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A Point About Fetal Hemoglobin Determination in Cord Blood*

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It has been shown by Pataryas and Stamatoyannopoulos that Hb A synthesis in vivo begins after the llth week of gestation,[1] and increases by the gestational age.[2, 3] This knowledge may help in the in trauterine diagnosis of hemoglobinopathies related to beta chain abnormalities after the first trimester.[1, 2, 3, 4]

Fetal hemoglobin "Hb F" concentration in term infants is about 53 to 89 % of the total hemoglobin,[5, 6] but the actual fetal hemoglobin being synthesized represents only about 60 % of the total hemoglobin synthesized at this time.[7] This quantity of Hb F being produced in term infants is significantly less than at 32 and 36 weeks gestation.[8]

The red blood cells at birth demonstrate marked variability in size and shape and also of their hemoglobin composition. It has been shown that young cells have a higher concentration of water and are larger than the old cells. In this study, young and old cells in the cord blood were separated by centrifugation and the Hb F content of these cells was evaluated by alkali denaturation and acid elution methods.

Material and Methods

15 ml of cord blood was collected in ACD containing tubes from the placental site of 41 full term infants in the delivery room. These samples were centrifuged at 4000g for 15 minutes in special cellulose tubes. Plasmas were decanted and special places at the bottom of the tubes were pierced and 1 cc of erythrocyte sediment was obtained; when only 1 cc of top red cells was left, these were placed in another tube marked "top".

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Hb F concentrations of "top" and "bottom" parts of red blood cells were determined separately by Singer et al's method.[9] In 7 specimens, one drop of upper and lower erthrocyte samples were diluted with 2 drops of their own plasma and smears were made for acid elution stain, according to Kleihauer et al's method.[10] 500 cells were counted from the upper and lower erythrocytes samples in the evaluation of Hb F containing cells.

Results

The mean Hb F values of the "upper" and "lower" samples were found to be 59.6 ± 10.8 % (SD) and 71.3 ± 8.8 % (SD), respectively, and the difference between them was statistically significant ($p<0.001$), (Figure 1, A).

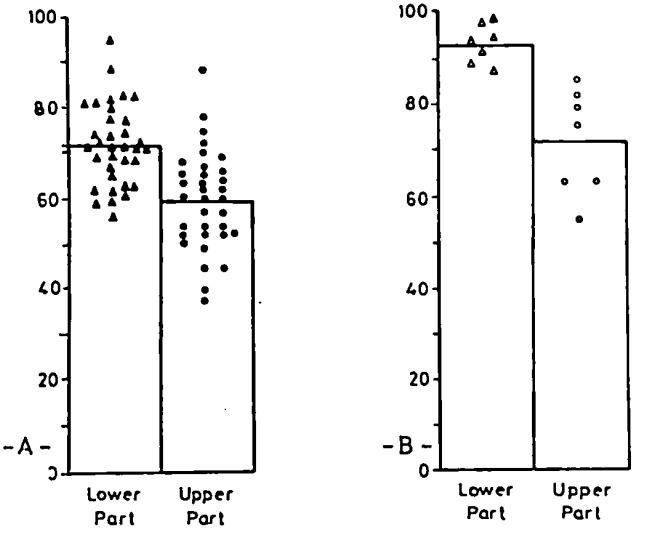

Figure 1

A. Fetal hemoglobin determination, from "upper" and "lower" specimens of cord blood, by alkali denaturation method.

B. By acid elution method.

By the acid elution technique, red blood cells containing Hb F of "upper" and "lower" parts were 71.57 ± 11.63 % (SD) and 92.57 ± 4.19 % (SD), respectively. The difference between them was also statistically significant, ($p<0.001$) (Figure 1. B). Some white blood cells, normoblasts and platelets were seen in the "upper" samples, which were not present in the "lower" specimens.

The upper red blood cells were more heterogenous from the standpoint of their hemoglobin composition and the cells seemed to be larger.

The lower red blood cells were somewhat smaller and stained darker in some areas, like sperocytes (Figures 2 and 3).

