CHAPTER 1

The Blood Film and Count

Blood

Blood is a life-sustaining fluid which circulates through the heart and blood vessels. It carries oxygen and nutrients to the tissues and waste products to the lungs, liver and kidneys, where they can be removed from the body. Usually when blood is removed from the body it forms a solid blood clot. However, if clotting is prevented by mixing with an anticoagulant, the blood separates, under the influence of gravity, into three layers (Fig. 1.1). The bottom layer is deep red in colour and is composed of red cells. The top layer is clear and pale yellow. It is called plasma and is composed of various salts and proteins dissolved in water. In between is a narrow layer called the buffy coat because of its buff or yellowish white colour. The buffy coat is composed mainly of cells of a variety of types, collectively known as white cells. In addition there are small cellular fragments, called platelets, which have a role in blood clotting.

The blood film

Although we can judge the proportions of red cells and white cells in a tube of sedimented blood, we get far more information if the blood is carefully mixed and a thin layer is spread on a glass slide to form a blood film. The blood cells are then preserved by exposure to the alcohol methanol, a process known as fixation. The fixed film of blood is stained with a mixture of several dyes so that the individual cells can be recognized when they are examined with a microscope. After staining, the colour of red

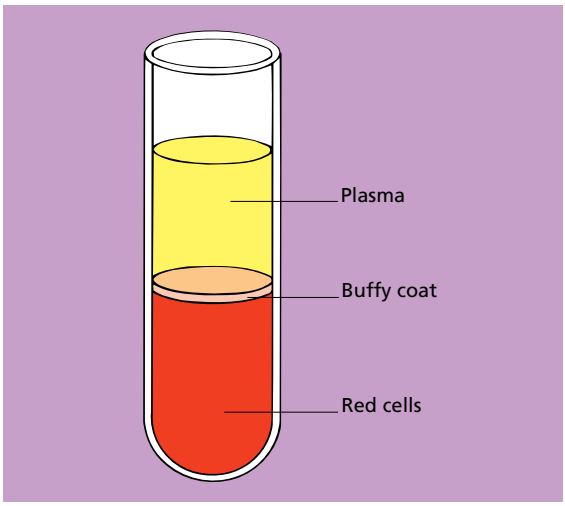

Fig. 1.1 Diagram of a tube of anticoagulated blood which has been allowed to sediment, showing the separation of blood into red cells, a buffy coat (white cells and platelets) and plasma.

cells is enhanced and the white cells and platelets, which would otherwise be transparent and colourless, have acquired a variety of colours which allow their detailed structure to be recognized. One of the commonest mixtures of dyes used to stain blood cells is the May–Grünwald–Giemsa (MGG) stain, named after its inventors. All the photographs in this book are of MGG-stained blood films.

Red cells

The most numerous cells in a blood film are the red cells, also known as erythrocytes. Normal red cells are disc-shaped but are thinner in the centre (Fig. 1.2). As a consequence, on a stained blood film, they have a circular outline and a paler central area (Fig. 1.3). Red cells owe their pinkish-brown colour to the presence of a complex protein, haemoglobin, which is their major constituent. Enhancement of their colour in a stained film is because haemoglobin takes up eosin, one of the dyes of the MGG stain. In the body it is haemoglobin of the red cells which, in the

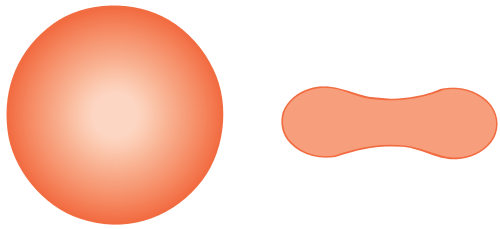

Fig. 1.2 A diagram of a red cell viewed from above and in cross-section.

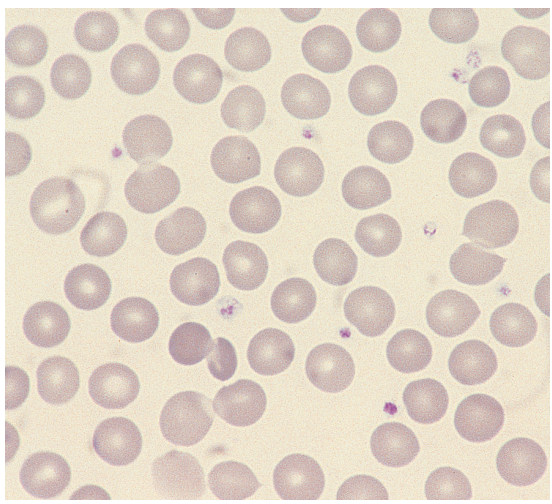

Fig. 1.3 Normal red cells (erythrocytes) showing little variation in size and shape, an approximately round outline and a small area of central pallor in some of the cells. The small lilac-staining structures between the red cells are platelets.

lungs, combines with oxygen from inspired air and transports it to tissues where it is needed for the metabolic processes supplying the energy needs of the body. Mature red cells in humans (although not in some other species) differ from most body cells in that they do not have a nucleus. Red cells are produced in the bone marrow and usually lose their nuclei when they are released into the blood stream.

White cells

In healthy people there are at least five types of white cell or leucocyte in the circulating blood. Unlike red cells, white cells have retained their nuclei. The cell is therefore made up of a nucleus and cytoplasm. The cytoplasm is the site of protein synthesis and other cellular functions. The nucleus is composed of chromatin, which is mainly deoxyribonucleic

