FOETAL HAEMOGLOBIN
IN HAEMOLYTIC DISEASE OF THE NEWBORN

BY

THOMAS E. OPPÉ* and IAN D. FRASER

From the Departments of Child Health and Pathology, University of Bristol
and Southmead General Hospital, Bristol

A difference in the proportions of foetal (Hb-F) and adult (Hb-A) haemoglobins between normal infants and those affected by haemolytic disease due to the rhesus factor was first reported by Jonxis (1948). His work was later extended by several investigators and the differences were confirmed. The hitherto reported studies have been concerned mainly with full-term infants and it was the purpose of the present investigation to gain information about the proportions of Hb-F and Hb-A in affected infants born prematurely. As a result we are able to present some original data concerning the problem and to offer an explanation of the results obtained which differs from those previously suggested.

Material and Methods

Cord blood samples were obtained at delivery from 37 infants affected by haemolytic disease due to the rhesus factor. Cord blood samples were also taken from 29 infants of similar maturities but not affected by haemolytic disease.

The samples were analysed for total haemoglobin concentration by the Medical Research Council grey wedge photometry and for Hb-F by the alkali-denaturation method of Singer, Chernoff and Singer (1951).

Maturity was assessed clinically and although birth weight was an important factor in assessment the cases were grouped according to the estimated gestational age. Cases delivered during the 35th to 36th week of pregnancy (10 cases) were termed 'premature', during the 37th to 38th week (15 cases) 'pre-term' and after the 38th week (12 cases) 'full-term'.

The severity of the haemolytic disease was assessed largely on the cord blood haemoglobin value. A total haemoglobin of 100% (14·8 g./100 ml.) or above placed the infant as 'mildly' affected; if the cord haemoglobin value was 78% (11·5 g./100 ml.) or below the infant was assessed as 'severely' affected. A classification of 'moderate' disease was made if the haemoglobin was between these levels. Clinical condition of the baby, maternal antibody titre and subsequent progress entered

into the evaluation of the babies, but in this series did not cause any major deviation from assessments of severity based on cord haemoglobin level.

Premature induction of labour was performed for clinical reasons and broadly followed the indications recommended by Tovey and Valaes (1959), the clinician in charge of the case making the decision without knowledge of this study.

Results

Fig. 1 shows the individual values for percentage Hb-F in the cord blood of the affected infants related to gestational age and severity of haemolytic disease. For comparison the means ±2 standard deviations of similar determinations on normal infants are also shown. It can be seen that with the exception of mildly affected infants at term, the percentage of Hb-F in the affected infants falls well below the expected value at all periods of gestation.
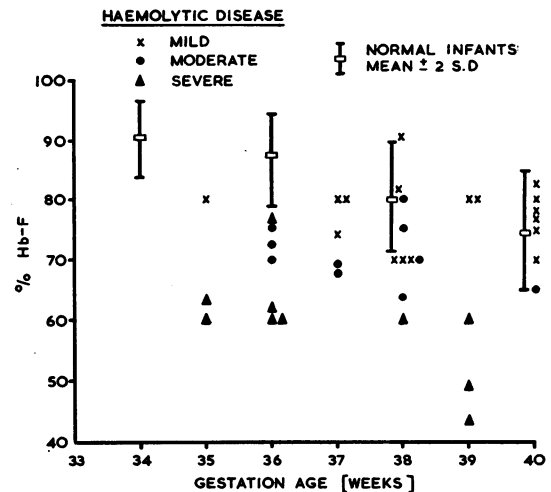

FIG. 1.—The results of individual determinations of percentage foetal haemoglobin in 37 infants affected with haemolytic disease compared with mean values of percentage foetal haemoglobin in normal infants.

* Present address: St, Mary's Hospital Medical School, London, W.2.

TABLE 1

	No. of Cases	Mean % Hb-F	Standard Deviation	S.E. of Mean
Mild	17	77·9	6·19	1·50
Moderate ..	10	71·0	4·85	1·53
Severe	10	59·8	8·20	2·59

The mean values for percentage foetal haemoglobin in the affected infants grouped by severity of disease but ignoring gestational age are given in Table 1.

The difference between these means by analysis of variance is significant at p = 0·001 level. A 't' test between the means of the mild and moderate cases gave t = 3·20 (p = 0·01). If gestational age were taken into account the significance would be even higher as the severer cases were more immature than the mild cases.