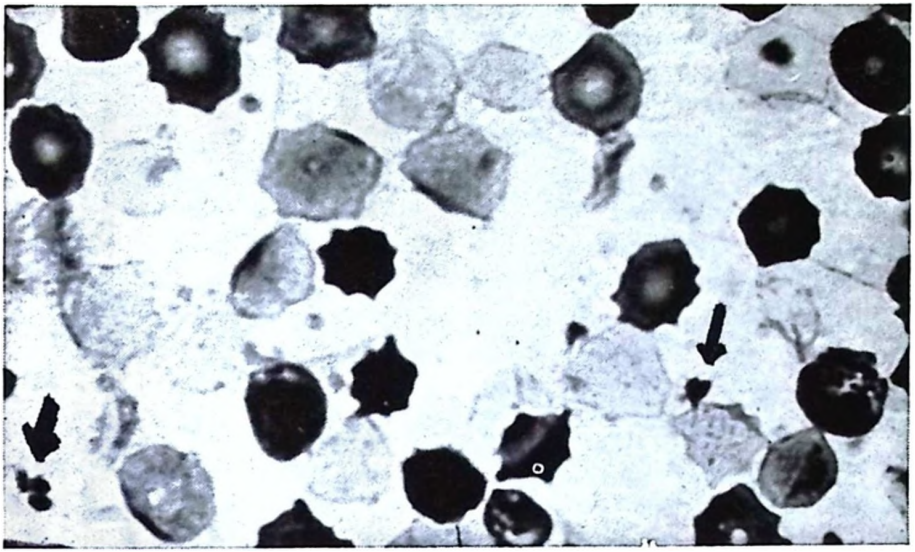

Figure 2
Ghost cells containing hemoglobin A are clearly seen from upper specimen smear, (arrows indicate platelets).

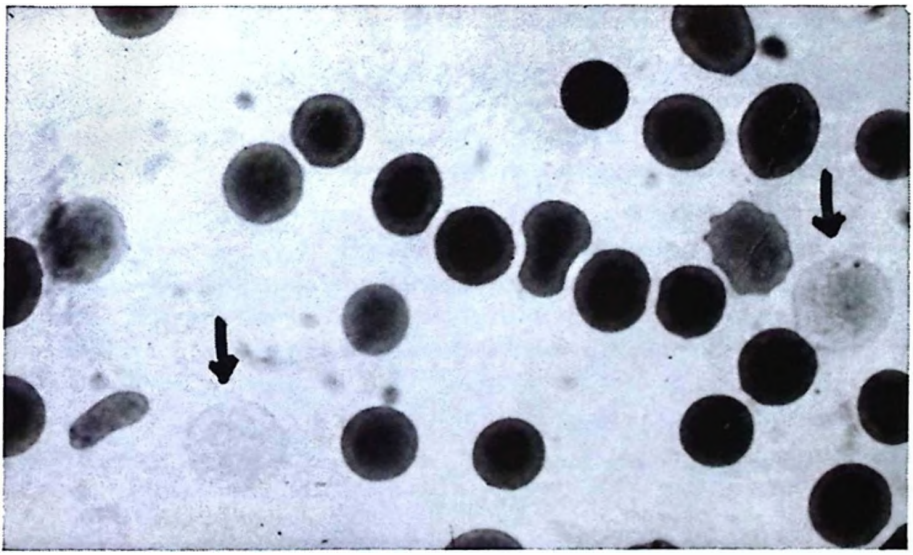

Figure 3
Smear prepared from the lower part of the specimen of Figure 2. The spherocytes are seen clearly; heterogeneity of red cells are less pronounced. (Arrows indicate HbA containing ghost cells, which are much fewer than in the upper parts.)

Comments

Fetal hemoglobin is found elevated in different conditions such as, beta-Thalassemia major, persistant fetal hemoglobin syndrome, sickle-cell anemia,[9] spherocytosis,[9] pernicious anemia,[11] leukemia,[12] dtrisomy,[13] etc. In general, fetal hemoglobin in the newborn could be separated from the adult type fetal hemoglobin elevation by the determination of the alanine-gylcine ratio in 136 amino acids of this hemoglobin.[14] But this determination would not be practical in the diagnosis of some conditions which might occur in the newborn period, such as maternal-fetal transfusions or in the evaluation of intrauterine transfusions in the erythroblastosis fetalis cases.

In these conditions, blood is obtained during delivery and several analyses are carried out on that specimen. In the light of this study, it should be emphasized that:

a. If large amounts of blood is obtained from the cord for different studies, the specimen should be used properly. For Hb F determination by alkali denaturation samples should not be obtained,only upper or lower parts.

b. The smears prepared for a fetal hemoglobin stain, are better prepared directly from the patient; but if it has to be made from the sample at the laboratory, it must be prepared before centrifugation. If the samples have waited for a long time, the smear must be made after the samples have been mixed.

It was also previously shown from this laboratory, that Hb F stain and alkali denaturation methods gave comparable results but the results should be evaluated accordingly, since the staining method will give higher values in the same specimens.[15]

Komazawa, Garcia and Oski,[16] have recently shown that the elution process does not alter the size of the cell, but larger erythrocytes contain a significantly lesser proportion of fetal hemoglobin and the total hemoglobin concentration of these cells is also decreased. Bard et al,[7] indicated that in term infants the actual Hb F being synthesized represents only 60 % of the total hemoglobin synthesized. It should also be pointed out that in this study, the Hb F concentration of the upper part was 59.6 percent.

Summary

Fetal hemoglobin concentrations of upper and lower part cord blood in 34 full term infants were determined by alkali denaturation method and was found significantly different, i.e. 59.6 % and 71.3 %, respectively. Smears from the same specimen were prepared and the red blood cells

were counted according to their Hb F staining, which showed the bottom specimens to have a significantly higher value than the upper part. Whenever large samples of cord blood is sent to the laboratories for Hb F determination, blood should be mixed before these determinations, or the results should be evaluated accordingly.

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