Immunologic Studies of Hemoglobins

III. Fetal Hemoglobin Changes in the Circulation of Pregnant Women

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THE PURPOSE of this investigation was to determine whether fetal red cells can be detected in the maternal circulation during the course of pregnancy or at delivery by following the changes in the amount of fetal hemoglobin present in the mother's blood. Recent studies on fetal hemoglobin utilizing a sensitive quantitative precipitin test have shown that over 50 per cent of normal adult bloods contain minute amounts of a hemoglobin component probably identical with fetal hemoglobin.1 In most of these people less than 0.1 per cent of their total hemoglobin mass is of the fetal type, although occasionally individuals with no apparent hematologic abnormalities are found with amounts up to 1.0 per cent. The fetal hemoglobin content in any one person is remarkably constant over periods of up to several months, seldom varying more than ±0.1 per cent by our determinations.

A group of pregnant women was therefore investigated to determine whether any changes occur in the fetal hemoglobin concentration in the maternal circulation during and after pregnancy. It was anticipated that if a significant rise in the amount of the embryonic pigment could be detected during pregnancy this observation would support, although not prove, the possibility of a transplacental transfer of fetal red cells. Contrary to expectations, our results indicated that in a small percentage of the women followed, increased amounts of fetal hemoglobin were found during the second trimester of pregnancy and that these quantities decreased during the remainder of the pregnancy and the post partum period.

MATERIAL AND METHODS

Blood was collected periodically from 42 white and 49 Negro pregnant women, all patients in the Washington University Clinics. A special effort was made to select women who belonged to the AB blood group because they lack the A and B iso-agglutinins and thus would be less apt to eliminate incompatible fetal cells. Twenty-five of the patients were Rh negative, selected so that any correlation between the appearance of fetal hemoglobin and changes in Rh antibody titer could be detected. Unfortunately, however, none of the 25 subjects had demonstrable anti-Rh antibodies. Blood was collected in some subjects as early as 200 days before delivery and as late as one day before delivery. A second blood sample was drawn usually within a day after delivery and in 24 women samples were also procured at the six week post partum examination.

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Fetal Hemoglobin Determination

The percentage of fetal hemoglobin was determined by the quantitative precipitin test previously described. Tests were generally run in duplicate or in triplicate, but at times as many as 10 to 12 determinations were carried out to ensure the accuracy of the results. A discussion of the errors involved in this procedure has been presented.

All specimens were analyzed shortly after being drawn. Since this experiment lasted ten months all diluted hemoglobin solutions were kept in a deep freeze at -10°C, and at the completion of the series all specimens showing significant changes in the Hgb F content were re-analyzed simultaneously with antiserum of the same lot. This was done to rule out alterations in the reactivity of the antiserum with age which would have given a false decrease in the percentage of fetal hemoglobin in these patients. Prolonged storage of the hemoglobin solutions at this temperature has not resulted in a loss in the percentage of fetal hemoglobin.

RESULTS

Ten of the 91 women studied were found to have significant changes in the fetal hemoglobin content of their circulating red cells (table 1). The remainder, not included in table 1, failed to develop significant differences in the per cent of Hgb F during the period of observation. Fetal hemoglobin could be detected in about half of these 81 individuals. The greatest changes were decreases of 0.09 per cent in two patients. Most of the others showed variations of ± 0.05 per cent between samples.

Data on those patients showing suggestive changes are shown in table 1 and figure 1. Fetal hemoglobin present is expressed as the percentage of the total hemoglobin. Seven women had decreases in fetal hemoglobin varying from 0.33 to 0.98 per cent during the period of observation. Patient no. 1 exhibited the highest amount of fetal hemoglobin in the series, 1.37 per cent, in the specimen obtained 106 days before term. The average of 11 determinations on blood drawn at delivery was 0.604 per cent; by the sixth week post partum the value had fallen to 0.388 per cent. This total decrease of 0.982 per cent is well beyond

