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The full blood count and blood film in healthy term and preterm neonates

Introduction

Haemopoiesis is the process that ensures life-long production of haemopoietic cells. In newborn infants the process has many distinct features that differ from those in older children and adults. These differences reflect both the ontogeny of haemopoiesis during fetal development and the unique interaction between the fetus and mother, as well as the effects of birth itself. The sequential changes in the sites and regulation of haemopoiesis during development also help to explain the natural history of many neonatal haematological problems.

Brief outline of the ontogeny of haemopoiesis

Haemopoiesis in humans begins in the yolk sac between 2 and 3 weeks post-conception (Fig. 1.1).[1,2] This is known as primitive haemopoiesis. Studies in other species, particularly in mice, indicate that the predominant cell types produced in the yolk sac are erythroid cells and macrophages.[2,3] While megakaryocytes and lymphoid cells may also be yolk sac-derived, the current consensus of opinion is that true, long-lived haemopoietic stem cells (HSC) arise from a region of specialised endothelium ('haemogenic' endothelium), which is localised to the ventral wall of the dorsal aorta in a region known as the aorto-gonado-mesonephros (AGM).[4–6] In humans, haemopoiesis begins in the AGM at around 5 weeks post-conception and is known as definitive haemopoiesis.[6–8] Haemopoiesis in the aortic wall is only transient, presumably because this region lacks the necessary physical space and specialised microenvironment to support expansion and differentiation of the HSC and progenitor populations required to meet the needs of the growing fetus.

By 6 weeks post-conception, HSC and progenitor cells have migrated to the fetal liver,[8,9] which remains the main site of blood cell production throughout fetal life[10,11] and AGM haemopoiesis ceases. The first signs of haemopoiesis in the bone marrow are evident from around 11 weeks post-conception.[8,12] Although fetal bone marrow is able to give rise to cells of all lineages, it is becoming clear that the predominant cell types produced in the bone marrow are B lymphocytes and their progenitor cells together with granulocytes, monocytes and their progenitors. Although erythropoiesis and megakaryopoiesis take

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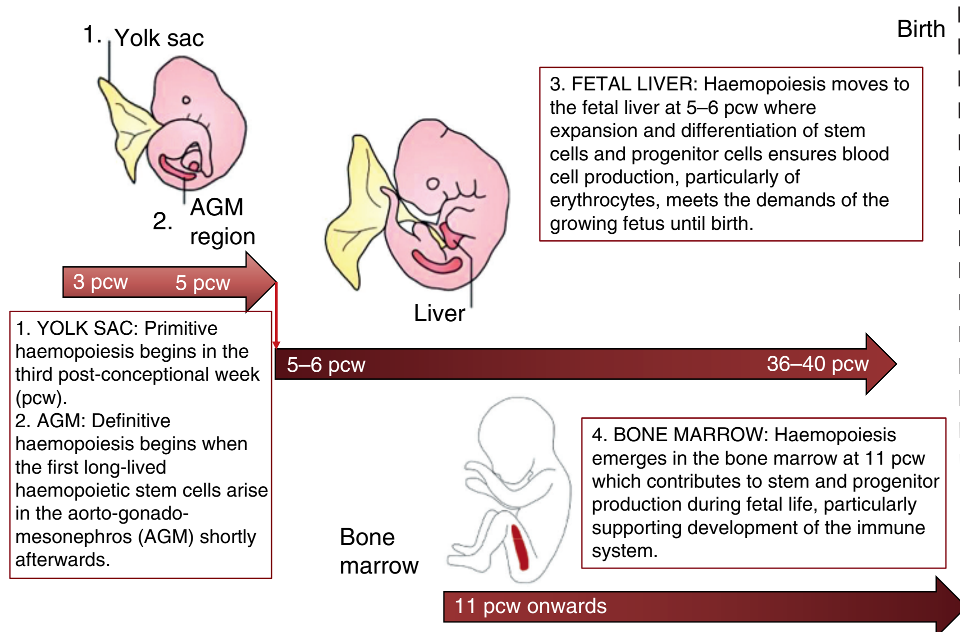

Fig. 1.1 Ontogeny of human haemopoiesis in embryonic and fetal life. AGM, aorto-gonado-mesonephros; pcw, post-conceptional week. Based on references 1 and 2.

place in the fetal bone marrow from the end of the first trimester, most red blood cell and megakaryocyte production takes place in the fetal liver until shortly before term.[8] Thus, for preterm infants, the liver is the main haemopoietic organ at and shortly after birth; this is likely to be a contributory factor in a number of disorders, including the haematological abnormalities seen in neonates with Down syndrome (see pages 154–160 and 206).

Properties of fetal haemopoietic stem and progenitor cells

Major advances in the immunological and molecular tools available to analyse haemopoietic stem and progenitor cells have allowed us to build up a much clearer picture of the process of haemopoiesis in fetal life and how this differs from adult life. Fetal HSC, like adult HSC, are the cells at the top of the haemopoietic hierarchy (Fig. 1.2). When HSC divide, they do so either through a process of 'self-renewal', where they generate more HSC (sometimes referred to 'symmetric cell division'), or through asymmetric division during which one of the two daughter cells differentiates into progenitor cells, which in turn generate the mature cells of all the haemopoietic lineages (Fig. 1.2).[9]

Fetal haemopoietic stem cells

Studies in mice, and more recently in humans, indicate that fetal HSC are markedly different from those in adult bone marrow.[9,13–16] Elucidating the nature of the differences between fetal and adult HSC, and the molecular mechanisms that underpin these differences, is likely to help our understanding of many of the haematological problems that

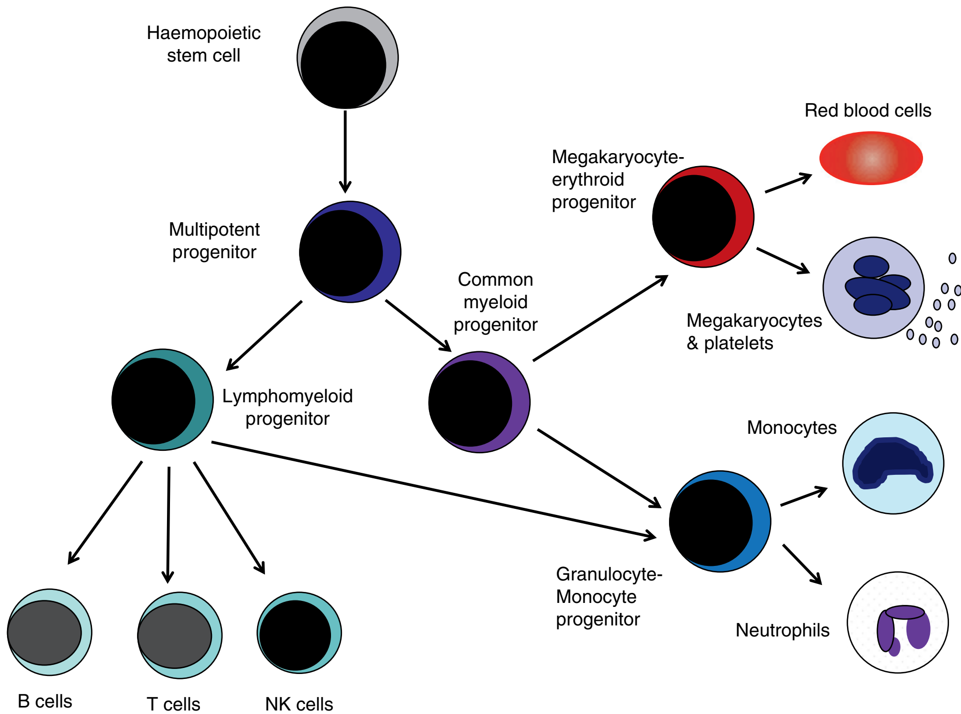

Fig. 1.2 A simplified scheme of the fetal haemopoietic stem and progenitor cell hierarchy showing the differentiation of multipotent and committed progenitor cells from haemopoietic stem cells. Details of the fetal-specific pathway of B lineage progenitor differentiation are shown in Fig. 1.3. Based on reference 9.

affect neonates and potentially open up new approaches to treatment. For example, the need for rapid expansion of haemopoietic cells to meet the needs of the growing fetus means that the numbers of fetal HSC have to increase more rapidly than at any other time of life. Furthermore, since HSC are responsible for life-long haemopoiesis, this process of HSC expansion needs to be precisely regulated to prevent either uncontrolled proliferation (and the risk of haematological malignancy) on the one hand or HSC 'exhaustion' (and the risk of bone marrow failure) on the other. These properties are thought to underlie the particular prevalence of certain haematological diseases in fetal and neonatal life, including Diamond–Blackfan anaemia and juvenile myelomonocytic leukaemia.[17]

There are differences both in the intrinsic properties of the HSC and in the regulatory signals produced by the haemopoietic microenvironment during fetal life.[16] One of the characteristic intrinsic differences in fetal HSC is the increased proportion of HSC that are actively cycling and undergoing a process of 'self-renewal' that results in expansion of the pool of long-lived HSC in fetal life.[18] This behaviour of fetal HSC contrasts dramatically with adult HSC which are largely quiescent cells that enter the cell cycle infrequently.[9,16,18] The amplification in fetal HSC numbers probably takes place mainly in fetal liver rather than in the bone marrow,[16,19] which may explain why so many haematological disorders in

neonates are accompanied by hepatomegaly. A second characteristic of fetal HSC is that they are primed to give rise to a higher proportion of erythroid and megakaryocytic progenitors compared with adult HSC, reflecting the requirement of the fetus for large numbers of red blood cells and the importance of adequate numbers of platelets to maintain vascular integrity.[9,17] Finally, fetal HSC exhibit different sensitivity to and dependence upon haemopoietic growth factors, such as insulin-like growth factors, compared with adult cells[20,21] and a different pattern of mature cell output.[9,16,18] Reflecting this, fetal HSC also have unique gene expression programmes,[13,15–17,22–26] which have recently been shown to be important in the leukaemic transformation events that lead to infant acute lymphoblastic leukaemia (ALL).[27]

