Blood formation in infancy

It is hard to believe that 27 years have passed since the first of Douglas Gairdner's papers on this subject was published in the *Archives*.¹ Although he was later to write about other aspects of neonatal haematology—such as the postnatal plasma shift^{2, 3} and the control of acidosis during exchange transfusion⁴—it is perhaps his 4 papers about red cell formation^{1, 5, 6, 7} which have best stood the test of time and are still 'required references' (on both sides of the Atlantic) in any article about the physiological anaemia of the newborn or the anaemia of prematurity.

In the first paper¹ the authors described the appearance of the bone marrow in normal infants during the first 3 months of life. Each baby was selected from those whose mother, having already had a normal baby, had volunteered to take part in the project. The examination of the marrow showed a decline in erythroid activity during the first one to 2 months which paralleled the fall in haemoglobin and was accompanied by a pronounced increase in marrow lymphocytes, a finding which could be misinterpreted by the unwary observer as true red cell hypoplasia. We still do not know if these 'lymphocytes' so prominent in the marrow at the nadir of erythroid activity are all truly lymphoid cells; some of them perhaps, residing in the null (non-T, non-B) fraction are stem cells or early erythroid precursors awaiting a surge of erythropoietin to nudge them into differentiation and multiplication.

In the next two papers^{5, 6} results of measurement of haemoglobin, red cell count, PCV, and reticulocytes during the first 3 months on the same group of normal term babies and in a large number of cord blood samples were reported. The relative macrocytosis of neonatal red cells, the low reticulocyte count, and the progressive decline in haemoglobin between 2 and 9 weeks were noted. The authors were not the first to make these measurements, but they related them to the changing bone marrow appearances to produce a classical description of the pattern of normal neonatal erythropoiesis. The advent of the electronic counter has made more accurate red cell counts and direct measurement of the MCV possible and the use of these techniques confirms the pattern of red cell values during the first months after birth.^{8, 9} Gairdner and his colleagues attributed the changing red cell picture after birth to decreased red cell production and to the effect of

body growth rather than to accelerated red cell destruction. They concluded that in the fetus and the infant, erythropoiesis was governed by the arterial oxyhaemoglobin level and that erythropoietic activity was regulated to ensure a level in the fetus and newborn of about 11 g of oxyhaemoglobin per 100 ml blood.

How do these conclusions look a quarter of a century later? It has been shown, using ⁵¹Cr-labelled autologous red cells that the fall in haemoglobin is due to an actual decrease in red cell mass rather than to expansion of plasma volume.¹⁰ The red cell of the term baby, examined in the isotope era, has a life span of about two-thirds of the adult cell,¹¹ that this is due to an intrinsic defect has been shown by infusion of DF^{32P}-labelled neonatal cells into normal adults.¹² There are now substantial descriptions about the metabolic characteristics of the fetal erythrocyte which include greater glucose consumption, reduced phosphofructokinase, and increased levels of other glycolytic enzymes and greater susceptibility to oxidant injury.¹³ However no relationship has been established between these metabolic abnormalities, which are not simply those of a young red cell population, and the cells' relatively short survival. In the last months of fetal life the estimated rate of red cell production is 3 to 5 times that of adult life¹⁴ and it has been suggested that this intense erythroid activity results in formation of short-lived macrocytes, so-called stress reticulocytes.¹⁵

Despite this evidence of decreased red cell survival, the major factor in the postnatal decline of haemoglobin is, as Gairdner surmised, decreased red cell production. The decline in marrow erythroid activity is reflected in decreased ⁵⁹Fe clearance and incorporation into red cells.¹⁶ It is now evident that red cell production is mediated through the effect of tissue oxygen tension on the output of the hormone erythropoietin.¹⁷ The site of erythropoietin production remains controversial. Although in postnatal life it is probably the kidney, fetal erythropoietin, the production of which is independent of the mother, may be made in the liver. The main effect of erythropoietin is to stimulate committed erythroid precursor cells to differentiate to proerythroblasts.¹⁸ Erythropoietin can be detected in the plasma of the fetus and in cord blood from the newborn infant, but levels decline rapidly

*DF^{32P} = di-isopropyl phosphofluoridate.

after birth until between 8 and 12 weeks when its reappearance heralds the resumption of erythroid activity in the bone marrow.^{19, 20} Anaemic and hypoxic infants continue to produce erythropoietin during the first weeks after birth and, as Gairdner observed, show no decline in marrow activity.