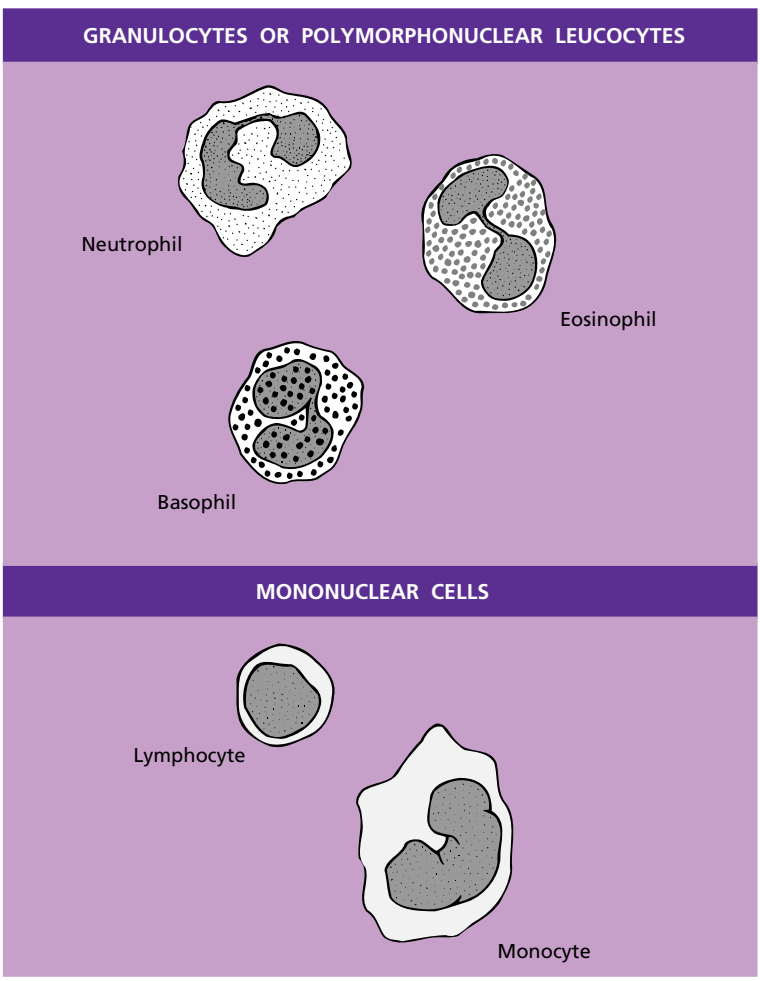

Fig. 1.4 A diagram showing how white cells are classified.

acid (DNA), carrying genetic messages. Genetic messages are transmitted from the nucleus to the cytoplasm by ribonucleic acid (RNA).

White cells are divided into granulocytes (also known as polymorphonuclear leucocytes) and mononuclear cells. There are three types of granulocyte and two types of mononuclear cell (Fig. 1.4). The names are not very logical but they have been in use for a long time and are generally accepted. Granulocytes are so named because their cytoplasm contains prominent granules. However, monocytes also have granules and so do some lymphocytes. The term polymorphonuclear leucocyte refers to the very variable nuclear shape which is typical of granulocytes. The term mononuclear cell means that the cell has only a single nucleus. However, this is true of granulocytes, as well as of the cells conventionally referred to as mononuclear. The functions of the various leucocytes are summarized in Table 1.1.

Neutrophils

Neutrophils (Fig. 1.5) have a nucleus which stains purple and is divided into two to five segments or lobes. The lobes are separated by a thin strand or filament of nuclear material. The nuclear chromatin is heterogeneous with some clumping. The cytoplasm of neutrophils is very pale blue and is packed with fine lilac-staining granules. The granules are referred to as

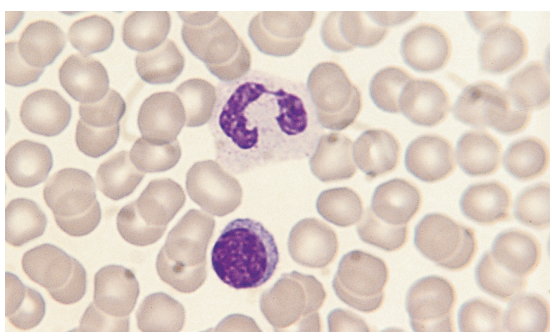

Fig. 1.5 A normal neutrophil with a bilobed nucleus and cytoplasm containing delicate lilac-staining granules. The other nucleated cell is a small lymphocyte.

Table 1.1 The functions of leucocytes.

Cell	Major function
Neutrophil	Is attracted to sites of infection by a process known as chemotaxis; ingests micro-organisms (a process known as phagocytosis) and destroys them
Eosinophil	The same functions as the neutrophil; in addition, helps control parasitic infections; has a role in allergic responses
Basophil	Has a role in immediate hypersensitivity reactions, allergic and inflammatory responses and in the control of parasitic infections
Lymphocyte	Mediates immune responses: B lymphocyte matures into a plasma cell, which secretes antibodies (humoral immunity); T lymphocyte attacks cells bearing foreign antigens and antibody-coated cells; can help or suppress B cells (part of cell-mediated immunity); Natural killer lymphocyte (NK cell) attacks foreign cells and tumour cells (part of cell-mediated immunity)
Monocyte	Phagocytoses and kills micro-organisms including mycobacteria and fungi, phagocytoses cells or organisms that have bound immunoglobulin or complement and phagocytoses dead and damaged cells; presents antigen to cells of the immune system; migrates to tissues where it differentiates, to become a long-lived phagocytic and antigen-presenting cell known as a macrophage

neutrophilic because they owe their colour to uptake of both the acidic and the basic components of the stain. In females a proportion of the neutrophils have a very small lobe, known as a 'drumstick', protruding from the nucleus (Fig. 1.6). It represents the inactive X-chromosome of the cell.

Neutrophils are produced in the bone marrow. They spend 6–10 hours in the blood stream before moving from capillaries into tissues. The major function of neutrophils is as tissue phagocytes. They move preferentially to sites of infection or inflammation where they ingest, kill and break down bacteria. The process of moving to sites of infection or inflammation

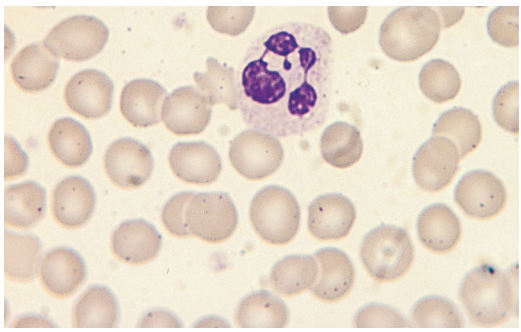

Fig. 1.6 A normal neutrophil from a female showing a nucleus with four lobes and a 'drumstick'.

is known as chemotaxis and occurs in response to activated complement components and chemical signals released by a variety of cells. The process of ingesting bacteria is known as phagocytosis.