The reported findings that infants affected by erythroblastosis have a proportionate increase in Hb-A is confirmed and, furthermore, it is apparent that this increase is present by the 36th week and is correlated with severity of disease.

However, the demonstration of a proportionate increase of Hb-A does not show whether it is due to an absolute increase in the amount of Hb-A or a diminished quantity of Hb-F. In Fig. 2 it is made clear that in part at least the proportionate increase in Hb-A is due to an absolute increase in concentration of adult haemoglobin. There the total haemoglobin concentration in grammes per cent. is plotted with the absolute contributions made by the two varieties of haemoglobin. The cases are grouped by estimated gestational age and by severity of disease.

The data from the premature infants (35-36 weeks) are the most instructive and normal infants are compared with affected infants irrespective of severity in Table 2.

TABLE 2

	No.	Mean Hb-A (g. %)	Standard Deviation
Normal	9	1·94	±0·678
Affected	10	3·48	±0·557

A t test between these means gives a value of t = 5·42 (p = 0·001). It is clear that the reduction in total haemoglobin caused by the haemolytic disease affects the Hb-F fraction and there is a significantly greater absolute concentration of Hb-A in the peripheral blood of affected infants than in normal premature infants.

A further analysis of the data is instructive. The absolute concentrations of Hb-F and Hb-A in the cord blood samples can be plotted against the individual total cord blood haemoglobin values. For convenience these two analyses are placed on the same graph in Fig. 3. It can be seen that there is a linear correlation between cord blood total haemoglobin and Hb-F, and a much less marked correlation with Hb-A.

The correlation coefficients were calculated and gave:
(a) total haemoglobin v Hb-F, r=0·968, p=0·001;
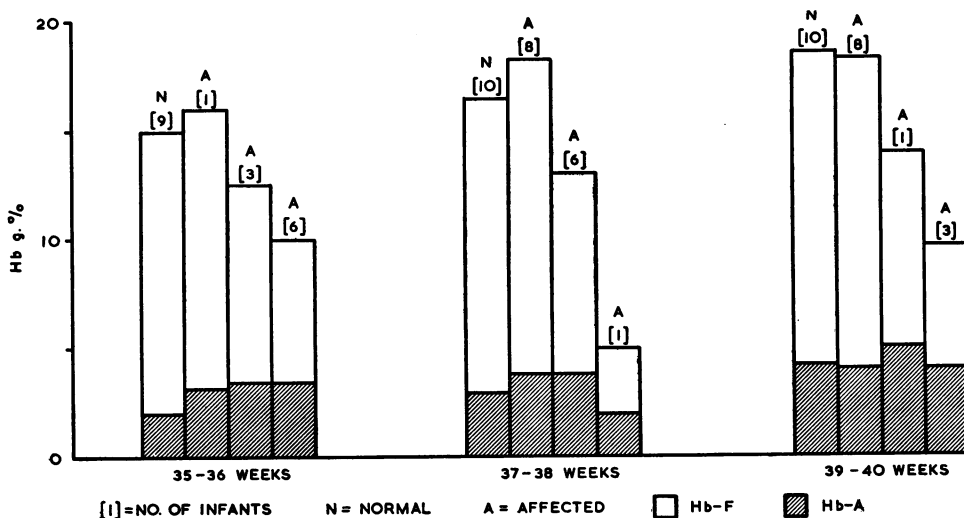(b) total haemoglobin v Hb-A, r=0·365, p=0·05.

FIG. 2.—Mean values for concentration of total haemoglobin and fractions of adult and foetal haemoglobin in the cord blood of premature, pre-term and full-term infants. At each gestational age unaffected infants are represented by the left-hand columns.
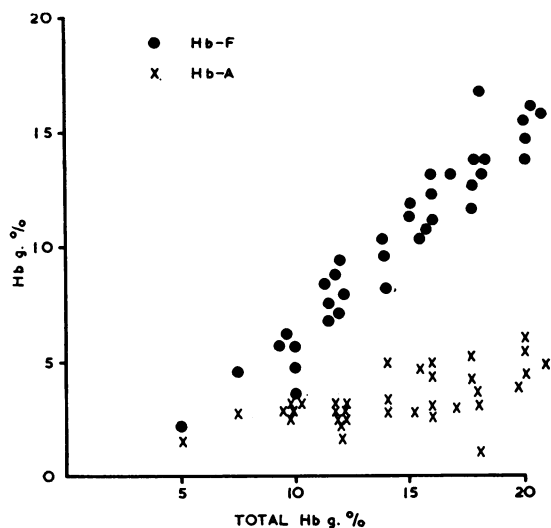
FIG. 3.—Concentration of total haemoglobin in affected infants compared with the fractions of foetal haemoglobin and of adult haemoglobin.