The postnatal decline in erythropoietin production and the subsequent fall in haemoglobin is therefore a physiological response to the rise in arterial oxygen saturation at birth and the resulting increase in oxygen delivery to the tissues. More slowly the left shifted haemoglobin-oxygen dissociation curve which, in the fetus, favours transfer of oxygen from the maternal to the fetal circulation, gradually shifts to the right, favouring increased oxygen delivery to the tissues without impairment of oxygen loading in the lungs. This shift of the curve is mediated by the presence of increasing amounts of adult haemoglobin with increased binding of the phosphate ester 2,3 diphosphoglycerate (23DPG). It has been calculated that at a mixed venous O₂ tension of 40 mmHg the 3-month-old infant delivers more oxygen to his tissues than a neonate despite a fall in Hb from 17.0 to 10.5 g/dl.²¹

The replacement of fetal by adult haemoglobin is a function of fetal maturity and is uninfluenced by the timing of birth. Haemoglobin A is synthesised in small amounts (5–10%) from 6 to 8 weeks' gestation, until 32–36 weeks when there is a sharp increase in the synthesis of Hb A and a decline in that of Hb F; the switch is not confined to one cell line making Hb A alone and is not restricted to either marrow or hepatic erythropoiesis.²² The mechanism of the 'switch' remains unknown, tantalisingly, since ability to reverse the process might provide an effective treatment of disorders of β -thalassaemia and sickle cell anaemia by endowing permanent possession of 100% haemoglobin F. Homozygous hereditary persistence of fetal haemoglobin and possession of haemoglobin variants with high oxygen affinity both appear compatible with normal growth and health despite possession of a permanently left-shifted haemoglobin-oxygen dissociation curve.²³

What of the earlier and more profound drop in haemoglobin which occurs in the preterm infant? Gairdner and his colleagues distinguished between this *early* anaemia of prematurity occurring between the 2nd and 4th months after birth and the *late* anaemia occurring after 5 to 6 months; the latter being clearly due to iron deficiency. They next addressed the problem of the early anaemia.⁷ Their attention, like that of many others, was partly diverted by haematinic red herrings. Cobalt, now known to stimulate production of erythropoietin, was given to 5 premature babies and caused reticulo-

cytosis and a rise in haemoglobin but was unacceptably toxic resulting in transient exophthalmos and goitre.

Gairdner also investigated the use of oral iron and found that giving it from the 3rd week mitigated but did not prevent the early anaemia of prematurity. It has since been shown that this anaemia is not associated with a low serum iron or raised TIBC and is not influenced by early administration of oral iron.²⁴ Iron stores in the preterm infant are influenced by birthweight and blood loss (particularly iatrogenic), and by feeding practices; iron is readily absorbed from breast milk and breast-fed infants attain greater iron stores than those fed artificially.^{25, 26} Nevertheless the preterm baby fed on breast milk, like the artificially-fed premature baby, is at risk of developing a late anaemia due to iron deficiency unless he receives additional iron. A recent prospective study of low birthweight infants used sequential measurements of Hb, iron, and TIBC and also serum ferritin, the most sensitive index of tissue iron stores. One half of the babies received supplementary iron. The same nadir of haemoglobin was reached at 2 months in both groups; by 3 months iron stores were significantly lower in the control group and by 6 months most babies in the control group, whether breast or bottle fed, were iron deficient and anaemic.²⁷

The problem of iron supplementation was compounded for a while by the vitamin E story. 12 years ago it was reported that a group of low birthweight infants developed haemolytic anaemia, thrombocytosis, and oedema associated with decreased plasma levels of vitamin E and increased sensitivity of the red cells to peroxide haemolysis.²⁸ A number of similar reports followed and it seemed possible that vitamin E deficiency might play a role in the anaemia of prematurity. However it is now apparent that this haemolytic anaemia is a result of highly unphysiological dietary manipulation. The lipid content of the red cell membrane reflects the lipid content of the diet and in low birthweight preterm babies fed certain proprietary milks rich in polyunsaturated fatty acids the membrane becomes a substrate at risk of peroxidation. Plasma vitamin E (antioxidant) levels are low in such babies and the addition of iron generates free radicals which initiate the process of peroxide haemolysis of the red cell membrane.^{29, 30, 31} Thus the haemolytic anaemia is a multifactorial disorder of artificially-fed low birthweight premature infants receiving early iron supplements: it is therefore preventable.