Eosinophils

Eosinophils (Fig. 1.7) have a nucleus that is usually bilobed and pale blue cytoplasm, which is packed with large refractile, orange–red granules. The granules are referred to as eosinophilic because they take up the acidic dye eosin. Eosinophils are produced in the bone marrow and circulate in the blood stream for

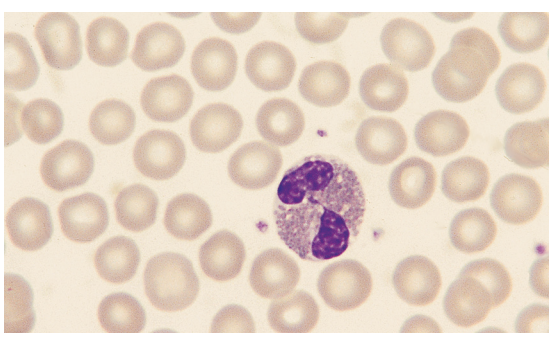

Fig. 1.7 A normal bilobed eosinophil. The granules are reddish-orange and pack the cytoplasm.

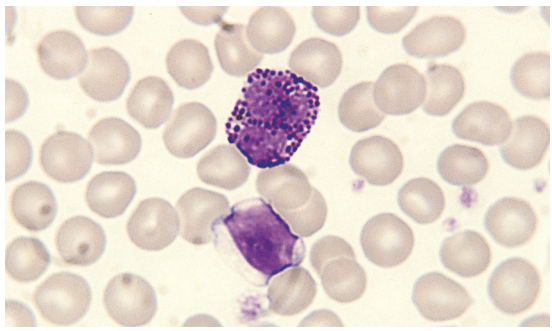

Fig. 1.8 A normal basophil. The nucleus has three lobes. The cytoplasm is packed with large purple granules. (The lower cell is a lymphocyte.)

about 6 hours before migrating to tissues. They respond to chemotactic stimuli, are phagocytic and can kill ingested organisms. They are important in the body's defences against tissue parasites, being able to discharge their granule contents extracellularly, seriously damaging large parasites. Eosinophils are also involved in allergic reactions.

Basophils

Basophils (Fig. 1.8) have a lobulated nucleus, which is often obscured by the large purple-staining granules which pack the very pale blue cytoplasm. The granules are referred to as basophilic because they take up basic components of the stain (such as methylene blue). In fact they stain metachromatically with basic stains, i.e. the granules react with a blue dye to produce a purple colour. Basophils are produced in the bone marrow and circulate in the blood in small numbers before migrating to tissue. They have a role in allergic and inflammatory responses.

Lymphocytes

Lymphocytes are the second most numerous circulating white cell after neutrophils. They are smaller than granulocytes with a round or somewhat irregular outline and pale blue, clear

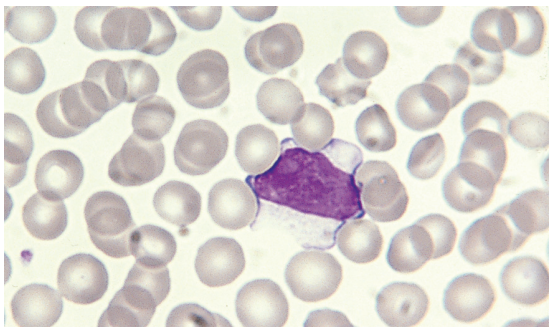

Fig. 1.9 A large lymphocyte with a less densely staining nucleus than occurs in a small lymphocyte and more plentiful pale blue cytoplasm. A nucleolus is apparent, top left in the nucleus.

cytoplasm. Some lymphocytes have a variable number of azurophilic (pinkish-purple) granules. Lymphocytes are divided into three morphological categories, depending on their size, the amount of cytoplasm and the presence or absence of cytoplasmic granules. These categories are small lymphocyte (Fig. 1.5), large lymphocyte (Fig. 1.9) and large granular lymphocyte (Fig. 1.10). Small lymphocytes are most numerous. The nuclear chromatin of lymphocytes may be dense and homogeneous (particularly in small lymphocytes) or more lightly staining and somewhat heterogeneous (particularly in large lymphocytes).

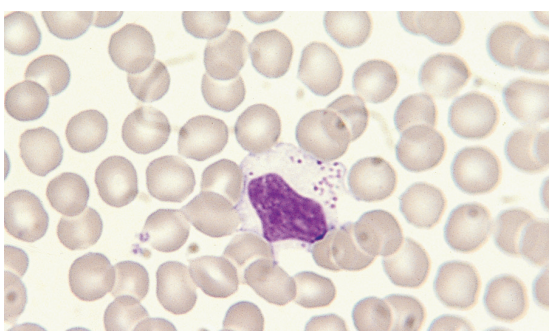

Fig. 1.10 A large granular lymphocyte showing a moderate number of prominent azurophilic granules in clear cytoplasm.

Occasional normal lymphocytes show a discrete but ill-defined paler structure within the nucleus, which is the nucleolus.

Lymphocytes are produced from lymphoid stem cells in the bone marrow and probably the thymus. Their function is in tissues such as lymph nodes, spleen, tonsils and the lymphoid tissue associated with mucous membranes. They circulate in the blood stream, enter lymphoid tissues and emerge again from lymphoid tissues into lymphatic channels, where they form one constituent of a clear fluid known as lymph. Lymphatics drain into the thoracic duct and ultimately into the blood stream. This process of continuing movement between tissues and the blood stream is known as lymphocyte recirculation. Lymphocytes function in the body's immune responses. They are divided into three functional types: B cells, T cells and natural killer (NK) cells. B cells differentiate in tissues into plasma cells, which secrete antibodies, thereby providing humoral immunity. T cells function in cell-mediated immunity as do NK cells. T cells also modulate B cell function. The functional categories of lymphocyte show little correlation with morphological categories except that large granular lymphocytes are either T cells or NK cells. However, other T cells cannot be distinguished morphologically from B cells. The functional categories of lymphocytes are of far more importance than the morphological categories.