Fetal haemopoietic progenitor cells

The different types of haemopoietic progenitor cell present in fetal life are shown in Figs 1.2 and 1.3. The overall scheme of differentiation of HSC is similar in fetal and adult life. However, recent studies have identified fetal-specific lymphoid progenitors, including early lymphoid progenitors (ELP) and PreProB progenitors that may be important not only to rapidly boost B cell production during the second trimester, but also to act as targets of leukaemic transformation in infant and childhood ALL.[9,27–29] ELP are found very early in fetal life (from around 6 weeks post-conception in the fetal liver and from around 11 weeks post-conception in bone marrow) but are very rare in adult haemopoietic tissues.[29] They are defined both by their immunophenotype ($CD34^{+}CD127^{+}CD19^{-}CD10^{-}$) and their ability to generate B, T and NK cells as well as a small number of myeloid cells.[8,29] PreProB progenitors are one of two types of committed B progenitor cell in fetal life; they lack expression of the CD10 molecule and, like ELP, are very rare in adult bone marrow. By contrast, the second type of B progenitor, the ProB progenitor, is $CD10^{+}$ and is the main, or sole, type of B progenitor found in adult bone marrow.[29] It is likely that ProB progenitors lie downstream of PreProB in B lymphoid differentiation and, consistent with this, they have been shown to have undergone complete V_H-D_H-J_H rearrangement of their immunoglobulin heavy chain (IgH) loci, in contrast to ELP and PreProB progenitors, which show only

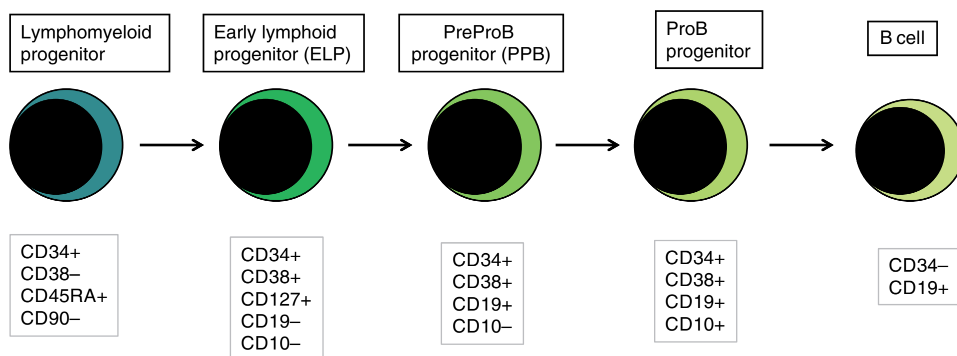

Fig. 1.3 Immunophenotypically defined progenitor populations along the B cell differentiation trajectory in the human fetus. The cell surface markers used to define these populations are shown below each cell type. Based on reference 9.

partial (D_H-J_H) IgH rearrangement.[8] The reasons for the existence of two types of B progenitor and a unique ELP cell in fetal life are unknown but it suggests that there are two pathways of fetal B cell production which may have different physiological roles.

Red blood cell production and development in the fetus and neonate

Normal erythropoiesis, the production of red blood cells, is crucial to early embryonic and fetal development. Most of our knowledge about the cells and genes involved in this process derives either from mouse models or from inherited anaemias, particularly in children. Almost all the characteristic features of red blood cells are different in the fetus and the newborn compared with their adult counterparts. These differences are even greater in preterm neonates and are directly relevant to our understanding of neonatal anaemias. The differences in erythropoiesis during fetal development are summarised in Table 1.1 and those that are important for our understanding of neonatal anaemias are discussed below.

Table 1.1 Features of fetal and neonatal red cells compared with adult red cells

Haemoglobin production	Embryonic haemoglobins (globin chains)
	Gower 1 ($\zeta_2\varepsilon_2$)
	Gower 2 ($\alpha_2\varepsilon_2$)
	Portland ($\zeta_2\gamma_2$)
	Fetal haemoglobin (globin chains)
	Fetal haemoglobin ($\alpha_2\gamma_2$)
	Adult haemoglobins (globin chains)
	Haemoglobin A ($\alpha_2\beta_2$) lower
	Haemoglobin A_2 ($\alpha_2\delta_2$) considerably lower
Red cell membrane	Gives resistance to osmotic lysis
	Altered expression of receptors (e.g. insulin)
	Increased lipid content and altered phospholipid profile
	More prone to oxidative damage
	Altered glucose transport
	Weak expression of A, B and I blood group antigens
	Increased variation in red cell shape (poikilocytosis)
	Red cell 'pocks' due to hyposplenism
Red cell metabolism	Glycolytic pathway
	Increased glucose consumption
	Altered enzyme levels, e.g. low 2,3-DPG and PFK
	Pentose phosphate pathway
	Increased susceptibility to oxidant-induced injury
	Lower level of glutathione peroxidase
	Reduced ability to generate NADPH

2,3-DPG, 2,3-diphosphoglycerate; NADPH, nicotinamide adenine dinucleotide phosphate; PFK, phosphofructokinase.

Erythropoietin production in the fetus and neonate

The principal cytokine responsible for regulating erythropoiesis in the fetus and newborn, as in adults, is erythropoietin (EPO).[30] Since EPO does not cross the placenta, EPO-mediated regulation of fetal erythropoiesis is predominantly under fetal control. The liver is the main site of EPO production in the fetus[31] and the only stimulus to production under physiological conditions is hypoxia with or without anaemia (reviewed in reference 32). Little or no EPO is produced under normoxic conditions, but hypoxia very rapidly triggers expression by up to 200-fold within 30 minutes, at least in hepatocyte cell lines.[33] This explains the high EPO levels in fetuses of mothers with diabetes mellitus or hypertension and in those with intrauterine growth restriction (IUGR) or cyanotic congenital heart disease;[34] EPO is also increased in fetal anaemia of any cause, including haemolytic disease of the fetus and newborn (HDFN). This, and the switch of EPO production from fetal liver to the neonatal kidney, may in part explain the physiological delay in triggering the production of new red blood cells, which is often not evident until the second month of life, even in healthy babies.

Haemoglobin synthesis and red blood cell production in the fetus and newborn

The rates of haemoglobin synthesis and red blood cell production fall dramatically immediately after birth and remain low for the first 2 weeks of life, probably in response to the sudden increase in tissue oxygenation at birth.[35] In healthy neonates the physiological rise in red cell production starts several weeks later, so that by 3 months of age a healthy infant, whatever the period of gestation at birth, should be able to produce up to 2 ml of packed red blood cells every day.[35] Studies in preterm neonates have estimated that over the first 2 months of life the maximal rate of red blood cell production may be closer to 1 ml/day. This is based on the observation that preterm babies receiving therapeutic EPO are unable to maintain their haemoglobin if more than 1 ml of blood per day is venesected for diagnostic purposes but can do so where sampling losses are less than this.[36]

The gestation-related changes in globin chain synthesis in the human embryo, fetus and neonate have been studied in detail and are summarised in Fig. 1.4.[37] The first haemoglobins, known as embryonic haemoglobins, are synthesised from approximately 2 or 3 weeks post-conception, predominantly in the blood islands of the yolk sac, by the erythroblasts and red blood cells generated there. There are three embryonic haemoglobins (see Table 1.1). ζ or α globin, encoded by adjacent genes in the α globin locus on chromosome 16, combine with ε or γ globin, encoded by genes in the β globin locus on chromosome 11, to produce haemoglobin Gower 1 ($\zeta_2\varepsilon_2$), haemoglobin Gower 2 ($\alpha_2\varepsilon_2$) and haemoglobin Portland ($\zeta_2\gamma_2$).

During normal human development, synthesis of embryonic haemoglobins is transient and largely restricted to yolk sac-derived erythroblasts which are larger than those generated once definitive haemopoiesis starts in the AGM and fetal liver (Figs 1.5 and 1.6) and express different transcription factor and epigenetic programmes. From 4 or 5 weeks post-conception, erythroblasts and red blood cells contain mainly haemoglobin F ($\alpha_2\gamma_2$), which remains the principal haemoglobin throughout fetal life. The factors that control the switch

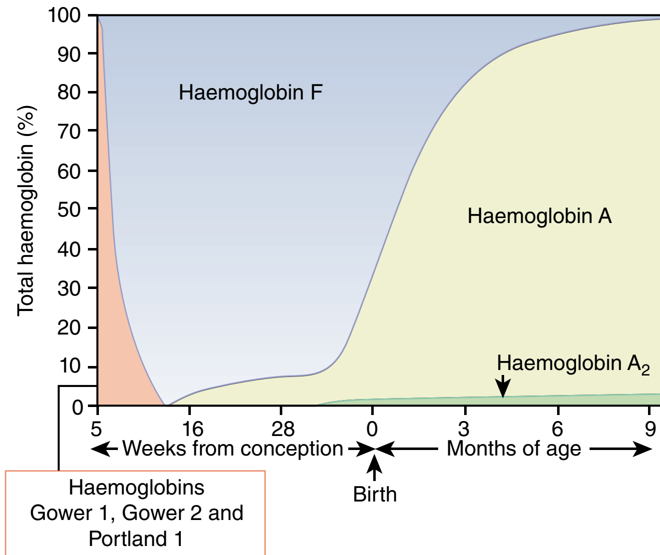

Fig. 1.4 Diagrammatic representation of the sites and rates of synthesis of different haemoglobins in the embryonic and fetal periods and during infancy. From Bain (2020)[37].

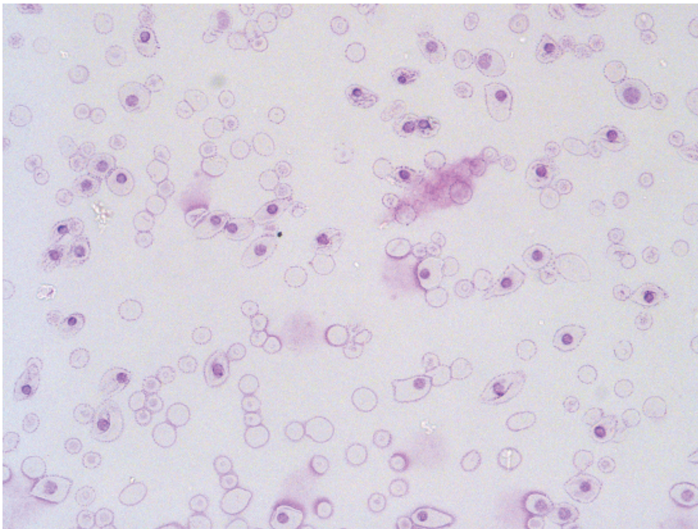

Fig. 1.5 First trimester (8 weeks) fetal blood film showing the large size of the erythroblasts (compare with Fig. 1.6) typical of those derived from the yolk sac. These erythroblasts contain mainly embryonic globins. Note the high proportion of red cells that are nucleated and the absence of white blood cells. May–Grünwald–Giemsa (MGG), ×40 objective.