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<th>Age yrs.</th>
<th>Race</th>
<th>Maternal Blood Type</th>
<th>Gra-vida</th>
<th>Para</th>
<th>Prepartum</th>
<th>Post Partum</th>
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Table 1.—Significant Changes in Fetal Hemoglobin Concentration During and After Pregnancy
the limits of error of our method. Patient no. 2 also had an abnormally large amount of fetal hemoglobin, 1.26 per cent; 72 days later, at delivery, the value was 0.96 per cent. Unfortunately, no subsequent samples were obtained. Patient no. 3 had 0.65 per cent Hgb F 72 days before delivery and only 0.161 per cent at the time of delivery; no further change occurred during the six week post partum period. Patient no. 4 showed a similar drop (0.493 per cent) but also was not available for a post partum follow-up study. Patient no. 5 had 0.65 per cent fetal hemoglobin 108 days before delivery. Twelve hours before and 30 hours after delivery her fetal hemoglobin level was 0.141 per cent. Three other patients were sampled immediately before and after delivery with no demonstrable increase in fetal hemoglobin. Patient no. 6 had 0.512 per cent fetal hemoglobin 143 days before delivery. Fifty-seven days before delivery she was hospitalized briefly for pyelonephritis; the value then had fallen to 0.265 per cent. At the time of delivery it was only 0.161 per cent. In patient no. 9, the fetal hemoglobin decreased from 0.375 per cent 209 days pre-partum to 0.263 per cent 100 days later; none at all could be detected during labor or six weeks thereafter.

In three patients the changes were not as striking. Patients no. 7 and no. 10 showed decreases of questionable significance, 0.288 and 0.25 per cent, during their relatively short prenatal observation periods, 37 and 72 days respectively. Unfortunately a follow-up sample was not obtained on no. 7. Patient no. 8, with prenatal and post-natal titer of 0.3 per cent was the only one whose decrease occurred only in the post partum period.

The greatest decreases in fetal hemoglobin were observed, for the most part, in those women whose blood had been sampled earliest in pregnancy. We,
therefore, chose another ten women at random whose expected dates of confinement were 90 to 120 days hence and drew two blood samples 30 days apart. Only one of these patients showed a significant amount of fetal hemoglobin (0.92 per cent) and the level had not changed 30 days later. These patients were not included in the previous series of 91 since they were not followed to term. Inspection of the hospital records of the ten women who showed significant fetal hemoglobin changes revealed nothing that could be correlated with these results. Blood type distribution and parity had no apparent influence; three of the women were nulliparous, two had one previous abortion and the rest were multiparous. Of possible but doubtful significance is the race distribution, eight of the ten being Negroes. Sickle cell tests were not done. All were Rh positive. The total hemoglobin values in the above 10 women ranged from 11 to 14 Gm. per cent.

**Discussion**

Since the discovery of the Rh factor in 1940 by Landsteiner and Wiener and the recognition of its role in erythroblastosis fetalis in 1941, by Levine et al., the mechanism whereby fetal erythrocytes cause sensitization has been under investigation by a number of workers. Levine has postulated that fetal red cells leak through the placenta during the course of pregnancy, since half of the women who deliver erythroblastotic infants as a result of their first pregnancy give no history of previous intramuscular injection or transfusion of blood. In support of this are the appearance of Rh antibody in a few untransfused primigravida and demonstrable increases in Rh antibody titer during pregnancy in sensitized women. Since the appearance of Rh antibodies and hemolytic disease are rare in the first born, Wiener believes that the chorionic villae are impermeable during pregnancy and that fetal erythrocyte transfer occurs by the rupture of chorionic villae during labor and at delivery. The increase in antibody titer between one and six weeks post partum is cited as supportive evidence of this but it is also possible that the Rh positive fetus may absorb antibodies, thus maintaining a low level of circulating antibody in the mother during the course of pregnancy.