Monocytes

Monocytes (Fig. 1.11) are the largest normal blood cells. They have lobulated nuclei and voluminous cytoplasm which is greyish-blue, is sometimes opaque and may be vacuolated or contain fine azurophilic granules.

Monocytes have an intravascular life span of several days. They function mainly in tissues where they differentiate into long-lived macrophages (sometimes called histiocytes). Monocytes and macrophages respond to chemotactic stimuli and are phagocytic. They are part of the body's defences against bacterial and fungal infections and also ingest and break down dead and dying body cells. They present antigen to lymphocytes.

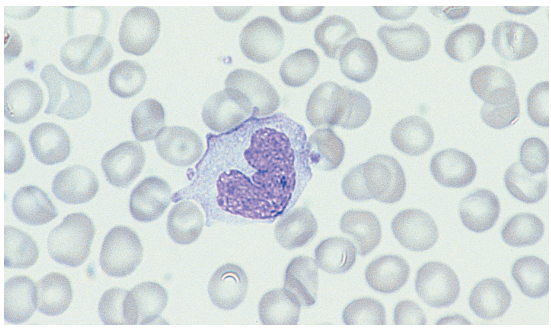

Fig. 1.11 A monocyte, showing a lobulated nucleus and voluminous, opaque cytoplasm containing very fine azurophilic granules. Several platelets are also visible. (Monocytes should not be confused with large granular lymphocytes. Lymphocytes have clear, pale blue cytoplasm and discrete, sometimes prominent granules whereas monocytes usually have more opaque, grey–blue cytoplasm with very fine granules.)

They secrete chemical messengers, known as cytokines, which influence the behaviour of other body cells, including blood cells and their precursors. Monocytes differentiate not only into macrophages but also into various specialized cells, specific to different organs, such as the Kupffer cells of the liver and the microglial cells of the brain.

Platelets

Platelets are produced within the vascular channels (sinusoids) of the bone marrow by the fragmentation of the protruding cytoplasm of large bone marrow cells known as megakaryocytes. They are thus not, strictly speaking, cells but rather are fragments of the cytoplasm of cells.

Platelets are considerably smaller than red cells and white cells (Fig. 1.11). They are pale blue with fine azurophilic granules which tend to be clustered in the centre of the platelet. When blood films are made, as is generally the case, from anticoagulated blood, the platelets are usually discrete and separate from each other, but in some circumstances they form clumps or aggregates.

Haemopoietic cells

Peripheral blood cells are produced in the bone marrow. Their precursors are referred to as haemopoietic cells (Fig. 1.12). The only significant function of haemopoietic cells is the production of mature end cells. Recognizable haemopoietic precursors are present in the circulating blood of healthy subjects but, except in the neonatal period and during pregnancy, they are quite uncommon and are not often noted in a blood film. They are much commoner in patients with leukaemia or other haematological disorders and in patients with severe infection or other serious systemic diseases.

Myeloblasts

Myeloblasts (Fig. 1.13) are very rare in the blood of healthy subjects. They are larger than lymphocytes but often smaller than monocytes. They have a high nucleocytoplasmic ratio and scanty to moderate amounts of cytoplasm, which varies from weakly to moderately basophilic. (Basophilic in this context indicates a blue colour consequent on the uptake of basic dyes.) The nucleus is approximately round, nuclear chromatin is diffuse and nucleoli may be apparent. In patients with leukaemia and related disorders, the cytoplasm may contain small numbers of azurophilic granules or other inclusions or vacuoles (see page 91). Myeloblasts are precursors of neutrophils, eosinophils and basophils.

Promyelocytes

Promyelocytes (Fig. 1.14) are rare in the blood of healthy people. They are larger than myeloblasts with more plentiful cytoplasm and consequently a lower nucleocytoplasmic ratio. The cytoplasm is more basophilic than that of a myeloblast and contains azurophilic (pinkish-purple) primary granules. Sometimes there is a more lightly staining zone in the cytoplasm adjacent to the nucleus, which represents the Golgi apparatus, where granules are produced. The nucleus is round or oval, is usually eccentric, shows some chromatin condensation and has a