Discussion

The results presented confirm the finding that in haemolytic disease there is an increase in the proportion of adult haemoglobin (Hb-A) in the peripheral blood and that this proportionate increase of Hb-A is due to a decrease in the concentration of foetal haemoglobin (Hb-F). We have shown further that these differences in haemoglobin constitution are apparent in affected infants born during the 35th to 36th weeks of gestation as well as nearer or at full-term. In our material there are significant correlations between the severity of the haemolytic disease (as estimated by anaemia at birth) and increase in percentage Hb-A; and between the total haemoglobin concentration and concentration of the Hb-F fraction. There is a somewhat less significant correlation between the total haemoglobin concentration and the Hb-A fraction. In these respects our results are essentially similar to those of Schulman and Smith (1954) and Brody and Engström (1960). However, in confirming Schulman and Smith's (1954) finding that in haemolytic disease there is an absolute increase in the concentration of Hb-A we are at variance with Brody and Engström (1960). It is of considerable interest that we found this to be so even in premature infants.

These discrepancies are more likely to be due to differences in the cases examined than variations in technique, because there is good agreement between investigators regarding other aspects of the same data. They are, however, important because much of the discussion regarding the interpretation of the changes found in haemolytic disease depends upon the controversial findings. Briefly it is maintained by Schulman and Smith (1954) that evidence of an increase in concentration of Hb-A indicates a preferential generation of Hb-A in the foetus under stress, while Brody and Engström (1960) on their data prefer to revert to the original hypothesis of Jonxis (1948) that there is selective destruction of cells containing Hb-F.

In our view the experimental data can be reasonably explained without recourse to either preferential generation of one form of haemoglobin or selective destruction of the other, and the contradictions can be reconciled by biological variations within the spectrum of the disease process. If it is assumed that in haemolytic disease of the newborn there is random destruction of vulnerable erythrocytes (Mollison, 1943), and that during the latter weeks of pregnancy there is a constantly increasing synthesis of Hb-A from a small fraction of the total haemoglobin synthesis at 34 weeks to a large proportion at term (Walker and Turnbull, 1955; Cook, Brodie and Allen, 1957; Brody and Nilsson, 1960; Beaven, Ellis and White, 1960), it is possible to construct a simple arithmetical model of the dynamics of haemoglobin formation at this time. The result of such an artificial construction in terms of haemoglobin differentiation in the peripheral blood is graphically illustrated in Figs. 4 and 5.

'Foetus A' (Fig. 4) represents a normal foetus during the last 10 weeks of gestation, in which the haemoglobin mass triples in volume and the synthesis of adult haemoglobin increases regularly from 10% of the total at 31 weeks to 70% at term. The assumed erythrocyte life is 100 days. It can be
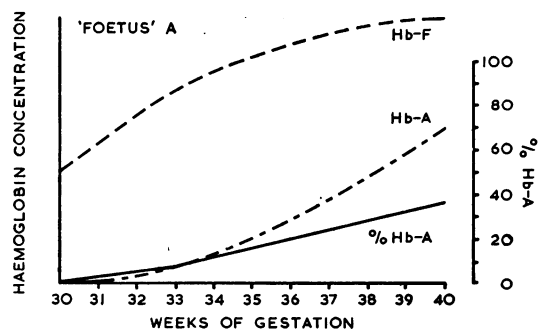

FIG. 4.—Representation of the concentrations of Hb-A and Hb-F and proportion of Hb-A in a hypothetical unaffected foetus. It is assumed that during the last 10 weeks of gestation the haemoglobin mass triples in volume and that the survival time of the foetal erythrocyte is 100 days. A constant increment in the synthesis of Hb-A is assumed.
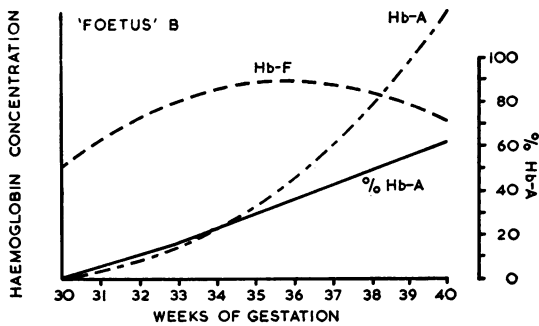
FIG. 5.—Representation of the concentrations of Hb-A and Hb-F and the proportion of Hb-A in a hypothetical affected foetus. It is assumed that during the last 10 weeks of gestation the haemoglobin mass triples in volume and that the survival time of the foetal erythrocyte is 20 days with a constant increment in the synthesis of Hb-A, similar to that in 'foetus' A (Fig. 4).

