

HAEMOGLOBIN AND RED CELLS IN THE HUMAN FOETUS

III.—FOETAL AND ADULT HAEMOGLOBIN

BY

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In 1866 Körber found chemical differences between haemoglobin obtained from human placental blood and that from normal adult blood. Since then it has been clearly demonstrated that there are many important differences between human and animal and between foetal and adult haemoglobins (Krüger, 1888; Bischoff and Schulte, 1926; Haurowitz, 1930, 1935; Trought, 1932; Brinkman, Wildschut and Wittermans, 1934; Hill, 1935; Brinkman and Jonxis, 1935; Jope and O'Brien, 1949).

Haselhorst and Stromberger (1930, 1932) showed that the blood of the human foetus possessed a higher affinity for oxygen than that of its mother, but Hill (1935) found that human foetal haemoglobin had a lower affinity than human adult haemoglobin. This apparent anomaly was explained by the work of McCarthy (1943), who showed that the affinity of the haemoglobins for oxygen was greatly modified when they were within a corpuscle. Adult corpuscles caused a marked decrease in the oxygen affinity of their contained haemoglobin, while foetal corpuscles had little effect on the affinity of foetal haemoglobin for oxygen. Recently, Allen, Wyman and Smith (1953) have re-studied the oxygen affinity of human haemoglobins. Unlike Hill (1935) and Haurowitz (1935) they found no difference between the affinity for oxygen of the haemoglobin of the human foetus and that of its mother, provided that the haemoglobin solutions were so treated that the concentrations of dialysable substances were identical. They also found that the haemoglobin from one foetus at 31 weeks had the same affinity as the haemoglobins of term foetuses and of their mothers.

Most investigators have been concerned with a study of the physical, chemical and other characteristics of human haemoglobins, and little information is available of the relative proportions of each type of haemoglobin in the blood of the human foetus at

various stages of gestation, although such information is available for the sheep foetus. Karvonen (1949) found that adult haemoglobin first appeared in the sheep foetus at or about the 105th day and that at term 50% of the haemoglobin was adult in type. Beaven, Hoch and Holiday (1951), in one human foetus, at 20 weeks' gestation, found that 6% of the haemoglobin was of the adult type and Schulman (1953) that there was at least 10% adult haemoglobin in the blood of premature infants. Many investigators have, however, studied the proportion of adult haemoglobin in the cord blood of the foetus at birth, at or near full term, and their results are shown in Table 1, from which it can be seen that

TABLE 1
PERCENTAGE OF ADULT-TYPE HAEMOGLOBIN
IN HUMAN CORD BLOOD AT BIRTH

Reference	Adult Haemoglobin (%)
Haurowitz (1930)	20
Brinkman <i>et al.</i> (1934)	20
Jonxis (1949)	2-25
Ponder and Levine (1949)	20-3
Beaven <i>et al.</i> (1951)	20
Zeisel (1951)	16-30
Chernoff and Singer (1952)	15-50
Schulman (1953)	29 (11-55)

the adult fraction contributes, on average, about 20% of the total haemoglobin, but that wide variations exist.

This investigation was planned to study the proportion of adult haemoglobin in the blood of the human foetus throughout gestation and was performed in association with our studies of oxygen levels, haemoglobin levels and red cell characteristics in the blood of the human foetus (Walker and Turnbull, 1953; Turnbull and Walker, 1954). Normal and clinically abnormal cases were studied, and the methods of selection of cases and of clinical classification were as described in previous papers.

Technique

At delivery 1 ml. of blood was removed from the umbilical vein into a syringe, the barrel of which had previously been wetted with a heparin solution. The proportion of adult haemoglobin was estimated (E.P.N.T.) by the technique of alkali denaturation originally described by Brinkman and Jonxis (1936) modified by Jonxis (1952).

Results

Fig. 1 shows the percentage of adult haemoglobin found in the blood of the human foetus throughout gestation in a series of 131 cases. Of these, 98 were normal and of the others, 18 were of pre-eclampsia, 17 of foetal distress, and in three abortion had threatened earlier in the pregnancy. It is seen from a study of the scattergram that no difference exists between the findings in the normal and abnormal cases.