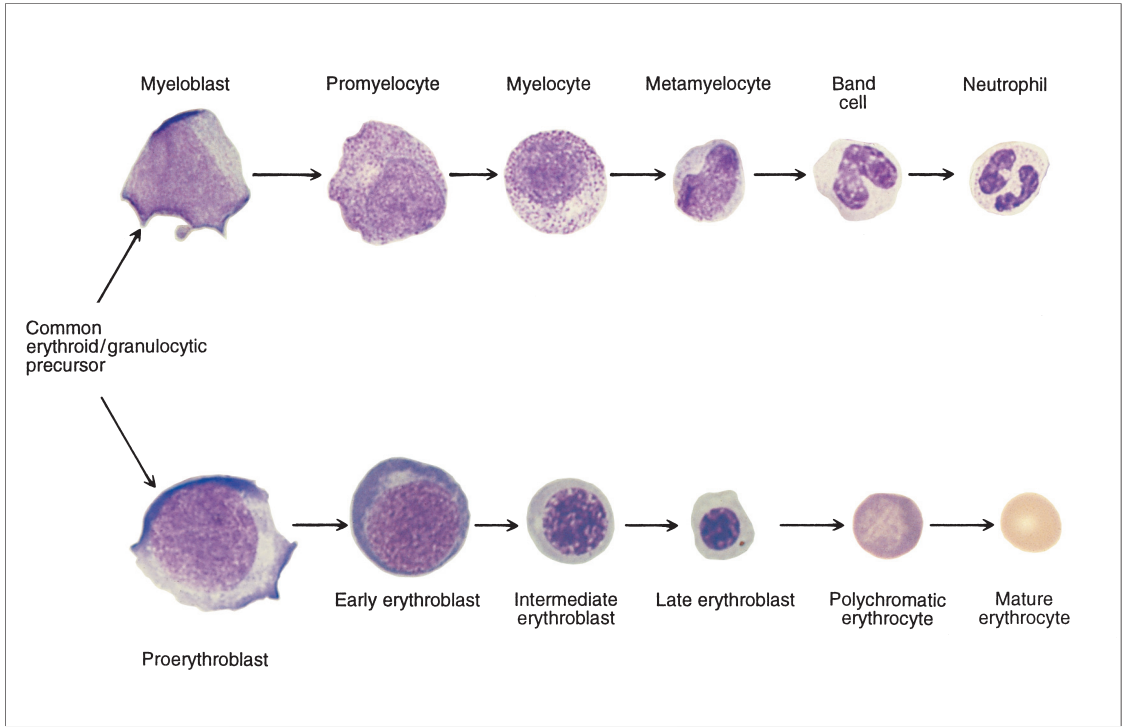

Fig. 1.12 A diagram showing the relationship of haemopoietic precursors to each other and to the end cells into which they differentiate. Proliferation of cells occurs simultaneously with maturation or differentiation so that one myeloblast is likely to give rise to 16 mature granulocytes and one proerythroblast to 16 red cells. Myeloblasts, promyelocytes and myelocytes are all cells capable of cell division or mitosis. Metamyelocytes and all later cells are non-dividing cells. All red cell precursors with the exception of late erythroblasts are dividing cells. Myeloblasts differentiate not only into neutrophils, as shown in the diagram, but also into eosinophils and basophils.

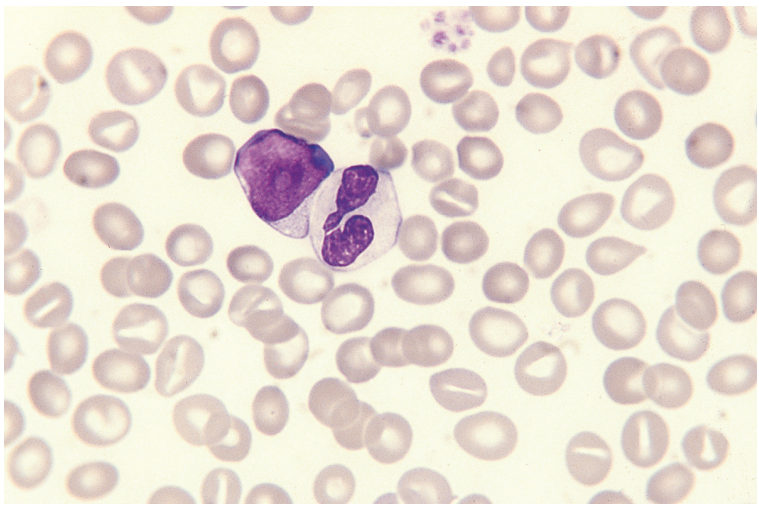

Fig. 1.13 A blood film of a patient showing a myeloblast and a neutrophil. The myeloblast has a high nucleocytoplasmic ratio, a diffuse chromatin pattern and a single nucleolus. The neutrophil is hypogranular.

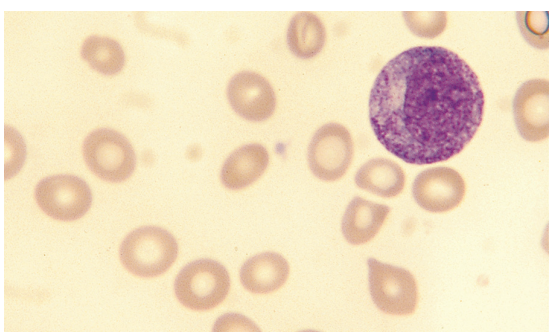

Fig. 1.14 A promyelocyte showing a lower nucleocytoplasmic ratio than that of a myeloblast, an eccentric nucleus, azurophilic granules and a Golgi zone to the left of the nucleus.

visible nucleolus. Because they have no specific (lineage-associated) granules, promyelocytes, which are precursors of neutrophils, eosinophils or basophils, cannot generally be distinguished from each other.

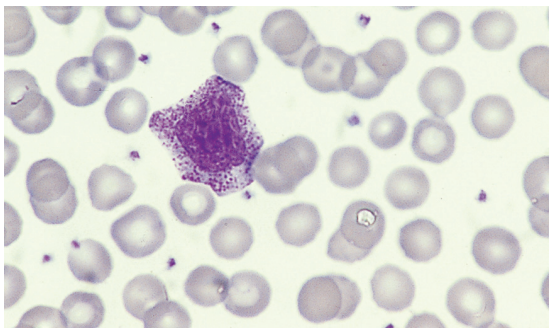

Fig. 1.15 A neutrophil myelocyte showing a smaller cell than a promyelocyte with some condensation of nuclear chromatin and no visible nucleolus. On microscopic examination it is apparent that such cells have primary and secondary granules with different staining characteristics.

Myelocytes

Myelocytes (Fig. 1.15) are uncommon in the blood of healthy subjects except in the neonatal period and during pregnancy. They are smaller than promyelocytes. They have not only azurophilic or primary granules but also secondary granules characteristic of specific lineages, i.e. neutrophilic, eosinophilic or basophilic granules. The myelocyte nucleus is round or oval and shows chromatin condensation; no nucleolus is apparent.

Metamyelocytes

Small numbers of neutrophil metamyelocytes (Fig. 1.16) are present in the blood of healthy subjects. Basophil and eosinophil metamyelocytes are not seen in the blood of healthy subjects. Metamyelocytes have similar characteristics to myelocytes but differ in that the nucleus is indented, U-shaped or C-shaped and the primary granules are usually no longer apparent.