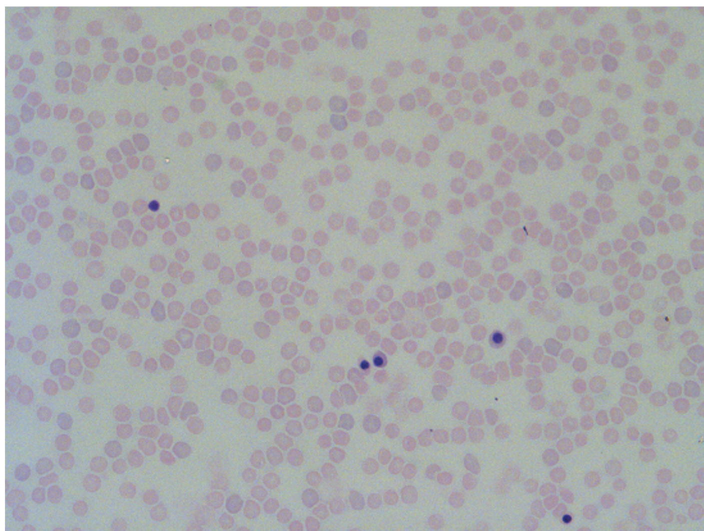

Fig. 1.6 Second trimester (14 weeks) fetal blood film showing typical erythroblasts derived from definitive haematopoiesis. These erythroblasts contain mainly fetal haemoglobin. Note the smaller size of the erythroblasts and the higher proportion of enucleated red cells compared with the first trimester (Fig. 1.5). MGG, ×40.

from primitive to definitive erythropoiesis are not yet clear due to the difficulties in studying this process at such an early stage of development. Understanding more about the mechanisms which normally silence expression of ζ globin would potentially open up new ways of treating α thalassaemia major,[38] an important cause of fetal and early neonatal death (see Chapter 2).

The production of adult haemoglobin (haemoglobin A; $\alpha_2\beta_2$) begins during the second trimester and remains at low levels until 30–32 weeks post-conception, when haemoglobin A production starts to increase concomitantly with a fall in haemoglobin F production. The net result is an average haemoglobin F in term babies of 70–80% and haemoglobin A of 25–30%.[39,40] After birth, haemoglobin F falls, to approximately 2% by the age of 12 months, with a corresponding increase in haemoglobin A. The molecular control of this change from haemoglobin F to haemoglobin A is termed globin switching. In recent years, there has been considerable research into the genes involved in globin switching (e.g. *BCL11A*) in order to identify strategies to delay or reverse this physiological switch after birth and so maintain haemoglobin F production for children affected by severe β globin disorders such as sickle cell disease or thalassaemia major.[41,42]

The timing of globin switching depends on post-conceptional age rather than postnatal age. In fact, in term babies there is little change in haemoglobin F in the first 15 days after birth, but in preterm babies who are not transfused, haemoglobin F may remain at the same level for the first 6 weeks of life before haemoglobin A production starts to increase. This delay in haemoglobin A production (i.e. the switch from γ globin production to β globin production) can make the diagnosis of β globin disorders in the neonatal period difficult, particularly in preterm infants. This is in contrast to α globin disorders,

which are almost invariably evident at birth since α globin chains are essential for the production of all but the very earliest embryonic haemoglobins (see Fig. 1.4 and Table 1.1).

Red blood cell lifespan and the red blood cell membrane in the fetus and neonate

Neonatal red blood cells, particularly in preterm babies, have a shorter lifespan than adult red blood cells. Red cell lifespan is inversely proportional to gestational age at birth. Studies over 50 years ago using isotopically labelled red blood cells estimated red blood cell lifespans for preterm infants at 35–50 days, compared with 60–70 days for term infants and 120 days for healthy adults.[35] More recent estimates, using mathematical modelling and transfusion of autologous cord blood cells, have also calculated the red cell lifespan in preterm neonates to be approximately 50 days.[43]

The reasons for a lower red cell lifespan in neonates, although not fully understood, are thought to include the many biochemical and functional differences in the membrane of neonatal versus adult red blood cells (see Table 1.1). Known differences between neonatal and adult red blood cells include increased resistance to osmotic lysis, increased mechanical fragility, increased total lipid content with an altered lipid profile, increased insulin-binding sites and reduced expression of blood group antigens such as A, B and I.[35] Together these differences translate into the characteristic morphological differences seen in neonatal blood films, particularly in preterm neonates (Figs 1.7–1.9), and are associated with accelerated red cell membrane loss[44] leading to reduced red cell lifespan. Indeed, the distinctive geometry of neonatal red blood cells and the membrane deformability of some of the irregularly shaped cells have been compared to the properties of red blood cells in the inherited red cell membrane disorders.[45]

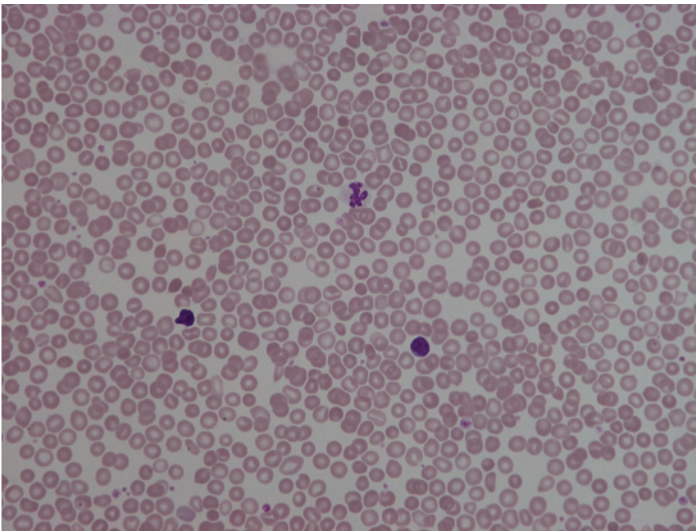

Fig. 1.7 Normal blood film at term. MGG, ×40.

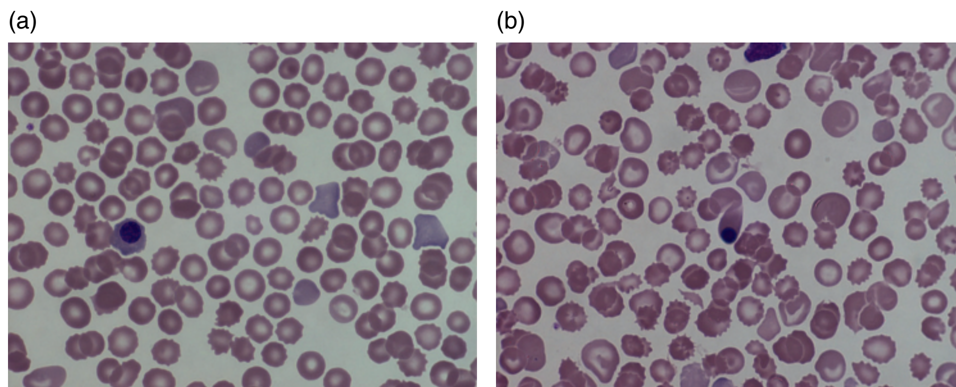

Fig. 1.8 Normal preterm red cells at different gestational ages: (a) baby born at 28 weeks' gestation showing echinocytes, polychromatic macrocytes and one nucleated red blood cell (NRBC); (b) baby born at 25 weeks' gestation showing numerous echinocytes, echinocytic fragments and one NRBC. Note that anisocytosis and poikilocytosis is greater at 25 weeks than at 28 weeks. MGG, ×100.

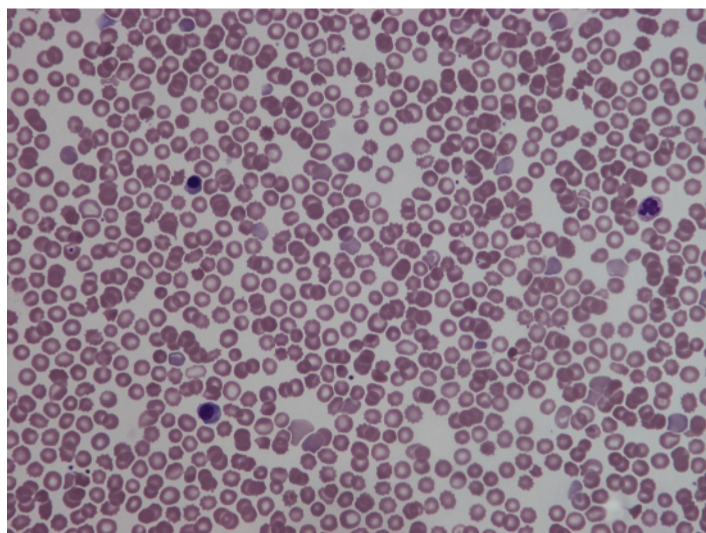

Fig. 1.9 Blood film of a normal preterm baby (born at 28 weeks' gestation) showing a degree of erythroblastosis. MGG, ×40.

Red blood cell metabolism in the fetus and neonate

There are major differences between the metabolism of fetal or neonatal red blood cells and that of adult red blood cells. These differences affect not only the functional properties of the red cells of healthy fetuses and neonates but also the clinical impact of inherited and acquired red cell disorders. Both the glycolytic pathway and the pentose phosphate pathway are affected (see Table 1.1). Overall, glycolysis and glucose consumption are lower in neonatal red blood cells than in adult red blood cells. This occurs despite the increased

activity of most glycolytic pathway enzymes, such as glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase and lactate dehydrogenase (LDH), and is thought to be the result of reduced phosphofructokinase activity. Neonatal red cells also have less ability to generate the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) via the pentose phosphate pathway and have lower levels of glutathione peroxidase than adult red blood cells. The net result is that neonatal red blood cells are more susceptible than adult cells to oxidant-induced injury.[46]

In addition, neonatal red blood cells have a lower level of methaemoglobin reductase (about 60% of that in adult red blood cells). Methaemoglobin levels are therefore slightly higher in neonates than in adults (mean 4.3 g/l in preterm neonates, 2.2 g/l in term neonates and 1.1 g/l in adults).[35] Neonates are also more likely to develop methaemoglobinaemia because they are susceptible to the toxic effects of chemicals, such as nitric oxide and local anaesthetics, that oxidise haemoglobin-derived iron more rapidly than the maximal possible rate of methaemoglobin reduction (see Chapter 2, Case 2.7).