Other investigators have studied the placenta in attempts to demonstrate gross defects which can account for the transfer of fetal erythrocytes to the mother. The occurrence of emboli of chorionic villae in the lungs of many women coming to autopsy in the post partum period has been one of the strongest arguments in favor of transfer of fetal tissue at delivery. Naeslund and Per Aren were able to demonstrate ruptured chorionic villae by serial microscopic sections of the placenta. These studies were done on placentas which had been delivered from below and the defects found could well be the result of the passage of the placenta through the birth canal. Other investigators have studied infarcts and other changes of erythroblastotic and normal placentas with no conclusions as to the mechanism of transfer.

Physiologic experiments have also been devised to support the possibility of placental permeability to intact erythrocytes. Dienst as early as 1905 obtained evidence for transfer of particulate matter from fetus to mother by injecting methylene blue and milk into the umbilical cord immediately after separation of
the infant by Caesarean section and recovering the milk from the uterine veins. Naeslund injected \( ^{32}P \)-labeled erythrocytes into maternal veins shortly before delivery and in one case out of six was able to demonstrate radioactivity in the cord blood erythrocytes. Much of the \( ^{32}P \) diffused out of the injected red cells, however, giving the maternal plasma a detectable amount of radioactivity. Since the maternal blood lakes in the placental sinusoids it seems possible that the \( ^{32}P \) could have reached the fetal circulation not by transfer of maternal erythrocytes but by diffusion from the maternal plasma into fetal erythrocytes in the chorionic villae. Hedenstedt and Naeslund transfused elliptocytes into women shortly before the onset of active labor and were able to demonstrate elliptocytes in the cord blood, immediately after delivery. Unfortunately it was technically impossible to obtain control cord blood samples before the introduction of the elliptocytes into the mother.

The use of a fetal hemoglobin antibody seemed to offer a suitable method for identifying fetal cells in the maternal circulation. Since our method determines the proportion of fetal hemoglobin in the total hemoglobin of washed maternal erythrocytes, increases in the percentage of circulating fetal hemoglobin during pregnancy could be explained on the basis of placental transfer of intact fetal erythrocytes. Furthermore, changes in the maternal blood volume would not affect the results because only relative concentrations of fetal to total hemoglobin are involved.

The specificity of the fetal hemoglobin antiserum has been discussed earlier; electrophoretic and solubility studies corroborate the conclusion that the alkali resistant hemoglobin used to prepare the antibody is fetal hemoglobin. Normally up to 1 per cent of adult hemoglobin may be of the fetal variety, and the distribution of values observed in this series agrees with that previously found.

It seems likely that the variations observed in seven of the ten women listed in table 1 are beyond the errors of this method. In the other three women the variations of 0.16, 0.23 and 0.25 per cent are well outside the fluctuations found in the other 81 women but are considered merely as suggestive decreases in fetal hemoglobin.

Our results indicate that erythrocytes containing fetal hemoglobin appear in the maternal circulation early in pregnancy, and slowly disappear during the remaining pregnancy and post partum periods. Unfortunately the studies were begun too late in the pregnancies to demonstrate the rise in the level of circulating fetal hemoglobin, but the subsequent drop to accepted normal values suggests that the observed elevations are valid.

These results can be explained by three possible mechanisms. First, a large amount of fetal cells could have entered the maternal circulation in a relatively short period of time and then slowly disappeared over the ensuing 80 to 120 days, the survival time of fetal erythrocytes. The greatest fetal hemoglobin decrease was 0.98 per cent in patient no. 1. Her total hemoglobin during pregnancy was about 12.5 Gm. per cent. Assuming that her blood volume was 4 liters, her total hemoglobin mass was about 500 Gm. Five Gm. (0.98 per cent) of this would be fetal hemoglobin. It has been observed that fetal blood contains at most ten to twelve Gm. per cent of hemoglobin during the first trimester of pregnancy. Assuming this to be true, about 40 cc. of fetal blood would have crossed the pla-
centa to account for the five Gm. of fetal hemoglobin introduced into the mother's circulation. At the end of the first trimester the fetus weighs about 150 Gm. and its blood volume is calculated at approximately 10 cc. Similar calculations on patient no. 9 with a fetal hemoglobin decrease of 0.375 per cent suggest that about 15 cc. would have been transferred during the first trimester. Thus the fetal hemoglobin could not have entered the maternal circulation rather suddenly from the fetus, unless maternal blood was in turn transferred to the developing infant.