Band cells

Neutrophil band forms (Fig. 1.17) are present as a minor population in the blood of healthy people. They are intermediate in characteristics between metamyelocytes and mature

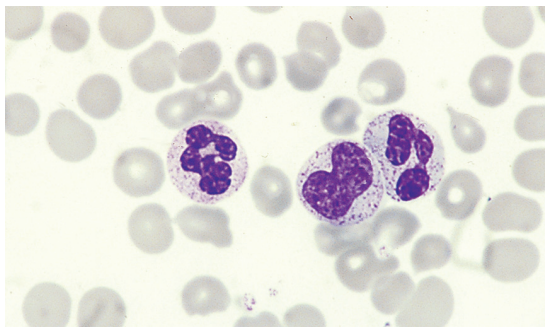

Fig. 1.16 A neutrophil metamyelocyte between two segmented neutrophils. The nucleus is indented.

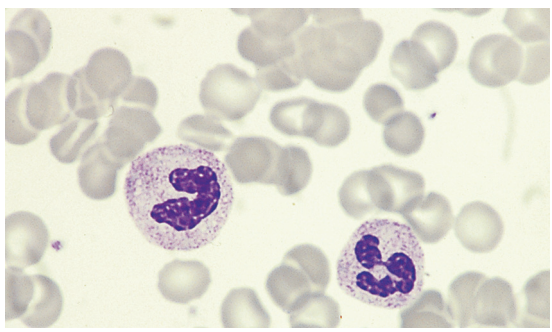

Fig. 1.17 A neutrophil band form (left) compared with a segmented neutrophil (right).

neutrophils. The nucleus has an irregular shape with some parallel edges so that it resembles a band or ribbon. It differs from a mature or segmented neutrophil in that the nucleus is not divided into distinct lobes or segments. Eosinophil and basophil band forms are quite uncommon.

Nucleated red blood cells

Nucleated red blood cells (NRBC) or erythroblasts (Fig. 1.18) are present in very small numbers in healthy people, except during the neonatal period. Those which are most likely to be released

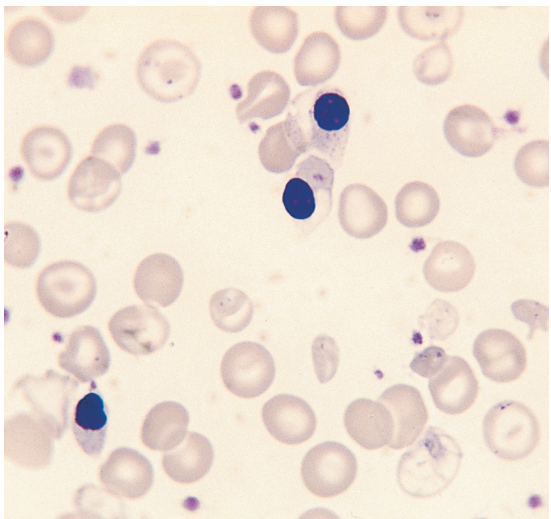

Fig. 1.18 Three nucleated red blood cells (NRBC) showing a small densely staining nucleus and cytoplasm which is pink because of the presence of haemoglobin.

into the blood stream are late erythroblasts. They can be readily recognized because the cytoplasm is at least partly haemoglobinized giving them a pinkish or lilac tinge. NRBC have a superficial resemblance to lymphocytes but can be distinguished from them not only by the colour of the cytoplasm but also by the lower nucleocytoplasmic ratio and the denser, more homogeneously staining nucleus.

The blood count

Haematology laboratories not only examine blood films. They also perform various measurements relating to the haemoglobin content of the blood, the characteristics of red cells and the number of red cells, white cells and platelets. These measurements are collectively referred to as a blood count or full blood count (FBC). During illness, abnormalities can develop in any of the cells in the blood. The purpose of performing a blood count and examining a blood film is to detect quantitative and qualita-

tive abnormalities in blood cells. Their detection often helps in diagnosis and in the treatment of the patient.

Haemoglobin concentration

If red cells are lysed, the haemoglobin is released from the red cells and forms a solution in the plasma. The haemoglobin concentration (Hb) can be measured biochemically by light absorption at a specified wave length after a chemical reaction which converts haemoglobin to cyanmethaemoglobin or to lauryl sulphate haemoglobin. Hb is measured in either grams per decilitre (g/dl) or grams per litre (g/l). A fall in the Hb is referred to as anaemia.

Haematocrit or packed cell volume

An alternative way of detecting anaemia is to centrifuge a tube containing an aliquot of blood and measure the proportion of the column of blood which is occupied by the red cells. Nowadays an equivalent measurement is made by various automated instruments using a quite different principle to get the same information. This test is called a packed cell volume (PCV) or a haematocrit (Hct). Some haematologists use these two terms interchangeably while others used PCV to refer to a measurement made after centrifugation and Hct for an estimate made by an automated instrument. This measurement is expressed as a decimal percentage, i.e. as litres/litre (e.g. 0.45).

Cell counts

Traditionally blood cells were counted by diluting a small quantity of blood in a diluent which could also stain the cells or, if white cells or platelets were to be counted, could lyse the more numerous red cells. The diluted blood was placed in a counting chamber of known volume and the number of cells present was counted microscopically. Such a method of counting blood cells is very labour-intensive and not suited to the large number of blood counts needed in modern medical practice. Nowadays blood cells are counted by large automated instruments.

A stream of cells in a diluent passes through a sensing zone. They are sensed either because they pass through an electric field or because they pass through a beam of light. Each cell passing through the sensing zone generates an electrical impulse, which can then be counted. Red cells are both relatively large and relatively numerous and so can be readily counted. White cells can be counted by lysing the more numerous red cells or by altering the red cells in some way so that they are 'invisible' to the instrument. Platelets are distinguished from other cells by their smaller size. Cell counts are expressed as the number of cells in a litre of blood. The red blood cell count (RBC) is expressed as a number $\times 10^{12}$ per litre (e.g. 5×10^{12}/l). The white blood cell count (WBC) and platelet count are expressed as a number $\times 10^{9}$ per litre (e.g. 7.5×10^{9}/l and 140×10^{9}/l). A white cell count of 7.5×10^{9}/l means that there are 7 500 000 000 cells in a litre of blood.