Iron metabolism in the fetus and neonate

Although stores of iron are adequate at birth in term babies born to well-nourished mothers, this is not always the case in preterm neonates. This is because the majority of fetal total body iron is stored during the third trimester. Estimates have shown that total body iron increases from 35–40 mg at 24 weeks' gestation to 225 mg at term, with the result that preterm neonates, especially those with IUGR, are born with lower iron stores than term neonates.[47,48] These amounts of iron are equivalent to 6 months iron store for a term neonate[49] but only around 2 months for extremely preterm neonates unless they are given supplementary iron. In addition, preterm neonates have an increased requirement for iron both because of their rapid growth rate and because of frequent phlebotomy.[50,51] Therefore, preterm neonates generally develop iron deficiency after 2–4 months if the recommended daily intakes are not maintained.[52] Administration of iron supplements to preterm babies leads to a slightly higher haemoglobin concentration (Hb) and improved iron stores, thereby reducing the risk of subsequent iron deficiency anaemia.[53] The recommended iron intake of preterm infants with a birthweight of 1500–2000 g is 2 mg/kg/day from 2 to 4 weeks of life using iron-containing human milk fortifier or preterm formula milk and/or iron supplements until at least 6 months of age.[54] For very low birthweight neonates, a higher daily iron intake (2–3 mg/kg/day) is usually recommended, starting at 2 weeks of age.[54]

The regulation of iron status in the neonate, even in those who are born extremely preterm, has recently been shown to depend on the action of hepcidin, erythroferrone (ERFE), ferroportin and EPO, as in adults.[55,56] Thus, hepcidin falls when iron deficiency develops, facilitating increased iron absorption, while hepcidin is upregulated when iron stores are sufficient, triggering degradation of the iron exporter ferroportin, which results in inhibition of iron absorption and mobilisation.[57] Like serum ferritin, hepcidin levels increase with gestational age and in parallel with the increasing iron stores.[58] Hepcidin and prohepcidin levels in term and preterm infants vary in response to inflammation, infection and red blood cell transfusion.[59–61] ERFE, an erythroid hormone, acts as a direct suppressor of hepcidin expression in the liver in response to EPO. Little is known about the role of

ERFE in regulating iron metabolism in neonates but some preliminary data suggest that although the components of the EPO–ERFE–hepcidin–ferroportin axis are present in neonates,[55] ERFE-mediated suppression of hepcidin is impaired, at least in preterm neonates.[62]

Normal values for red blood cell parameters in the fetus and neonate

Haemoglobin concentration and red blood cell indices

Typical normal values for red cell variables in the fetus and neonate are shown in Tables 1.2 and 1.3.[63–68] At birth, the Hb in term infants is high (140–215 g/l), which compensates for the low oxygen concentration in the fetus. The range of normal values for Hb and haematocrit have been updated to reflect the changes in neonatal medicine, including the lower limit of gestation of neonates admitted for neonatal intensive care. Jopling *et al.* published the results of a retrospective study of archived laboratory measurements of Hb and haematocrit from approximately 25 000 neonates analysed on the same equipment in a single group of hospitals between 2002 and 2008.[69] They found an approximately linear increase in both Hb and haematocrit between 22 weeks' and 40 weeks' gestation calculated from

Table 1.2 Impact of gestational age at birth on the principal blood count parameters in healthy neonates*

Gestation at birth	Term (≥37 weeks)	30–36 weeks	26–29 weeks	<26 weeks
Erythropoiesis				
Hb (g/l)	140–215	130–215	115–200	115–185
Hct (l/l)	0.43–0.65	0.40–0.42	0.30–0.58	0.30–0.57
MCV (fl)	98–115	100–117	103–130	104–133
MCH (pg)	32.5–39	33.5–40.5	33.5–43	34.5–44.5
NRBC				
/100 WBC	≤5	≤25	≤25	≤25
$\times 10^9$/l	<1.0	1.0–2.0	2.0–3.0	2.0–3.0
Leucocytes ($\times 10^9$/l)				
Neutrophils				
0–72 hours	3.0–28.0	1.0–25.0	1.0–25.0	1.0–25.0
72–240 hours	2.7–13.0	1.0–12.5	1.3–15.3	1.3–15.3
Monocytes	0.45–3.3	0.20–2.50	0.2–2.20	0.2–2.50
Eosinophils	0.12–1.20	0.06–1.10	0.03–0.90	0.01–0.80
Lymphocytes	3.0–11.0	3.0–11.0	2.5–11.0	3.0–12.0
Blast cells (%)	<5	<8	<8	<8
Platelet count ($\times 10^9$/l)	140–450	140–450	140–450	140–450

* Values for Hb, Hct, MCV and MCH are based on reference ranges in Christensen *et al.*[63] Values for leucocytes and NRBCs are based on reference ranges in references 64–67 and our own hospital laboratory data (unpublished); values for peripheral blood blast cells and platelets are based on Roberts *et al.*[68]
Hb, haemoglobin concentration; Hct, haematocrit; MCH, mean cell haemoglobin; MCV, mean cell volume, NRBC, nucleated red blood cell.

Table 1.3 Impact of postnatal age on Hb and Hct values in healthy term and preterm neonates*

	Postnatal age		
	Birth	**2 weeks**	**4 weeks**
Gestation at birth 35–42 weeks			
Hb (g/l)	140–215	110–180	100–170
Hct (l/l)	0.43–0.65	0.32–0.55	0.27–0.48
Gestation at birth 29–34 weeks			
Hb (g/l)	130–215	100–170	80–135
Hct (l/l)	0.40–0.42	0.30–0.48	0.24–0.42

* Values are based on reference ranges in Christensen *et al*. 2009.[63]
Hb, haemoglobin concentration; Hct, haematocrit;

samples collected within 6 hours of birth. In contrast to adults, no differences in Hb or haematocrit were seen between male and female neonates.

In the absence of red cell transfusion, the Hb and haematocrit fall over the first few weeks of life due to the physiological reduction in red blood cell production. In term babies, the average Hb falls from 180 g/l at birth to 140 g/l at the age of 4 weeks,[69] reaching a nadir of around 100 g/l at 2 months of age. Studies of healthy preterm infants carried out almost 50 years ago reported a more rapid fall in Hb than in term babies, reaching a mean of 65–90 g/l at 4–8 weeks postnatal age (reviewed in reference 35). However, these differences are difficult to interpret because of the variable clinical course of preterm infants and the effects of red cell transfusion, particularly in neonates of less than 26 weeks' gestation at birth. More recently, data from non-transfused neonates have confirmed the lower Hb nadir in preterm neonates, with a mean Hb 28 days after birth of approximately 105 g/l in neonates with a gestational age at birth of 29–36 weeks and 130 g/l for term neonates.[69]

The mean cell volume (MCV) of red blood cells in healthy neonates at birth is higher than that in older children and adults and is inversely proportional to gestational age (Table 1.2).[63,68] The average MCV of a term neonate is about 105 fl, while extremely preterm neonates with a gestational age of less than 26 weeks typically have an MCV averaging about 120 fl.[63] Similarly, the mean cell haemoglobin (MCH) at birth is higher in preterm neonates than in term neonates, averaging 40 pg in a preterm neonate of less than 26 weeks' gestation and about 36 pg in a term neonate.[63] The MCV, but not MCH, has been reported to be significantly lower in black preterm compared with white preterm neonates, although this may reflect a higher prevalence of α thalassaemia trait in these neonates as this was not specifically investigated.[70] In contrast to the MCV and MCH, the mean cell haemoglobin concentration (MCHC) does not change significantly during gestation[70] and, unlike in older children, changes in MCHC are not very useful for diagnostic purposes in neonates. In term babies, the MCV and MCH fall slowly over the first few weeks of life, with a lower limit of normal of 77 fl and 26 pg, respectively.[63] The same pattern is seen in preterm neonates although the high frequency of red cell transfusions means that reliable data are not available.

Two newly available automated red cell parameters generated as part of a full blood count, MicroR and HYPO-He, have recently been evaluated in neonates.[71] In adult red blood cells, MicroR provides an automated measure of the percentage of red blood cells with an MCV of <60 fl and HYPO-He measures the percentage of red blood cells with an MCH of <17 pg. Bahr and colleagues created new reference ranges for neonates based on analysis of more than 11 000 blood counts and used them to show that a combination of MicroR and HYPO-He was more sensitive than MCV/MCH in identifying iron deficiency at birth.[71] Further validation of these results in prospective studies and assessment of the impact of gestational age will be needed to determine the value of these new parameters in the diagnosis of neonatal iron deficiency and anaemia.

Reticulocytes and circulating nucleated red blood cells

The reticulocyte count falls rapidly after birth as erythropoiesis declines. In term babies it then starts to increase at 7–8 weeks of age, reaching 35–200 × 10^9/l (1–1.8%) at 2 months of age; in preterm babies it increases at 6–8 weeks of age.[35] Neonatal reticulocytes, like mature neonatal red blood cells, have a larger volume and lower Hb than adult reticulocytes.[35] Manual reticulocyte counts have now largely been replaced by automated reticulocyte counts based on cell size and ribonucleic acid (RNA) content. Automated reticulocyte counts also provide a measure of the fraction of reticulocytes with the highest RNA content (Immature Reticulocyte Fraction [IRF]), which has been used in neonates to determine whether or not there is increased erythropoietic activity, for example in response to EPO treatment or as a diagnostic aid to haemolysis.[72–74]

Normal ranges for the numbers of circulating nucleated red blood cells (NRBC) in neonates have been compiled by Christensen *et al.*[66] and are generally higher in preterm neonates (2–3 × 10^9/l) than in term neonates (about 1 × 10^9/l) (see Table 1.2). Although modern analysers are increasingly able to generate accurate absolute NRBC counts, a useful guide from examination of blood films is that the presence of up to 5 NRBC/100 white blood cells in a term baby and up to 25 NRBC/100 white blood cells in a preterm baby can be considered normal for the first 1–2 days of life. In fact, there seems to be no difference between results obtained when the manual and automated methods are compared.[75] The number of NRBC in the peripheral blood falls rapidly over the first week of life and these cells are no longer seen after the second week of life. By contrast, circulating erythroblasts are increased in a variety of conditions in neonates and their presence can therefore be useful diagnostically because they usually indicate increased erythropoiesis driven either by anaemia or by chronic intrauterine hypoxia, for example due to IUGR (Table 1.4 and Fig. 1.10). It should be noted that NRBC also appear in the peripheral blood as part of a leucoerythroblastic picture, most typically in response to acute perinatal hypoxia (Fig. 1.11).[76,77]

Red blood cell morphology

Even in freshly taken samples, the morphology of neonatal red blood cells is distinctly different to that of adult cells.[35,78] The most typical morphological feature that differs from what is observed at other times of life is the presence of echinocytes (see Fig. 1.8). In healthy neonates, the proportion of echinocytes in blood films made from samples collected during the first week of life is inversely proportional to gestational age at birth.