Another possibility is that the fetal erythrocytes slowly leaked across the placenta during the first half of pregnancy and gradually raised the maternal level of Hgb F. This slow process would thereby allow the fetus to compensate for the loss of red cells. Both of these possibilities are unlikely since the chorionic villae are much thicker earlier in pregnancy, still having the Langhans layer present. Since the slope of the curves in figure 1 offers no evidence that fetal erythrocytes cross the placental barrier during the last trimester when the villae are thinner and more susceptible to rupture, it seems more probable that the fetal hemoglobin found was produced by the mother in response to the stress of pregnancy. Indeed, it has previously been proposed that the fetal hemoglobin produced in various hemolytic anemias is due to a persistent or reactivated fetal erythropoietic mechanism which attempts to compensate for excessive demands upon the adult erythropoietic tissues. Although the absolute nutritional requirements for the early embryo are much smaller than for the fetus of the last trimester, hormonal changes occur very rapidly, the most noteworthy being in the concentration of chorionic gonadotropin. It may be postulated that the acuteness of these changes produces an early stress which reactivates a fetal erythropoietic system with resultant production of erythrocytes containing fetal hemoglobin. Later the mother may adjust to the pregnancy in that she forms less fetal hemoglobin and slowly loses from her circulation fetal hemoglobin-containing red cells formed in the early stages of pregnancy.

Those who favor leakage of fetal cells across the placenta during pregnancy stress the fact that only small amounts of blood are required for Rh sensitization. Our technic is not sensitive enough to rule out this possibility.

Since this study was completed, Chown has presented one case of neonatal anemia due to fetal placental hemorrhage into the maternal sinusoids. From the data presented, at least 90 ml. of blood was transfused to the maternal circulation. The values for the amounts of fetal hemoglobin are open to considerable doubt because the methods used are insensitive to less than 10 per cent hemoglobin F. Qualitatively, however, there seems to be little question that at least some transfer of blood took place. That this mechanism must be an uncommon one is seen from the data in the present study which employed technics far more sensitive and would have detected the entrance of ten or more ml. of blood into the mother's circulation.

**SUMMARY**

Changes in the concentration of fetal hemoglobin in maternal blood during pregnancy and after delivery were studied in 91 pregnant women. Significant elevations of fetal hemoglobin were detected in the blood of ten women during
the second trimester of pregnancy; these values fell toward normal during the ensuing pregnancy and post partum periods. In no instance could a rise in fetal hemoglobin be detected after delivery. The significance of these observations is discussed; it is suggested that acute hormonal changes early in pregnancy may be responsible for the reactivation of a fetal erythropoietic anlage with the resultant production by the mother of erythrocytes containing fetal hemoglobin. The method used was not sensitive enough, however, to detect minute amounts of fetal blood which may have crossed the placental barrier.

**SUMMARIO IN INTERLINGUA**

Alterationes del concentracion de hemoglobinsa fetal in le sanguine materne durante le pregnantia e post parto esseva studiate in 91 feminas gravide. Significant elevaciones del nivello de heirioglohina esseva detegite in le sanguine de 10 feminas durante le secunde trimestre del pregnantia. Iste valores se re-abassava verso le norma durante le subsequente periodos pregnancial e post-partal. Un augmento del hemoglobina fetal post parto non esseva detegite in ulle caso. Nos discute le signification de iste observationes e presenta le hypothese que acute alterationes hormonal durante le prime phases del pregnantia es responsabile pro le re-activation de un primordio fetal de erythropoiese con le resultato del production per le matre de erythrocytos continentem hemoglobina fetal. Nonobstante, nos debe signalar que le metodo usate non esseva satis sensibile pro detegere minime quantitates de sanguine fetal que ha possibilemente transversate le barriera placental.

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