Serum and red-cell folate levels are relatively high in the newborn but may fall in the preterm infant within a few weeks to levels which are low by adult standards. Despite this, megaloblastic anaemia is

extremely rare and there is no evidence that folate supplementation influences the early anaemia of prematurity. It has been suggested that folate supplements should be given to very immature babies or to those who are at risk of dietary deficiency.³² Breast feeding should obviate this risk, since plasma and red-cell folate levels in breast-fed infants are higher than in adult controls.³³ It seems therefore, that breast milk is best haematinically as in every other respect.

The early anaemia of prematurity now known to be uninfluenced by haematinics, often exacerbated by venepuncture, is, like that of the mature baby, associated with reticulocytopenia and decreased marrow activity.^{7, 34} Erythropoietin, measured by bioassay, was detected in the urine of premature infants at birth but was then undetected until the haemoglobin declined to a nadir at 5 to 7 weeks. Hypoxic premature infants continued to produce erythropoietin.³⁵ It is claimed that radioimmunoassay is a more sensitive technique for measuring erythropoietin than bioassay, but the reliability of immunoassay is at present controversial. Erythropoietin was detected using radioimmunoassay in low concentration in the plasma of premature infants. There was a significant inverse correlation between plasma erythropoietin and haemoglobin and an even more significant inverse correlation between erythropoietin and oxygen-unloading capacity of the blood. This is hardly surprising since measurement of oxygen-unloading capacity takes account not only of the haemoglobin but also of the position of the haemoglobin-oxygen dissociation curve both of which affect oxygen delivery to the tissues. In infants with right shifted curves (that is, those who had been transfused and had relatively large amounts of adult haemoglobin) the haemoglobin fell lower before an erythropoietin response was observed.³⁶ These results suggest that premature infants respond appropriately to a decrease in oxygen-unloading capacity by increased erythropoietin excretion.

The reason for the earlier fall in haemoglobin in the premature baby is not entirely clear. One possible factor may be decreased red cell survival; the scanty data available suggest that red cell survival in the preterm baby is shorter than in the mature infant.¹¹

Stockman and Oski have recently reviewed the present state of knowledge of the anaemia of the newborn, much of it derived from their own work. They suggest that low oxygen consumption in the preterm baby allows a more profound drop in haemoglobin before reactivation of erythropoiesis, and consider that the 'anaemia' of prematurity is compatible with adequate tissue oxygenation.³⁷ However, most recent work on oxygen consumption

indicates that O₂ consumption is higher in the preterm baby^{38, 39} than in more mature infants.

The assumption that anaemia in the preterm newborn is physiological has been challenged in a recent paper in the *Archives* in which are reported results of measurement of haemoglobin, reticulocytes, P50 (partial pressure of oxygen at which Hb is 50% saturated), and available-oxygen. The figure for 'available-oxygen' is derived from the haemoglobin concentration and P50, and is calculated to be the amount of oxygen capable of being released per dl of blood. These values were correlated with the clinical features at the nadir of the anaemia. In babies of 33 weeks' gestation the clinical features of anaemia appeared to correlate better with available oxygen than with haemoglobin or P50 values; surprisingly this correlation did not hold for more mature babies, even those of 33-34 weeks' gestation. In some babies with relatively high haemoglobin but low available oxygen who were judged clinically anaemic, symptoms of anaemia improved after transfusion.⁴⁰

The clinical features of anaemia are as nonspecific in the infant as in the adult, but it is difficult to define a more objective way of assessing them. Simple comparisons of 'available-oxygen' in the preterm baby and the adult do not allow for the fact that cardiac output is much higher in the newborn infant than in the adult.

For the moment, rather than embarking on a policy of liberal transfusion with all its attendant dangers, we should continue to observe the generally accepted criteria⁴¹ while trying to determine more precisely which babies may benefit from transfusion.

It is good that the *Archives*, under Douglas Gairdner's editorship, has continued to provide a forum for debate about blood formation in infancy. To argue whether anaemia is 'physiological' or not is surely a question of semantics. Recent research has at least reminded us of the importance of concentrating not only on oxygen uptake and arterial oxygenation, but also on oxygen unloading at the tissues.

I am grateful to Professor Alastair Bellingham and Dr Edmund Hey for their helpful comments.

