosis was not seen, and there was only a suggestion of compensatory erythrocytosis in the fetuses. Our data do not deny the advantage to the normal fetus of high oxygen affinity, but they do attest to the flexibility of maternal–fetal oxygen transport. The practical importance of these observations is not limited to pregnancy in carriers of rare hemoglobins, because similar compensatory mechanisms probably occur when oxygen transport might be limited by maternal anemia.

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