Table 1.4 Causes of increased numbers of circulating nucleated red blood cells (erythroblasts) in term and preterm neonates

Response to anaemia: haemolytic disorders
Haemolytic disease of the newborn (especially due to anti-D and anti-c)
α thalassaemia major (occasionally, haemoglobin H disease)
Severe congenital dyserythropoietic anaemia (e.g. due to *KLF1* mutations)
Rare severe red cell enzyme deficiencies (e.g. pyruvate kinase deficiency or glucose phosphate isomerase deficiency)
Rare severe red cell membranopathies (e.g. hereditary stomatocytosis or autosomal recessive hereditary spherocytosis)
Response to anaemia: blood loss (mainly acute)
Fetomaternal haemorrhage
Placental abruption
Large cephalohaematoma
Haemorrhage into major organs, e.g. liver
Twin-to-twin transfusion (donor twin)
Response to hypoxia
Chronic *in utero* hypoxia:
Intrauterine growth restriction
Maternal hypertension
Maternal diabetes mellitus
Down syndrome (mechanism unclear)
Acute perinatal hypoxia:
Hypoxic ischaemic encephalopathy (leucoerythroblastic)
Neoplasms
Transient abnormal myelopoiesis in Down syndrome*
Congenital leukaemia (non-Down syndrome)
Other
Recovery phase of parvovirus B19 infection

* The increase in circulating erythroblasts is even higher in Down syndrome neonates with transient abnormal myelopoiesis than in Down syndrome neonates in general.

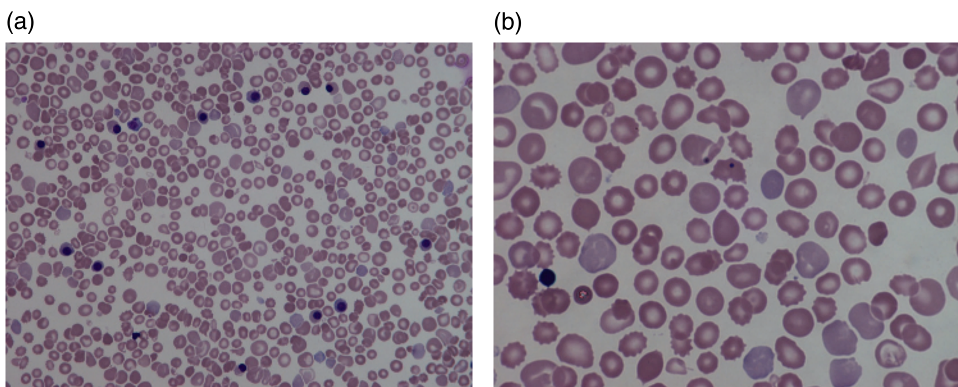

Fig. 1.10 Blood film showing features of hyposplenism resulting from intrauterine growth restriction: (a) low power showing anisocytosis, poikilocytosis, target cells and some fragments (MGG, ×40); (b) high power, two Howell–Jolly bodies are apparent (MGG, ×100).

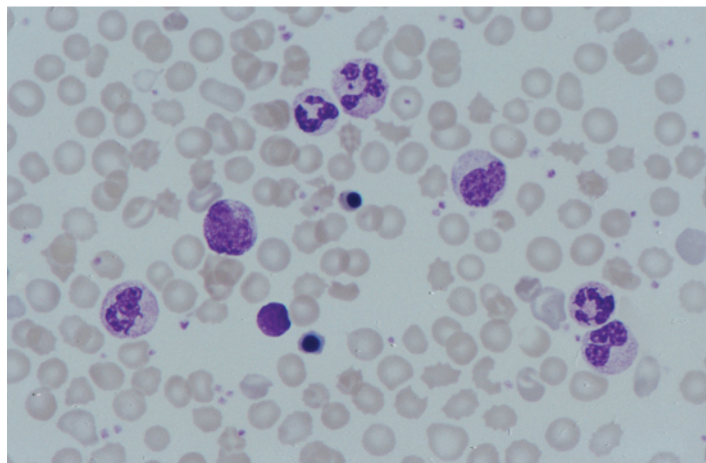

Fig. 1.11 Blood film from a preterm neonate (born at 25 weeks' gestation) on the day of birth showing a leucoerythroblastic picture with NRBC, a myelocyte and promyelocyte as well as toxic granulation of the neutrophils and red cell morphology typical of an extremely preterm neonate. This appearance is typical of perinatal hypoxia. MGG, ×100.

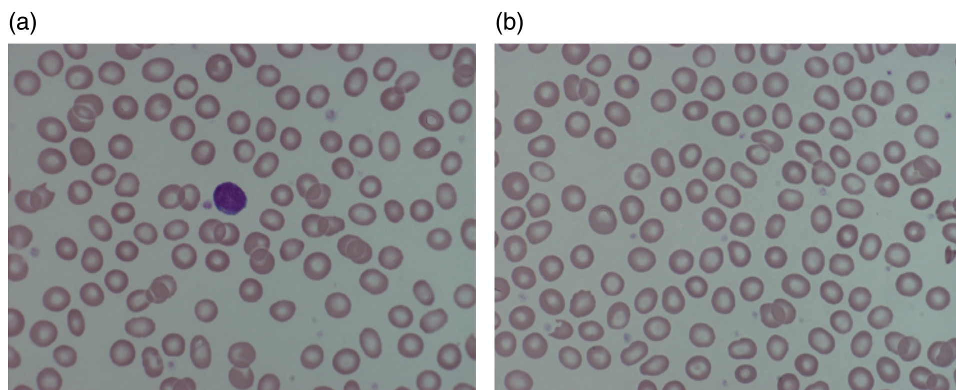

Fig. 1.12 Impact of postnatal age on red cell morphology; blood films from: (a) healthy term baby; (b) neonate at a postnatal age of 4 weeks. MGG, ×100.

Echinocytes gradually disappear from the peripheral blood film over the first few weeks of life so that even very preterm neonates will have few circulating echinocytes by 4 weeks of age (Fig. 1.12). This, together with the universal presence of echinocytes in very preterm neonates, strongly suggests that the changes reflect the unique differences in the cell membrane and metabolism of fetal red blood cells. Indeed, echinocytes are not a useful indicator of red cell pathology in neonates. Instead, other morphological indicators of red cell pathology, such as spherocytes, elliptocytes, target cells and occasionally acanthocytes, are a more reliable diagnostic guide (see Chapter 2).

The presence of a small proportion (typically <5%) of spherocytes and target cells in the first few days of life is normal, particularly in preterm babies, possibly reflecting a degree

of functional hyposplenism in the neonate. Consistent with this, these features are more marked in neonates with IUGR, when they are usually accompanied by the presence of Howell–Jolly bodies[34] (see Fig. 1.10). The presence of schistocytes in neonatal blood films may cause confusion. When they are present in large numbers in the first few days of life they may be an indicator of a microangiopathic process, as seen in disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura or Kasabach–Merritt syndrome. On the other hand, the presence of large numbers of schistocytes (10–20%) is very common in well babies for several months after the first few weeks of life (personal observation) and may simply reflect residual damaged fetal erythrocytes yet to be cleared from the circulation (see Fig. 1.12). Care should therefore be taken not to overestimate the significance of schistocytes in well babies with otherwise normal blood counts or mild physiological anaemia.

Blood volume

The normal blood volume at birth varies with gestational age and the timing of clamping of the umbilical cord.[79] In healthy term infants, the average blood volume is 80 ml/kg (range 50–100 ml/kg); however, this can be increased by up to 25% by late clamping of the cord.[79] Estimates of the blood volume in preterm infants show a slightly higher range of 85–106 ml/kg, largely due to an increase in plasma volume.[80–82]

Folic acid and vitamin B_{12}

In term and preterm babies of normally nourished mothers, stores of folic acid and vitamin B_{12} are adequate at birth and are maintained after birth in term neonates.[83] However, infants that are breastfed and born to mothers who are vitamin B_{12} deficient, either due to vitamin B_{12} malabsorption or because of a strict vegetarian diet, are at high risk of developing severe vitamin B_{12} deficiency at 4–8 months of life. The prevalence of vitamin B_{12} deficiency at birth varies in different countries from less than 1 in 100 000 in the USA to 1 in 3000–5000 in European countries where neonatal screening studies have been performed.[84–87] Although these infants are asymptomatic in the neonatal period, they develop progressive anaemia and neurological problems over the following months.[88] Although the anaemia is rapidly reversible with intramuscular vitamin B_{12}, persistent neurological impairment has been reported,[89] leading some to recommend neonatal screening for presymptomatic identification of vitamin B_{12} deficiency.[90] In preterm infants, folic acid reserves are lower at birth and are depleted more quickly than in term neonates, causing deficiency after 2–4 months if the recommended daily intakes are not maintained. The recommended daily intake for folic acid in preterm neonates is 25–100 μg/kg.[91] As preterm formula milks and breast milk fortifiers provide sufficient folic acid to prevent folate deficiency in preterm infants,[92] further supplementation is not required unless there is chronic haemolysis.

Leucocytes in the fetus and newborn

The leucocytes that form the human blood and immune system in the developing fetus start to appear in the peripheral blood during the first trimester.[93] Monocytes and lymphocytes appear in fetal blood by 8 weeks post-conception, although initially in very low

numbers. This is followed by the appearance of neutrophils and eosinophils from around 14–16 weeks post-conception, once haemopoiesis begins to be established in the bone marrow,[9,16] increasing by the end of the third trimester to the lower end of the values reported for leucocyte counts in term neonates. Blast cells are a normal feature in fetal blood, particularly in the second trimester, but are not usually greater than 10%.[94]

Apart from alterations in the numbers of white blood cells in response to infection, leucocyte disorders are not common in neonates. Nevertheless, some diagnostic dilemmas do present in the neonatal period, particularly when there is neutropenia or a rare disorder such as congenital leukaemia is suspected. Careful evaluation of leucocyte morphology can not only help to make an early diagnosis of bacterial infection but can also suggest the type of bacterial infection and provide rapid clues to the presence of congenital viral infection or a rare genetic or metabolic disorder (see Chapter 3). In addition, automated leucocyte differential counts are often inaccurate in neonatal samples, particularly in very preterm neonates, so that validation from a blood film is important.