In the four samples examined from foetuses at the 11th and 12th weeks' gestation the haemoglobin was entirely of the alkali-resistant foetal type. The adult type was first noted in the two cases at the 13th week when it constituted 1 and 2% of the total haemoglobin. The proportion increased gradually during the early weeks of pregnancy until at the 22nd to 24th weeks the level had risen to 10%. The six cases studied from then until the 35th week suggest that, during that period, the amount of adult haemoglobin remains fairly steady at or about that level. After the 35th week the readings begin to spread out, and, although in a few cases the level

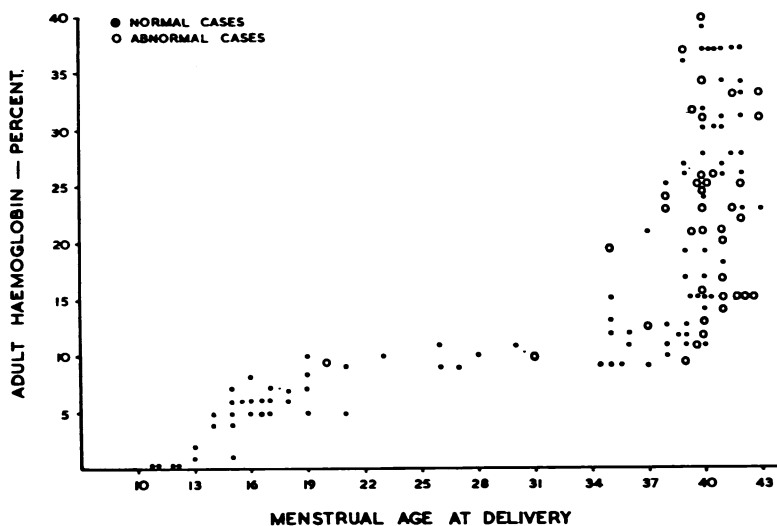
remains at or about 10% until the 41st week, the great majority of readings are much higher. At 40 weeks the proportion of adult haemoglobin ranges from 11 to 39% with a mean value of 22.2%. When pregnancy proceeds beyond the 40th week the proportion of adult haemoglobin in the foetal blood appears to increase and the mean value at 41 and 42 weeks was 30.7%.

Relation between Adult and Foetal Haemoglobin and Anoxia

The most significant finding in our study, and in those of other investigators quoted in Table 1, is the marked variation in the percentage of adult haemoglobin found in the blood of individual foetuses at term. Most investigators, including ourselves, find a mean reading at or about 20%, but individual bloods may have 5 to 55% of their haemoglobin as the adult type. We have shown previously (Walker and Turnbull, 1953) that the haemoglobin level in the cord blood of the individual foetus at term may vary from 15 to 21 g. per 100 ml. depending on the degree of anoxia to which the foetus had been subjected in the days preceding birth. We considered that the wide scatter in readings in the percentage of adult haemoglobin in the cord blood at birth might be, in some way, related to the degree of anoxia and therefore to the total haemoglobin level (foetal+adult) in each unit volume of blood. This approach was, however, complicated by the probability that increasing foetal age was also responsible for an alteration in the proportions of the two haemoglobins.

The findings in 71 cases delivered between the 39th and 43rd weeks of gestation were first recalculated so that the results in each case were expressed in g. per 100 ml. of blood (for example, if there was 20% adult haemoglobin in a blood with a total haemoglobin of 20 g. per 100 ml. then the results were expressed as total haemoglobin 20 g., foetal 16 g., adult 4 g.). The findings were then submitted to statistical analysis to study the effects of foetal age and total haemoglobin independently on the amount of each type of haemoglobin in a unit volume of blood.

FIG. 1.—Percentage of adult-type haemoglobin in cord blood of the human foetus in normal and abnormal pregnancy.



The regression equations of adult (A) and foetal (F) haemoglobin on menstrual age (W) and total haemoglobin (Hb) were calculated. The equations were:

$$A = 0.545 W - 0.023 Hb - 17.60$$

$$\pm 0.166 \quad \pm 0.150$$

$$F = -0.545 W + 1.022 Hb + 17.59$$

$$\pm 0.165 \quad \pm 0.150$$

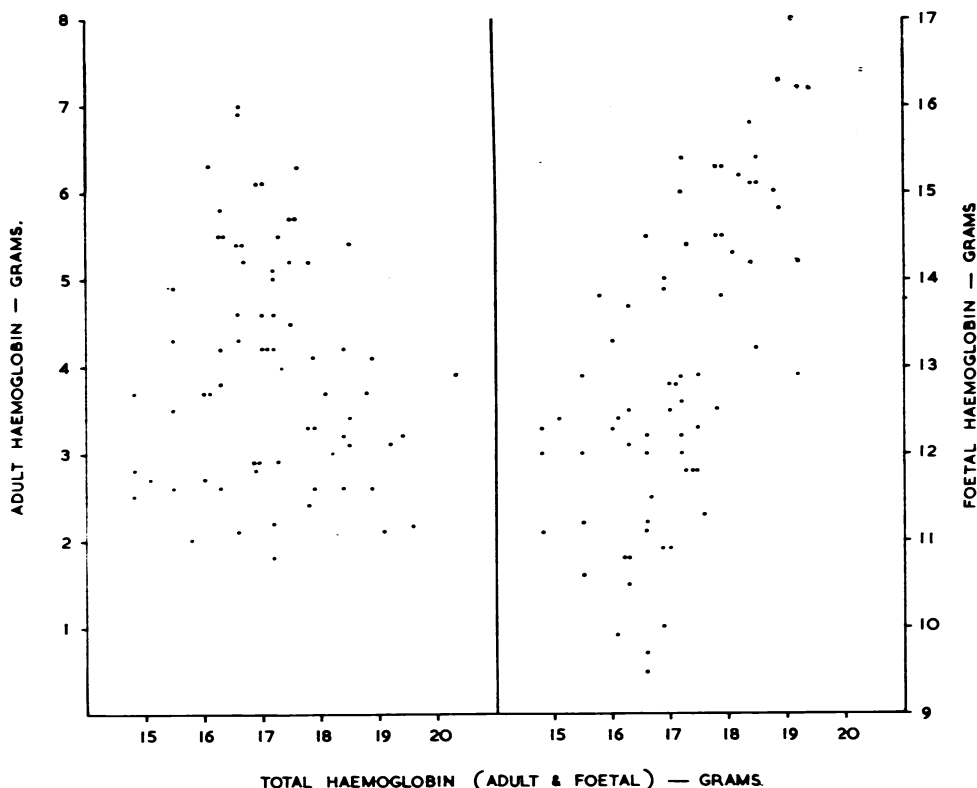
There is a highly significant ($P < 0.01$) effect of time on both adult and foetal haemoglobin, the former increasing and the latter decreasing as the foetus ages. When this effect is taken into account, differences in adult and total haemoglobin appear to be completely unrelated, but there is a highly significant ($P < 0.001$) regression of foetal haemoglobin on total haemoglobin (Fig. 2).

foetus, increasing as the foetus grows older. Although the amount of foetal haemoglobin normally decreases with increasing age of the foetus, the great increase in total haemoglobin (over 15 g.) which might be found at this stage in response to anoxia, arises entirely from an increase in the amount of foetal haemoglobin.

Discussion

The production of adult haemoglobin is part of the normal maturation of the blood. In the human foetus adult haemoglobin appears very early in gestation. The amount increases till at mid-pregnancy some 10% of the haemoglobin is of the adult type. It would appear from our findings and those of Schulman (1953) that up till the 35th or 36th weeks there is no great increase in production

FIG. 2.—The correlation between foetal haemoglobin, adult haemoglobin and total haemoglobin when the foetal and adult haemoglobin levels are adjusted to eliminate the effect of gestational age.



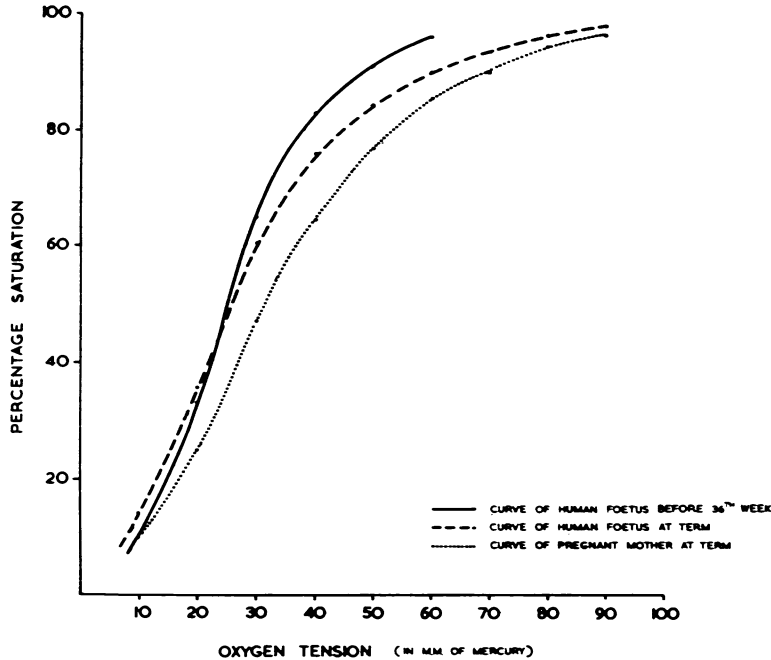
These two equations strongly support the suggestion that, over the period 39 to 43 weeks, the amount of adult haemoglobin in each unit volume of the cord blood depends only on the age of the

apart from the amount necessary to keep pace with increasing blood volume. After this time, however, adult haemoglobin increases greatly in amount and foetal haemoglobin production begins to decrease.