References

- ¹Gairdner, D., Marks, J., and Roscoe, J. D. (1952). Blood formation in infancy. I. The normal bone marrow. *Archives of Disease in Childhood*, **27**, 128-133.
- ²Gairdner, D., Marks, J., Roscoe, J. D., and Brettell, R. O. (1958). The fluid shift from the vascular compartment immediately after birth. *Archives of Disease in Childhood*, **33**, 489-498.
- ³Clark, A. C., and Gairdner, D. (1960). Postnatal plasma shift in premature infants. *Archives of Disease in Childhood*, **35**, 352-354.

- ⁴Gandy, G., Partridge, J. W., and Gairdner, D. (1968). Control of acidosis during exchange transfusion with citrated blood. *Archives of Disease in Childhood*, **43**, 147–150.
- ⁵Gairdner, D., Marks, J., and Roscoe, J. D. (1952). Blood formation in infancy. II. Normal erythropoiesis. *Archives of Disease in Childhood*, **27**, 214–221.
- ⁶Marks, J., Gairdner, D., and Roscoe, J. D. (1955). Blood formation in infancy. III. Cord blood. *Archives of Disease in Childhood*, **30**, 117–120.
- ⁷Gairdner, D., Marks, J., and Roscoe, J. D. (1955). Blood formation in infancy. IV. The early anaemia of prematurity. *Archives of Disease in Childhood*, **30**, 203–211.
- ⁸Matoth, Y., Zaizov, R., and Varsano, I. (1971). Postnatal changes in some red cell parameters. *Acta paediatrica Scandinavica*, **60**, 317–323.
- ⁹Saarinén, U. M., and Siimes, M. A. (1978). Developmental changes in red blood cell counts and indices of infants after exclusion of iron deficiency by laboratory criteria and continuous iron supplementation. *Journal of Pediatrics*, **92**, 412–416.
- ¹⁰Bratteby, L. E. (1968). Studies on erythrokinetics in infancy. XI. The change in circulating red cell volume during the first five months of life. *Acta paediatrica Scandinavica*, **57**, 215–224.
- ¹¹Pearson, H. A. (1967). Life span of the fetal red blood cell. *Journal of Pediatrics*, **70**, 166–171.
- ¹²Bratteby, L. E., Garby, L., and Wadman, B. (1968). Studies on erythrokinetics in infancy. XII. Survival in adult recipients of cord blood red cells labelled *in vitro* with di-isopropyl fluorophosphate DF32P. *Acta paediatrica Scandinavica*, **57**, 305–310.
- ¹³Oski, F. A., and Komazawa, M. (1975). Metabolism of the erythrocytes of the newborn infant. *Seminars in Hematology*, **12**, 209–221.
- ¹⁴Bratteby, L. E., Garby, L., Groth, T., Schneider, W., and Wadman, B. (1968). Studies on erythrokinetics in infancy. XIII. The mean life span and the life span frequency function of red blood cells formed during foetal life. *Acta paediatrica Scandinavica*, **57**, 311–320.
- ¹⁵Zipursky, A. (1965). The erythrocytes of the newborn infant. *Seminars in Hematology*, **2**, 167–203.
- ¹⁶Garby, L., Sjölin, S., and Vuille, J. C. (1963). Studies on erythrokinetics in infancy. III. Disappearance from plasma and red-cell uptake of radio-active iron injected intravenously. *Acta paediatrica Scandinavica*, **52**, 537–553.
- ¹⁷Stohman, F., Jr (1962). Erythropoiesis. *New England Journal of Medicine*, **267**, 342–348, 392–399.
- ¹⁸Wickramasinghe, S. N. (1975). *Human Bone Marrow*, pp. 212–227. Blackwell: Oxford.
- ¹⁹Halvorsen, S., and Finne, P. H. (1968). Erythropoietin production in the human fetus and newborn. *Annals of the New York Academy of Sciences*, **149**, 576–577.
- ²⁰Mann, D. L., Sites, M. L., Donati, R. M., and Gallagher, N. I. (1965). Erythropoietic stimulating activity during the first ninety days of life. *Proceedings of the Society for Experimental Biology and Medicine*, **118**, 212–214.
- ²¹Delivoria-Papadopoulos, M., Roncovic, N. P., and Oski, F. A. (1971). Postnatal changes in oxygen transport of term, premature, and sick infants: the role of red cell 2,3-diphosphoglycerate and adult hemoglobin. *Pediatric Research*, **5**, 235–245.