Red cell indices

Red cells can vary in their size and in the amount of haemoglobin contained in an individual cell. Abnormalities in both these cell characteristics are common in certain inherited abnormalities and when people are sick. Diagnostically useful information can be obtained by measuring them. Traditionally the size of red cells was estimated by dividing the PCV by the number of cells in the blood to give a mean cell volume (MCV). The haemoglobin content of individual cells was estimated by dividing the Hb by the RBC to give a mean cell haemoglobin (MCH). The Hb of individual cells was estimated by dividing the Hb by the PCV to give a mean cell haemoglobin concentration (MCHC). Nowadays, not only is the PCV estimated electronically but the size of a red cell can be calculated from the height of the electrical impulse which is generated when the cell passes through a light beam or through an electrical field. As the automated instruments also measure the total Hb of the blood, it is a simple matter for the red cell indices to be produced automatically as part of the blood count. Instruments can be designed to measure the MCV and calculate the PCV/Hct from the MCV and the RBC or, alternatively, to measure the PCV/Hct

and calculate the MCV from the PCV/Hct and the RBC. The formulae which relate the various red cell indices to each other are as follows:

$$\text{MCV} = \frac{\text{PCV}\ (\text{l/l}) \times 1000}{\text{RBC}\ (\text{cells/l}) \times 10^{-12}} \qquad (1)$$

e.g. if the PCV is 0.33 and the RBC 4.1×10^{12}/l, then

$$\text{MCV} = \frac{0.33 \times 1000}{4.1} = 80.5\,\text{fl}\ (\text{femtolitres})$$

(In understanding this formula and the following ones, it should be noted that if the RBC is 4.1×10^{12}/l then 4.1 is the RBC/l $\times 10^{-12}$.)

$$\text{MCH} = \frac{\text{Hb}\ (\text{g/dl}) \times 10}{\text{RBC}\ (\text{cells/l}) \times 10^{-12}} \qquad (2)$$

e.g. if the Hb is 12.3 g/dl and the RBC is 4.1×10^{12}/l then

$$\text{MCH} = \frac{12.3 \times 10}{4.1} = 30\ \text{pg}\ (\text{picograms})$$

$$\text{MCHC} = \frac{\text{Hb}\ (\text{g/dl})}{\text{PCV}\ (\text{l/l})} \qquad (3)$$

e.g. if the Hb is 12.3 g/dl and the PCV/Hct is 0.33 then

$$\text{MCHC} = \frac{12.3}{0.33} = 37.3\,\text{g/dl}$$

If an instrument measures the RBC rather than the PCV/Hct, then the formula is

$$\text{RBC/l} \times 10^{-12} = \frac{\text{PCV}\ (\text{l/l}) \times 1000}{\text{MCV}\ (\text{fl})} \qquad (4)$$

e.g. using the same figures as above

$$\text{RBC/l} \times 10^{-12} = \frac{0.33 \times 1000}{80.5} = 4.1$$

Normal ranges

In order to interpret blood counts it is necessary to know what is normal. This is usually done by reference to either a normal range or a reference range. A reference range is more strictly defined than a normal range but both represent the range of test results which would be expected in healthy people of the same age and sex (and, if relevant, of the same ethnic origin) as the person being investigated. Conventionally, both types of range are expressed as the central 95% of test results that would be expected in healthy people. The reason for excluding the top 2.5% and the bottom 2.5% is that there is usually an overlap between test results of healthy people and of those who are sick. A 95% range has been chosen to avoid either classifying too many healthy subjects as abnormal or missing relevant abnormalities in patients who are sick. It is clear that for any one test 5% of healthy subjects will have results falling outside the 'normal' range. Conversely, a patient who is sick may have a test result which is abnormal for him or her but which is still within the normal range. For example, a man may have a large gastrointestinal haemorrhage, causing his Hb to fall from its normal level of around 16 g/dl to 14 g/dl. The latter – 14 g/dl – is within the range expected for a healthy adult man but for this particular patient it is abnormal. This is because the range of test results expected in a group of healthy people is much wider than the range expected if the same test is repeated day after day in the same person. Usually we have no way of knowing what is 'normal' for a particular individual and so we have to resort to comparing his or her test results with a normal range.

The statistical distribution of test results differs for different tests. Many tests, e.g. the Hb, show a normal or Gaussian distribution. This means that if the distribution of the test results is plotted on graph paper a bell-shaped curve is obtained (Fig. 1.19a). If this is so, the 95% range can be calculated by estimating the mean ± 2 standard deviations. Other test results, e.g. the WBC (Fig. 1.19b), have a skewed distribution which only becomes bell-shaped if the test results are plotted on logarithmic graph paper. Test results with this type of distribution require special statistical treatment to derive the normal range.

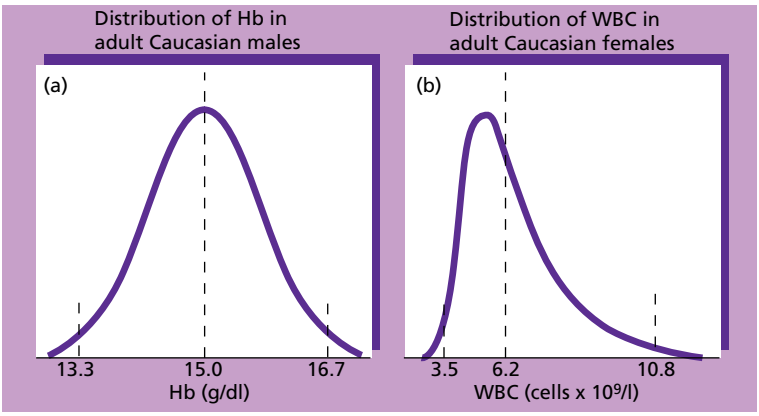

Fig. 1.19 Smoothed histograms showing (a) the normal distribution of Hb and (b) the log normal distribution of the white cell count.

Some normal ranges applicable to healthy people are shown in Tables 1.2–1.4. However, it should be noted that the test results for some haematological variables, e.g. the MCV, vary according to the method of measurement and it is desirable for laboratories to derive their own normal ranges for their own automated instruments by obtaining blood samples from a large number of healthy people. In the case of children, it is always difficult to obtain blood samples from large numbers of healthy individuals of various ages. As a consequence, published normal ranges for children are not as reliable as those for adults.