In contrast to the gestation-related differences in red cell morphology, leucocyte morphology (neutrophils, monocytes, eosinophils, basophils and lymphocytes) is the same in healthy neonates of any gestation as in adults.

Leucocyte production and function in the fetus and neonate

All of the same types of normal leucocyte found in older children can be seen in peripheral blood films of term and preterm neonates (Fig. 1.13), although their frequencies vary from those in older children and also vary both with gestational age and with postnatal age (see Table 1.2). Blast cells are the only cells commonly seen in neonatal blood films that are not usual in blood films from healthy older children or adults (Fig. 1.14).

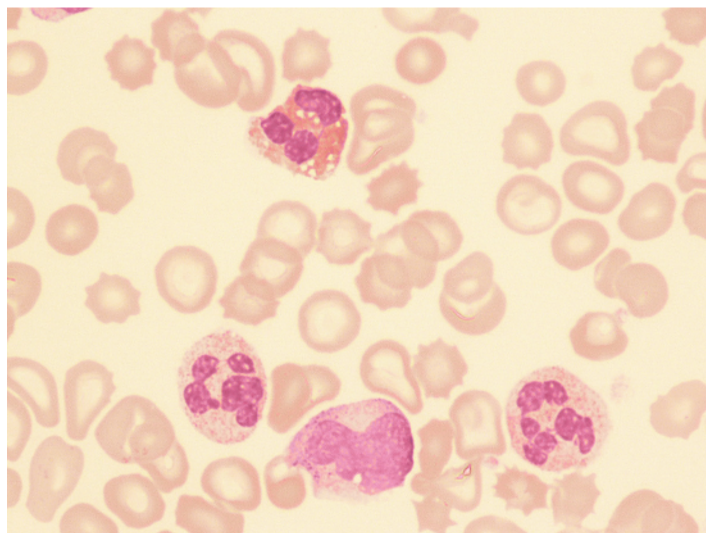

Fig. 1.13 Blood film of a preterm neonate showing normal white cells – two neutrophils, an eosinophil and a monocyte. MGG, ×100.

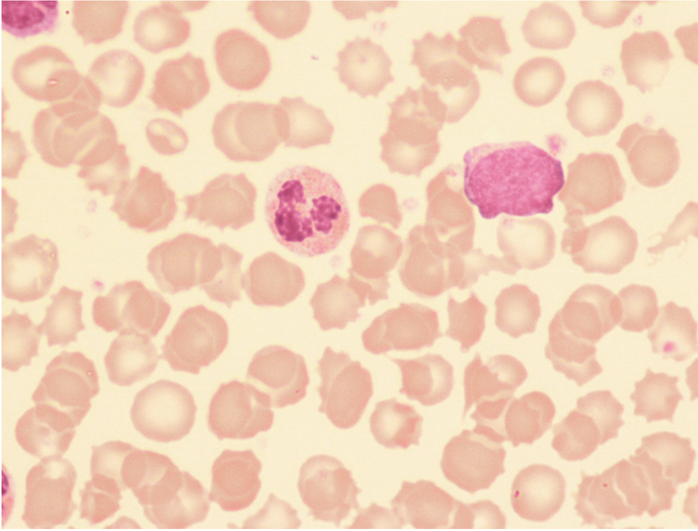

Fig. 1.14 Blood film of a healthy preterm neonate born at 25 weeks' gestation showing a neutrophil and a blast cell. MGG, ×100.

There is increasing recognition that leucocyte function in the fetus and newborn differs from that in adults[95] and that this is almost certain to contribute to the increased susceptibility to infection in neonates, particularly in those that are extremely preterm.[96] As the adaptive immune system only properly develops with antigen exposure after birth, neonates are specifically dependent on the innate immune system as a first line of defence against bacterial and fungal pathogens in particular.[97] Remarkably, all of the cellular components of the innate immune system are established in the human fetus over a small number of weeks late in the first trimester and early second trimester of fetal life.[16]

Neutrophils

At birth, neutrophils are the most plentiful of the circulating leucocytes in healthy neonates, constituting 50–60% of the total circulating white cells, in both term and preterm infants.[64,65,67] The absolute neutrophil count at birth is slightly higher in preterm neonates (averaging about 15×10^9/l) than in term neonates (averaging about 10×10^9/l),[64] and in most preterm neonates (<28 weeks' gestation at birth), the peripheral blood also often contains neutrophil precursors (metamyelocytes, myelocytes and promyelocytes) in small numbers, even when the baby is well (Fig. 1.15).

Neutrophils are produced almost entirely in the bone marrow during fetal life.[16] The numbers of circulating neutrophils in fetal blood samples taken in the first and second trimester are low ($0.1–0.2 \times 10^9$/l),[94] most likely because so much of the bone marrow at this gestation is devoted to expanding B cell production.[8] Thereafter, as granulopoiesis is established in the fetal bone marrow, the numbers of neutrophils gradually rise to reach over 2×10^9/l at the end of the third trimester. Nevertheless, the absolute neutrophil mass per kilogram is less than 25% of adult values in term neonates and slightly lower (20% of adult values) in preterm neonates.[95] This means that reserves of mature neutrophils in the

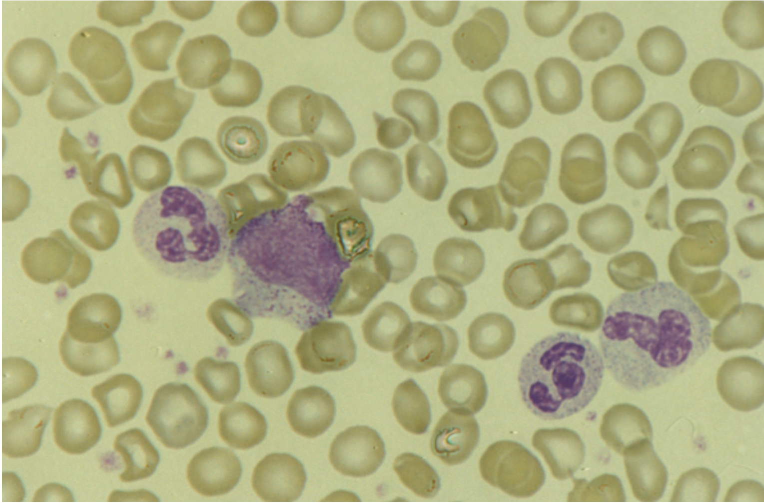

Fig. 1.15 Blood film of a well preterm neonate showing a myelocyte and three normal neutrophils. MGG, ×100.

bone marrow (sometimes referred to as the neutrophil storage pool) are rapidly depleted in neonates. This explains the frequent occurrence of severe neutropenia in preterm neonates as an immediate response to acute bacterial infection or necrotising enterocolitis (NEC) (Fig. 1.16) (see Chapter 3).[98–100] In contrast, adults have large reserves of neutrophils and neutrophil progenitors in the bone marrow, which can be rapidly recruited to boost neutrophil numbers in response to sepsis.

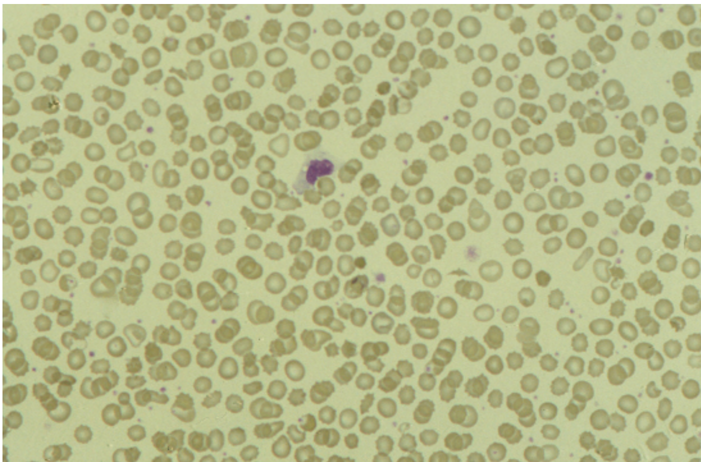

Fig. 1.16 Blood film of a preterm neonate with early clinical signs of necrotising enterocolitis showing severe neutropenia and moderate thrombocytopenia. Note the cytoplasmic vacuolation in the single neutrophil and the presence of large platelets and some schistocytes suggestive of disseminated intravascular coagulation (DIC). MGG, ×40.

Normal ranges for neutrophil counts in neonates were updated by Schmutz *et al.* in 2008.[64] These vary by gestational age at birth (see Table 1.2) and also by postnatal age, particularly over the first 24 hours. In healthy term babies the neutrophil count peaks at around 15.0 × 10^9/l (range 7.5–28.5 × 10^9/l) 6–12 hours after birth, with a very similar pattern also seen in healthy preterm babies over 28 weeks' gestation.[64] Normal ranges for very low birthweight neonates less than 28 weeks' gestation are difficult to establish because of the high frequency of medical problems, including infection, in these babies. In practice, the main clinical value of neutrophil normal ranges is to identify neonates with clinically significant neutropenia, especially those with severe congenital neutropenia (SCN), where prompt diagnosis is essential to prevent life-threatening complications. In SCN, the neutrophil count will nearly always be persistently low, although transient and moderate rises sometimes occur in the setting of infection. As a result, serial neutrophil measurements, as well as assessment of a blood film, are essential to rule out acquired disorders associated with neutropenia persisting for more than a week, for example cytomegalovirus (CMV) infection.

Monocytes

There are few monocytes in early second-trimester fetal blood (<0.06 × 10^9/l) but the numbers slowly increase to reach 0.1 × 10^9/l towards the end of the second trimester.[101] Normal ranges for monocyte counts at birth in term and preterm babies are shown in Table 1.2. These show a gradual rise from a mean of 0.75 × 10^9/l in preterm neonates less than 28 weeks' gestation at birth to 1.5 × 10^9/l in term babies.[65] Monocytes play a key role in the innate immune response of neonates to pathogens. Investigations have shown that although the ability of neonatal monocytes to phagocytose microorganisms is not impaired, other aspects of monocyte function in neonates are impaired compared with adult monocytes.[102,103] For example, neonatal monocytes have reduced expression of a number of functionally important cell surface molecules compared with adult monocytes, including HLA-DR, CD80 and L-selectin, which leads to a reduced ability to present antigens efficiently and to migrate to sites of inflammation.[104] In addition, many studies have reported differences in the pattern of pro- and anti-inflammatory cytokine production in neonatal monocytes, which may impair the antimicrobial activity not only of the monocytes themselves, but also of neutrophils,[105–108] although their ability to respond appropriately to bacille Calmette–Guérin (BCG) vaccine appears to be preserved.[109]

Eosinophils

Eosinophils are barely detectable in fetal blood until the middle of the second trimester.[101] Recent data indicate that there is no eosinophil production in fetal liver[9] and that circulating eosinophils are likely to be derived entirely from the bone marrow. The numbers of eosinophils in neonates gradually increase with gestational age from a mean of 0.02 × 10^9/l in preterm infants less than 28 weeks' gestation to 0.06 × 10^9/l in term neonates.[65] Normal ranges for neonatal eosinophil counts are shown in Table 1.2.