If, in the later weeks, the influence of anoxia is superimposed on the normal growth and maturation stimuli, extra haemoglobin is demanded and it would appear that this extra demand is met by the

by the production of foetal haemoglobin the gradient of pressure would be more easily increased because of a shift to the left in the dissociation curve of the foetal blood.

FIG. 3.—Dissociation curves of human foetal and maternal blood—from Sachs and Likhnikzaya (1938) and Leibson *et al.* (1935).



production of foetal haemoglobin. It would seem rational to suggest that such emergency supplies could best be obtained by maintaining production from centres producing foetal haemoglobin which normally would have been closing down, since adult haemoglobin centres are probably, at this time, already working at maximum capacity. This postulate assumes the existence of independent centres of production, perhaps side by side in each haemopoietic tissue or perhaps independent chemical processes existing side by side in the same centres.

Even though the method by which the foetus can rapidly obtain extra haemoglobin is not so simple as this, the production of extra foetal rather than adult haemoglobin by the anoxic foetus is physiologically advantageous. Under conditions of anoxia the haemoglobin level of the foetal blood rises. The main physiological result of this rise is to increase the gradient of pressure from the maternal to the foetal blood so that the oxygen content in the foetal blood may be maintained as near normal levels as possible despite a fall in the percentage saturation. If the increase in haemoglobin is achieved mainly

The Dissociation Curve.
In Fig. 3 are shown the average oxygen dissociation curves of the blood of the human foetus up to the 36th week of pregnancy (Sachs and Likhnikzaya, 1938), of the blood of the human foetus at term, and of the blood of pregnant women at term (Leibson, Likhnikzky and Sax, 1936). Those curves have been selected for discussion in preference to those of Eastman, Geiling and De Lawder (1933) or Darling, Smith and Asmussen (1941), as all the curves shown were obtained by the same workers, under similar conditions, and probably represent the relationships more clearly.

We have shown that up till the 36th week only some 10% of the haemoglobin is adult in type,

and the dissociation curve shown in Fig. 3 is well to the left of the curve of the blood at term (at least between 20 and 60 mm. oxygen pressure). At term, on average, 20% of the haemoglobin is adult, and the curve has shifted to the right so that, at 50% saturation, there is only half the gap between foetal and maternal curves that was between the curves before 36 weeks. It is interesting to note that Leibson *et al.* (1935) found that the curves in the blood of individual foetuses at term were not the same, and they actually suggested that 'the wide variation in the foetal dissociation curves lead us, however, to suppose that the foetal form of haemoglobin in the blood of the foetus before parturition has in different cases different qualitative and maybe quantitative significance'. The variation in the individual curves found by them could be explained by our finding of a great variation in amounts and proportions of foetal and adult haemoglobins in the blood of individual foetuses.

It would be interesting to study the dissociation curve of human foetal blood before the 20th week when little or no adult haemoglobin is present. It

appears, however, that when the proportion of adult haemoglobin rises after the 36th week the dissociation curve moves to the right and changes its shape, becoming more and more like the curve of normal adult blood and closer to the curve of the mother's blood. In the sheep, Barron (1951) found that the dissociation curve of the blood of the foetus begins to shift to the right and change its shape only after the 120th day, and Barron (1954) considers that the change in shape and position of the curve is due to the appearance at about 115 to 120 days and increased production of the adult type haemoglobin as shown by Karvonen (1949).

We have shown that under conditions of anoxia there is an increased production of foetal haemoglobin. Provided that such foetal haemoglobin is contained within foetal corpuscles, the effect would therefore be to cause the dissociation curve to shift to the left and alter its shape towards the early foetal position. The gradient of pressure from mother to foetus would therefore be improved at saturation over 40% (foetal) and oxygen pressures over 20 mm. mercury. Moreover, if the curve of Sachs and Likhnikzaya is correct in detail, transfer from the foetal blood to its own tissues would improve at saturation below 30%, both characteristics of the new curve being to the advantage of the foetus under deficient conditions of oxygen supply from the mother.