seen that the proportion of Hb-A rises steadily as does the concentration of Hb-A, whereas the concentration of Hb-F rises only slowly after the 36th week. This, in spite of the artificial linearity, is representative of the known facts in normal infants. 'Foetus B' (Fig. 5) represents an affected infant over the same time period and with the same rate of growth and synthesis of Hb-A. However, the assumed erythrocyte life is 20 days and it is further assumed that the red cells are destroyed randomly. Comparison of the two figures shows that the calculated 'affected' baby displays the same differences in haemoglobin differentiation as are found between normal and erythroblastotic infants.

This simple representation of the dynamics does not take into account the failure of the severely affected baby to produce adequate haemoglobin to maintain growth and compensate for haemolysis. It is probably the relationship between the erythropoietic capacity of the baby and the variation in survival time of the red cells combined with variation in rates of Hb-A synthesis which complicate interpretation of the experimental data and account for the discrepancies found.

Until further more direct measurements of the variables involved are available it seems reasonable to interpret the differences in foetal and adult haemoglobins in haemolytic disease as due to the effects of random haemolysis and blood regeneration occurring at a time when there is a normal increase in the synthesis of adult haemoglobin. It seems unnecessary to propound either a selective destruction of cells containing predominantly Hb-F or to suggest that stress on the erythropoietic organ induces preferential synthesis of adult haemoglobin.

Summary

Determination of foetal haemoglobin in the cord blood samples of 37 infants suffering from haemolytic disease and comparing them with normal infants of similar gestational age has shown the following:

(1) An increased proportion of Hb-A in the affected infants which is related to the severity of the disease.

(2) A decrease in the concentration of Hb-F which is related to the total haemoglobin concentration.

(3) An increase in the concentration of Hb-A which is poorly correlated with total haemoglobin concentrations.

(4) These effects are present from the 35th week of gestation.

An explanation of these findings is offered suggesting that they are the expected results of haemolysis and increased erythropoiesis at a time when there is regularly increasing biosynthesis of the Hb-A fraction.

REFERENCES

Beaven, G. H., Ellis, M. J. and White, J. C. (1960). Studies on human foetal haemoglobin. II. Foetal haemoglobin levels in healthy children and adults and in certain haematological disorders. *Brit. J. Haemat.*, 6, 201.

Brody, S. and Engström, L. (1960). Foetal and adult haemoglobin in newborn infants with erythroblastosis foetalis. *Acta paediat., Stockh.*, 49, 868.

—— and Nilsson, B. A. (1960). Foetal and adult haemoglobin mass in relation to foetal development. *J. Obstet. Gynaec. Brit. Emp.*, 67, 827.

Cook, C. D., Brodie, H. R. and Allen, D. W. (1957). Measurement of fetal hemoglobin in newborn infants. Correlation with gestational age and intrauterine hypoxia. *Pediatrics*, 20, 272.

Jonxis, J. H. P. (1948). Foetal haemoglobin and erythroblastosis. *Nature (Lond.)*, 161, 850.

Mollison, P. L. (1943). The survival of transfused erythrocytes in haemolytic disease of the newborn. *Arch. Dis. Childh.*, 18, 161.

Schulman, I. and Smith, C. H. (1954). Fetal and adult hemoglobins in hemolytic disease of the newborn. *Amer. J. Dis. Child.*, 87, 167.

Singer, K., Chernoff, A. I. and Singer, L. (1951). Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. *Blood*, 6, 413.

Tovey, G. H. and Valaes, T. (1959). Prevention of stillbirth in Rh haemolytic disease. *Lancet*, 2, 521.

Walker, J. and Turnbull, E. P. N. (1955). Haemoglobin and red cells in the human foetus. III. Foetal and adult haemoglobin. *Arch. Dis. Childh.*, 30, 111.