Lymphocytes

There are few studies of lymphopoiesis in the human fetus. Although B lymphocytes are found in low numbers in fetal liver and fetal blood by 8 weeks' gestation[110] and gradually increase in number during the second trimester,[111] the bone marrow is the main site

of B lymphopoiesis in fetal life.[8] By the second trimester, both fetal liver and fetal bone marrow B cells are polyclonal with equally diversified IgH chain repertoires, although at this stage the main source of IgM natural immunity seems to reside in the fetal liver as the majority of bone marrow B cells are still immature.[112] Two types of fetal B cell have been described in mice (B1 and B2 cells), with B1 cells being specific to fetal life and hypothesised to mainly play a role in innate immunity as they have limited Ig production capacity.[113] Putative B1 B cells have also been described in human fetal liver and bone marrow and in cord blood,[114] but their developmental origin and function are still to be defined.

T cell progenitors are first detected in the thymus at 9 weeks post-conception and mature T cells by 12–13 weeks post-conception followed by T cells appearing in the spleen and lymph nodes by 24 weeks.[115] Regulatory T cells, which are critical for promoting self-tolerance in fetal life, are detected in the thymus at 12 weeks post-conception.[116,117] T lymphocytes are detectable in fetal blood, marrow and thymus during the second trimester[118] and T cell development is largely complete by birth.[119] By term, T lymphocytes form 40–45% of circulating mononuclear cells, with a CD4:CD8 ratio of around 5:1, slightly higher than that in adult blood (3.1:1). The normal ranges for the total lymphocyte count in neonatal blood are the same in term and preterm neonates (see Table 1.2) and remain stable over the first month of life in healthy neonates.[67]

Blast cells

Blast cells, usually resembling myeloblasts, are a normal feature on neonatal blood films (see Fig. 1.14). Their numbers are increased in preterm compared with term babies and in babies with severe infection. A study that evaluated blood films in 123 healthy neonates found an upper limit of 4% for the frequency of circulating blasts in neonatal blood, whereas for sick babies up to 8% blasts were occasionally seen.[68] The causes of increased blast cells in neonates are discussed in Chapter 3 (see Table 3.6).

Leucocyte function in the fetus and neonate

The clinical importance of the immaturity of the innate immune system in neonates is best demonstrated by the fact that the pattern of infections in neonates closely mimics that seen in children with SCN. While neutrophil numbers at birth are similar to those in older children and adults, the lack of neutrophil reserves discussed above is aggravated by the impaired function of neonatal neutrophils.[95] First, recruitment and migration of neonatal neutrophils to sites of infection is impaired compared with adult neutrophils. The processes involved are complex and several aspects are defective in neonatal neutrophils, including chemotaxis, adhesion, rolling and transmigration, which together result in an impaired ability to leave the circulation and enter the tissues.[120–122] Secondly, neonatal neutrophils have an overall reduced ability to generate neutrophil extracellular traps (NETs), which are an important component of the innate immune response that limits the dissemination of a variety of pathogens.[123–125] NETs are extracellular, web-like structures composed of a variety of antimicrobial molecules, such as elastase, myeloperoxidase, lactoferrin and defensins, and are key for protection against infection in neonates, trapping, neutralising and killing bacteria, fungi, viruses and parasites.[126] Thirdly, neonatal neutrophils have lower levels of various antimicrobial granule proteins, such as lactoferrin and bactericidal/

permeability-increasing protein (BPI), particularly in preterm neonates.[95] Recent data suggest that lactoferrin may be particularly important for converting neonatal neutrophils and monocytes to myeloid-derived suppressor cells (MDSC), which are now recognised as being critical in controlling diseases associated with deregulated inflammation in neonates, including NEC.[127,128] Finally, although term neonates are able to phagocytose both Gram-positive and Gram-negative bacteria normally, in preterm neonates phagocytosis of bacteria and of *Candida albicans* is less efficient.[102,129]

Platelets and megakaryocytes in the fetus and neonate

Platelets appear in the circulation at 5–6 weeks' gestation and reach values of 150×10^9/l by the end of the first trimester[110,118] and 175–250 $\times 10^9$/l during the second and third trimesters.[93,130,131] Although some studies have suggested that there is a linear increase in the platelet count with increasing gestational age, a large study of more than 5000 fetal blood samples showed that there was no further significant increase in fetal platelet count through the second and third trimesters.[131] Thus, a platelet count of less than 150×10^9/l can be considered abnormal, even in the most preterm neonate.

Developmental megakaryopoiesis and thrombopoiesis

As for most of the other types of blood cell, there are many differences between neonates and adults in the processes that regulate megakaryocyte production (megakaryopoiesis) and platelet production (thrombopoiesis).[132,133] These differences are particularly marked in preterm neonates and likely to contribute to the frequent occurrence of thrombocytopenia in sick neonates; they are important to consider in the investigation and treatment of neonatal thrombocytopenia (see Chapter 4).

The principal cytokine regulating platelet production in the fetus and newborn, as in adults, is thrombopoietin (TPO).[34] Circulating levels of TPO, which is produced in the liver from early in fetal life,[134,135] are higher in healthy term and preterm neonates than in adults.[136,137] This does not seem to be a compensatory mechanism since TPO-induced signalling is upregulated in cord blood megakaryocytes compared with adult megakaryocytes.[133] Fetal megakaryocytes are also smaller and of lower ploidy than their adult counterparts, which may be the reason that they not infrequently circulate in the peripheral blood in preterm neonates (Fig. 1.17) and that cord blood-derived megakaryocytes produce approximately 50% fewer platelets per cell[138] (reviewed in references 139 and 140). Furthermore, unlike adults, thrombocytopenic neonates can only increase their megakaryocyte number, and not size, in response to consumptive thrombocytopenia.[141] Nevertheless, fetal megakaryocytes appear to be cytoplasmically mature and express increased amounts of messenger RNA for the transcription factor GATA1 and increased surface glycoprotein 1b compared with adult megakaryocytes.[133] These functional differences in fetal and neonatal megakaryocytes are now known to be accompanied by increased expression of genes associated with a number of signalling pathways, including those mediated by transforming growth factor β (TGFβ), insulin-like growth factor (IGF) and Janus kinase 2 (JAK2), in fetal compared with adult megakaryocytes.[142]

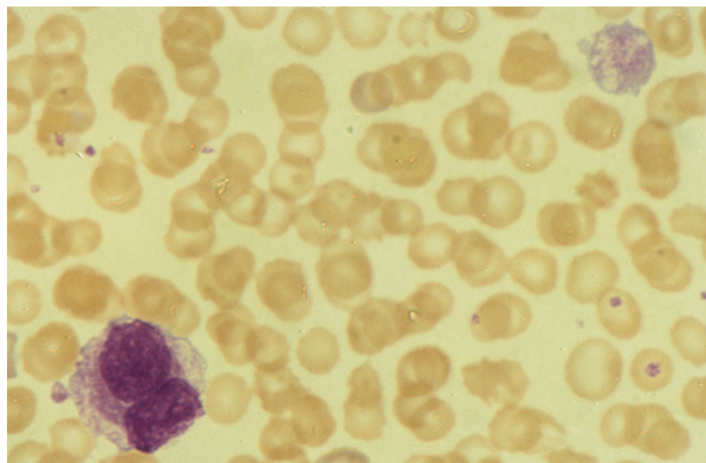

Fig. 1.17 Blood film of a preterm neonate born at 24 weeks' gestation showing a circulating megakaryocyte and giant platelet. MGG, ×100.

There are also developmental differences in megakaryocyte progenitor cells. The numbers of these cells are high early in fetal life and fall towards term and are higher in healthy preterm than term babies.[132,143,144] Additionally, fetal megakaryocyte progenitors have more proliferative potential *in vitro* than those derived from adults, perhaps related to stronger activation of the JAK2 and mTOR pathways in response to TPO stimulation as found in cord blood megakaryocytes.[133] These properties may explain how fetal and neonatal platelet counts are maintained at levels similar to those of adults, at least in the healthy fetus and neonate. The observation of increased numbers of reticulated (young) platelets in fetal compared with adult peripheral blood is consistent with this hypothesis.[145]

Platelet numbers in the neonate and fetus – normal values

Since platelet counts in fetal blood reach normal adult values by the end of the second trimester, it is reasonable to consider that platelet counts of less than 150×10^9/l represent thrombocytopenia in a healthy neonate regardless of gestation at birth. This conclusion has been challenged by a large retrospective analysis of 47 000 neonates, in which a reference range of platelet counts at different gestational ages was determined by excluding the highest and lowest 5th percentile of all observed counts. By this method, the lowest limit of platelet counts was found to be 104×10^9/l for infants of less than 32 weeks' gestation, compared with 123×10^9/l for neonates of greater than 32 weeks' gestation.[146] However, as the study included all neonates, regardless of clinical status, these counts may simply reflect the high frequency of thrombocytopenia in neonatal units, where sepsis and placental insufficiency are common causes for admission, rather than representing a new physiological definition of normal platelet counts in the newborn. Indeed, the mean platelet count was above 200×10^9/l regardless of gestation at birth, consistent with accepted normal ranges for the platelet count in neonates.[143]

Several studies have investigated the clinical relevance of newer automated platelet parameters in neonatal medicine, particularly the immature platelet fraction (IPF). Overall, these studies support the conclusion that in neonates, as in adults, the IPF% provides a measure of the proportion of immature platelets and a surrogate measure of platelet production although the results are variable.[147] MacQueen and colleagues established reference ranges for the IPF% in their hospitals based on more than 20 000 automated results from nearly 9000 neonates.[148] They reported a higher IPF% in the most premature infants (less than 32 weeks' gestation). Consistent with this, they also found that IPF% values were higher in neonates who developed consumptive thrombocytopenia compared with those were considered to have reduced platelet production, suggesting that further clinical studies of the value of the IPF% in neonates might be useful, given the practical difficulties of bone marrow aspiration in the newborn.