- ²²Wood, W. G. (1976). Haemoglobin synthesis during human fetal development. *British Medical Bulletin*, **32**, 282–287.
- ²³Stamatoyannopoulos, G., Bellingham, A. J., Lenfant, C., and Finch, C. A. (1971). Abnormal hemoglobins with high and low oxygen affinity. *Annual Review of Medicine*, **22**, 221–234.
- ²⁴Brozovic, B., Burland, W. L., Simpson, K., and Lord, J. (1974). Iron status of low birthweight infants and the response to oral iron. *Archives of Disease in Childhood*, **49**, 386–389.
- ²⁵McMillan, J. A., Landaw, S. A., and Oski, F. A. (1976). Iron sufficiency in breast fed infants and the availability of iron from human milk. *Pediatrics*, **58**, 686–691.
- ²⁶Saarinén, U. M., Siimes, M. A., and Dallman, P. R. (1977). Iron absorption in infants: high bioavailability of breast milk iron as indicated by the extrinsic method of iron absorption and by the concentration of serum ferritin. *Journal of Pediatrics*, **91**, 36–39.
- ²⁷Lundström, V., Siimes, M. A., and Dallman, P. R. (1977). At what age does iron supplementation become necessary in low birthweight infants? *Journal of Pediatrics*, **91**, 878–883.
- ²⁸Oski, F. A., and Barnes, L. A. (1967). Vitamin E deficiency: a previously unrecognized cause of hemolytic anemia in the premature infant. *Journal of Pediatrics*, **70**, 211–220.
- ²⁹Melhorn, D. K., and Gross, S. (1971). Vitamin E-dependent anemia in the premature infant. I. Effects of large doses of medicinal iron. *Journal of Pediatrics*, **79**, 569–580.
- ³⁰Melhorn, D. K., and Gross, S. (1971). Vitamin E-dependent anemia in the premature infant. II. The relation between gestation age and absorption of vitamin E. *Journal of Pediatrics*, **79**, 581–588.
- ³¹Williams, M. L., Short, R. J., O'Neal, P. L., and Oski, F. A. (1975). Role of dietary iron and fat on vitamin-E deficiency anemia of infancy. *New England Journal of Medicine*, **292**, 887–890.
- ³²Hoffbrand, A. V. (1970). Folate deficiency in premature infants. *Archives of Disease in Childhood*, **45**, 441–444.
- ³³Ek, J., and Magnus, E. M. (1979). Plasma and red blood cell folate in breast fed infants. *Acta paediatrica Scandinavica*, **68**, 239–243.
- ³⁴Seip, M., and Halvorsen, S. (1956). Erythrocyte production and iron stores in premature infants during the first months of life. *Acta paediatrica Scandinavica*, **45**, 600–617.
- ³⁵McIntosh, S. (1975). Erythropoietin excretion in the premature infant. *Journal of Pediatrics*, **86**, 202–206.
- ³⁶Stockman, J. A., III, Garcia, J. F., and Oski, F. A. (1977). The anemia of prematurity. Factors governing the erythropoietin response. *New England Journal of Medicine*, **296**, 647–650.
- ³⁷Stockman, J. A., III, and Oski, F. A. (1978). Physiological anaemia of infancy and the anaemia of prematurity. *Clinics in Haematology*, **7**, 3–18.
- ³⁸Hill, J. R., and Robinson, D. C. (1968). Oxygen consumption in normally grown, small for dates, and large for dates new-born infants. *Journal of Physiology*, **199**, 685–703.
- ³⁹Hey, E. N. (1969). The relation between environmental temperature and oxygen consumption in the new-born baby. *Journal of Physiology*, **200**, 589–603.
- ⁴⁰Wardrop, C. A., Holland, B. M., Veale, K. E. A., Jones, J. G., and Gray, O. P. (1978). Nonphysiological anaemia of prematurity. *Archives of Disease in Childhood*, **53**, 855–860.
- ⁴¹Davies, P. A., Robinson, R. J., Scopes, J. W., Tizard, J. P. M., and Wigglesworth, J. S., editors (1972). *Medical Care of Newborn Babies*. Clinics in Developmental Medicine No. 44/45, pp. 236–237. Heinemann Medical: London.

JUDITH M. CHESSELLS
 Department of Haematology,
 The Hospital for Sick Children,
 Great Ormond Street,
 London WC1N 3JH