Effect on the Individual Foetus and Infant. *In utero* up till the 36th week the foetal blood can pick up oxygen easily, and at low capillary saturations transfer oxygen easily to its tissues. If, however, the infant is born much before the 36th week, nearly 90% of its haemoglobin is of the foetal type. Adult haemoglobin production lags behind for some weeks and foetal haemoglobin and cell production gradually cease, although a little more slowly than in the mature foetus (Jonxis, 1949; Schulman, 1953). The foetus is therefore certain to show a marked degree of anaemia in the first few weeks of life (Schulman, 1953), an anaemia which is very difficult to influence by iron therapy, as the ability to produce adult type haemoglobin is not yet adequately developed. In addition the dissociation curve of its blood is well to the left of that of the normal-term foetus and adult. Its blood can become saturated with oxygen easily, but there must be a very small gradient of transfer to its own tissues at the higher capillary saturations demanded by functioning tissues in an extra-uterine existence.

In the infant born after the 41st week of gestation two influences are very clearly at work. Adult haemoglobin is being formed in ever increasing

amounts, but in view of the anoxia suffered by most of those infants there has been a marked rise in the haemoglobin level due mainly to an increase in foetal haemoglobin production. The summation of these opposite influences produces a blood with some 30% adult haemoglobin. The dissociation curve of the blood in the post-mature foetus and infant will therefore be much further to the right than that in the blood of the foetus at 40 weeks with the same haemoglobin level, as the post-mature foetus has much more adult haemoglobin. The blood of the foetus in the 42nd and 43rd week will therefore pick up oxygen much less easily from the maternal blood, but such a foetus, if born alive, will adapt much more easily to extra-uterine life and be more able to supply oxygen to its own tissues.

Relation of Haemoglobin and Red Cells. We have suggested that the dissociation curve of the blood of the foetus will vary in position and shape with the proportion of adult to foetal haemoglobin. We have suggested that the influx of extra foetal haemoglobin will be to the advantage of the anoxic foetus near term because of a consequent shift of the dissociation curve towards the early foetal position. The characteristic affinity for oxygen of foetal and adult blood depends entirely on the fact that the haemoglobins are contained within corpuscles (McCarthy, 1943), as there is no difference between the affinities of the haemoglobins themselves (Allen *et al.*, 1953). It seems clear, therefore, as suggested previously by Jonxis (1949) that foetal and adult haemoglobins are separately contained in foetal and adult corpuscles. It is interesting to note that, in Cooley's anaemia, in which the ability to produce adult haemoglobin is genetically absent, the cells are typically foetal in morphology. There is some indirect evidence from our findings to support the theory of distinct cell types. In a previous paper (Turnbull and Walker, 1954) we have shown that cells of the typical adult size (less than 7.5μ) present in small numbers in the blood by the 10th week rise in number and proportion throughout pregnancy very like the changes in adult haemoglobin which we first detected at the 13th week. Gilmour (1941) has shown that definitive adult haemopoiesis is well established by this time. We have also shown previously that, in the latter weeks, foetal bloods with high haemoglobin levels have red cells of greater mean volume than bloods with normal haemoglobin levels, suggesting that the increase in haemoglobin promoted by anoxia, which we have shown to be achieved by the production mainly of foetal haemoglobin, is achieved by the production of extra red cells of greater volume, i.e., foetal cells.

We have found that in the blood of the foetus in the late weeks, some 35% of the cells are at least $9\ \mu$ in diameter, and we suggest that those cells along with some small cells are specific foetal cells containing foetal haemoglobin. The normal adult mean cell volume and mean cell diameter are reached only after some months of life, by which time cells over $9\ \mu$ have disappeared from the blood and true foetal haemoglobin is unlikely to be found in the blood of the normal infant (van Creveld, 1932; Jonxis, 1949).

Summary

The proportion of adult haemoglobin has been measured in the blood of the human foetus from the 10th to the 43rd week of pregnancy. Adult haemoglobin was first detected at the 13th week and rose until, at the 22nd to 24th weeks, 10% of the haemoglobin was adult. After the 35th week the proportion of adult haemoglobin rose steadily and at 42 weeks a mean value of 30.7% was found.

The rise in the proportion of adult haemoglobin is primarily a function of the growth and maturation of the foetal tissues, and in the well oxygenated foetus in the late weeks production of foetal haemoglobin steadily lessens.

The extra haemoglobin produced in the late weeks under the stimulus of anoxia is mainly or entirely of the foetal type.

Provided the two haemoglobins are contained separately in foetal and adult corpuscles, differences in the proportion of adult and foetal haemoglobins will greatly influence the position and shape of the oxygen dissociation curve of the blood. A shift to the left and a change in shape are greatly to the advantage of the anoxic foetus.

The effect clinically on the premature and post-mature infant is briefly indicated.

Further indirect evidence is submitted to support

the theory that the two haemoglobins are contained in separate foetal and adult corpuscles.

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