Neonatal platelet function

Studies of neonatal platelet function *in vitro* have consistently shown hyporeactivity of neonatal compared with adult platelets to a wide range of platelet agonists, including adenosine diphosphate (ADP), thrombin and thromboxane and especially so to collagen and adrenaline (epinephrine).[132,149] In keeping with this, the number of α-adrenergic receptors on neonatal platelets has been found to be 50% lower than on adult platelets.[150,151] By contrast, expression of the main platelet adhesion receptors appears to be similar in neonatal and adult platelets, with the exception of glycoprotein (Gp) IIb/IIIa, which is expressed at lower levels in neonatal platelets.[152] Importantly, follow-up studies have shown that previously impaired platelet responses in neonates are similar to adult responses within 2 weeks of birth.[152,153] Another point of note is that most studies of neonatal platelet function have been carried out on cord blood platelet-rich plasma. However, more recent studies on whole blood have shown that healthy neonates have robust primary haemostasis overall, despite apparently hypofunctional platelets as assessed using standard techniques in platelet-rich plasma. This may, at least in part, be due to the higher haematocrit and higher levels of von Willebrand factor in neonates. In addition, measures of *in vitro* platelet activation and aggregation in cord blood do not match those obtained when studying peripheral blood.[154] These observations have led some investigators to conclude that the function of neonatal platelets should not be viewed as impaired but, instead, that neonatal platelets function as a component of a generally well-balanced neonatal haemostatic system.[149]

Practical problems in interpreting neonatal blood counts and films

Sample quality/artefacts

One of the commonest practical difficulties in interpreting neonatal blood counts and blood films occurs because of the much higher haematocrit in neonates. First, there is a higher frequency of clotted samples, which most likely reflects the challenge of collecting free-flowing blood samples from neonates, especially those who are very low birthweight and/or preterm. Secondly, the high haematocrit often leads to poor-quality 'thick' or

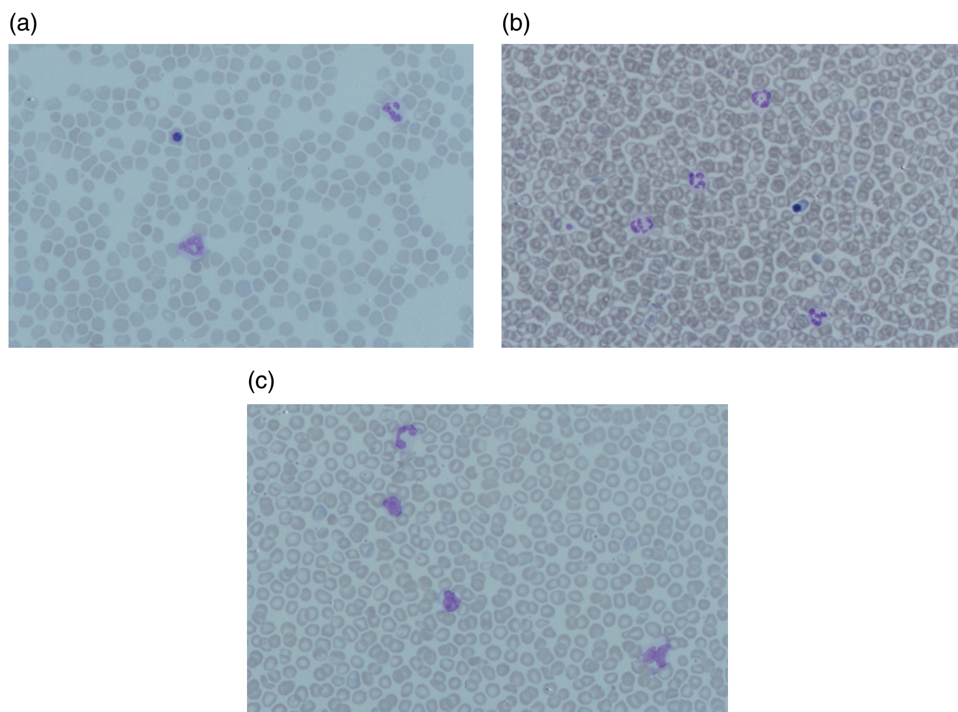

Fig. 1.18 Blood film of a healthy term neonate with a normal blood count and no evidence of haemolysis, showing artefactual changes in red cell morphology apparent on different sections of the blood film. (a) Thin end of the blood film showing apparent 'spherocytes' that are not present on the well-spread section of the film (compare with Fig. 1.18c). A normal neutrophil, monocyte and NRBC can also be seen. (b) Thick end of the same blood film showing overlapping red cells, which make it difficult to interpret the red cell morphology, and apparent target cells and hypochromia that are not present on the well-spread section of the film (compare with Fig. 1.18c). Normal neutrophils and an NRBC are also shown. (c) Good section of the blood film showing normal red cell morphology. Normal white blood cells are also shown. MGG, ×40.

unevenly spread films, which may give the misleading impression of the presence of abnormal red cells, such as spherocytes or target cells, which are not seen when the correctly spread section of the film is reviewed (Fig. 1.18). These effects can often be mitigated by dilution of the sample prior to analysis and preparation of the blood film. Another practical problem is the difficulty of distinguishing the typical changes seen on aged blood samples anticoagulated with ethylene diaminetetra-acetic acid (EDTA) from the normal red cell features typical of preterm babies. It is therefore particularly important to make blood films on neonatal samples as soon as possible.

Site of sampling

The site of sampling also influences blood count analyses in neonates. For example, in the first few hours of life the Hb of venous samples is lower than that of heel-prick samples collected simultaneously,[155] sometimes by up to 20–40 g/l.[35] This difference is greater in

preterm neonates and falls with increasing postnatal age, such that by the fifth day of life there is almost no difference in Hb between a well-taken heel-prick sample and a venous sample.[155] Similarly, the haematocrit and red blood cell count at birth are lower in venous blood compared with capillary blood samples collected simultaneously, while there is no difference in the other red cell indices by site of sampling.[155] Neonatal heel-prick samples have also been shown to have white cell, neutrophil and lymphocyte counts about 20% higher than arterial or venous samples; counts are most likely to approximate to those of venous blood if there is a free flow of blood and if early drops, excluding the first, are used for the count.[35] In contrast, the platelet count and mean platelet volume (MPV) are lower in capillary samples than in venous samples.[155,156]

Gestational age and postnatal age

Although the Hb, haematocrit and MCV vary little over the first week of life, there is a gradual loss of other distinctive neonatal red cell features after the first week of life. In particular, the numbers of circulating nucleated red cells fall, as mentioned above, and echinocytes are gradually replaced by red cells with the more typical appearance of adult red cells. Consideration of the postnatal age of a baby is most important when interpreting blood films to help in the identification of infection or the cause of anaemia. For example, maternal chorioamnionitis may cause extremely high neutrophil counts, often with associated toxic granulation, in the newborn infant despite the absence of active infection in the neonate (Fig. 1.19). It seems likely that this is due to maternal cytokines crossing the placenta, although this has not been specifically demonstrated. Importantly, virtually identical changes observed on neonatal blood films after the first week of life are highly likely to reflect active infection in the neonate as any maternal cytokine-driven changes will have resolved by this time.

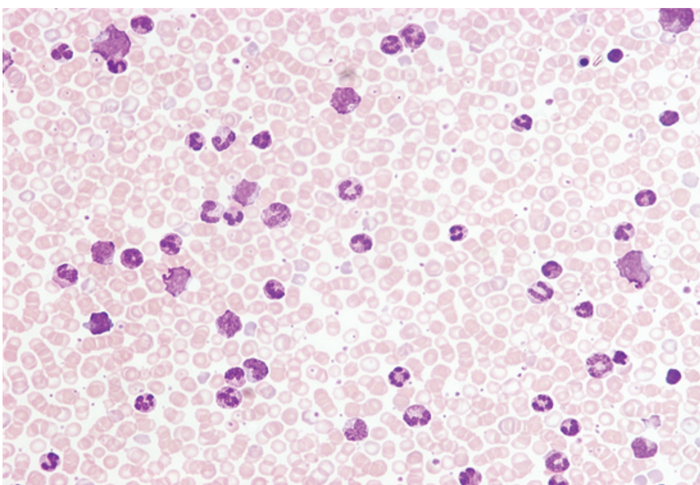

Fig. 1.19 Blood film in maternal chorioamnionitis showing neutrophilia, left shift (myelocytes and promyelocytes) and toxic granulation. MGG, ×40.

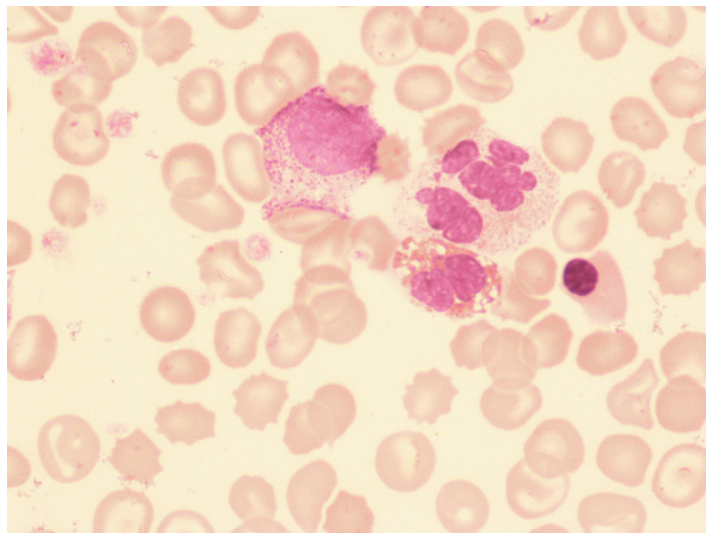

Fig. 1.20 Leucoerythroblastic blood film in a preterm neonate with hypoxic ischaemic encephalopathy showing a myelocyte, an NRBC, an eosinophil and a macropolycyte, likely to be a tetraploid cell. MGG, ×100.

Pregnancy-associated complications and mode of delivery

In addition to being influenced by maternal chorioamnionitis, the white cell count (WBC) at birth is also affected by the mode of delivery, being lower after an elective caesarean section than after either vaginal delivery or caesarean section performed after labour has commenced.[64] Hypoxic ischaemic encephalopathy (HIE) following perinatal hypoxia (e.g. due to the cord being round the neck of the baby) also causes an increased WBC; in this case the blood film is leucoerythroblastic and the neutrophils show varying degrees of toxic granulation, often making it difficult to identify the presence of coexisting infection based on the haematological findings alone (Fig. 1.20).

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