How to examine a blood film

Blood films should be examined in a systematic way. First the film should be examined without using the microscope, to make sure it is well spread (not too thick, too long or too short) and that its staining characteristics are normal. A film that is a deeper blue than other films stained in the same batch is usually indicative of an increase in the concentration of plasma proteins. This can be diagnostically important since it is often caused by multiple myeloma (a plasma cell malignancy) (see page 87) or by chronic inflammatory disease.

Table 1.2 Normal ranges for healthy Caucasian adults.

	Males	Females
WBC × 10^{-9}/l	3.7–9.5	3.9–11.1
RBC × 10^{-12}/l	4.32–5.66	3.88–4.99
Hb (g/dl)	13.3–16.7	11.8–14.8
PCV (Hct) (l/l)	0.39–0.5	0.36–0.44
MCV (fl)	82–98	
MCH (pg)	27.3–32.6	
MCHC (g/dl)	31.6–34.9	
RDW	9.5–15.5*	
	11.6–13.9†	
HDW	1.82–2.64†	
Neutrophils × 10^{-9}/l	1.7–6.1	1.7–7.5
Lymphocytes × 10^{-9}/l	1.0–3.2	
Monocytes × 10^{-9}/l	0.2–0.6	
Eosinophils × 10^{-9}/l	0.03–0.06	
Basophils × 10^{-9}/l	0.02–0.29	
Large unstained cells (LUC) × 10^{-9}/l	0.09–0.29	
Platelets × 10^{-9}/l	.143–332	169–358

RDW, red cell distribution width; HDW, haemoglobin distribution width. The differential white cell counts and the platelet counts are for Technicon H.1 series automated instruments. The ranges are wider for manual differential counts, particularly for monocytes, eosinophils and basophils. Platelet counts are very dependent on the method used for counting and should be assessed only in relation to a normal range derived for the instrument or method in use.

* Coulter S Plus IV.

† Technicon H.1 series.

Next the film is examined microscopically at low power (e.g. with a ×25 objective) so that a large part of the film can be scanned rapidly to detect any abnormal cells present in small numbers. Finally the film is examined at a higher power (e.g. with a ×40 or ×50 objective) so that the detailed structure of cells can be assessed. The great majority of films can be evaluated perfectly adequately without using high power (i.e. a ×100 oil immersion objective). High power can be reserved for making a detailed assessment of films that show significant abnormalities requiring further assessment. In examining a film be sure to look

Table 1.3 Normal ranges for Afro-Caribbean and Africans for those haematological variables where the ranges differ from those of Caucasians.

	Males	Females
West Indians		
WBC × 10^{-9}/l	2.8–9.5	3.3–9.8
Neutrophils × 10^{-9}/l	1.0–5.8	1.4–6.5
Platelets × 10^{-9}/l	122–313	149–374
Africans		
WBC × 10^{-9}/l	2.8–7.2	3.2–7.8
Neutrophils × 10^{-9}/l	0.9–4.2	1.3–4.2
Platelets × 10^{-9}/l	115–290	125–342

It should be noted that the lower RBC, Hb, PCV and MCV observed in Afro-Caribbean and Africans are likely to be consequent on a high prevalence of thalassaemia trait and haemoglobinopathies rather than on other ethnic differences. It is therefore appropriate to use Caucasian reference ranges for red cell variables for Afro-Caribbean and Africans.

Table 1.4 Approximate 95% ranges for red cell variables and for automated* total and differential white cell counts for Caucasian infants and children.

Age (years)	RBC (× 10^{-12}/l)	Hb (g/dl)	MCV (fl)
Birth	3.5–6.7	14–24	100–135
1	4.1–5.3	11–14	71–84
2–5	4.2–5.0	11–14	73–86
6–9	4.3–5.1	11–14	75–88
9–12	4.3–5.1	11.5–15.5	76–91

Age (years)	WBC	Neutrophil count	Lymphocyte count	Monocyte count	Eosinophil count
Birth	5–23	1.7–19	1–11	0.1–3.5	0.05–2
1	5.6–17.5	1.5–7	2.5–9	0.15–1.3	0.06–0.6
2–5	5–13	1.5–8.5	1.5–5.5†	0.15–1.3	0.08–1.2
6–9	4–10	1.5–6	1.5–4	0.15–1.3	0.08–1
9–12	4–10	1.5–6	1.5–4	0.15–1.3	0.04–0.8

* Ranges will be wider for manual differential counts than for automated counts.

† The lymphocyte count is up to 8 × 10^9/l in 2-year-olds, up to 5.5 × 10^9/l in 3- and 4-year-olds and up to 4.5 × 10^9/l in 5-year-olds.

specifically at red cells, white cells and platelets so that no abnormality is inadvertently overlooked. Be sure to look at the edges and tail of the film where abnormal cells may be found.

Finally, decide if a differential count is needed. Nowadays this will often have been performed by an automated instrument but you may need to verify its accuracy and in leukaemia you may need to carry out a manual differential count, i.e. one performed with the aid of a microscope.

Learning to look at blood films

When learning to recognize cells for the first time it is useful to compare cells seen down the microscope with photographs. Examining films on a double-headed microscope with an experienced laboratory worker is also very valuable. To learn to recognize high and low WBC and platelet counts, start by comparing the film appearance with the count on an automated instrument. After you have had some experience try to estimate what the count will be before you look at the test results. Later you will need to be able to do this fairly accurately so that you can recognize erroneous instrument counts. Similarly, start by looking at films with high and low MCVs and compare the size of the red cells with neutrophils and lymphocytes until you can recognize large and small red cells. When you have had some experience try to estimate the approximate MCV before you look at the test results. Eventually you will be able to judge the MCV, at least to within 5–10 fl.

Recognizing problems with the blood sample

Before carrying out a detailed assessment of a blood film it is important to detect any abnormal characteristics of the specimen which might interfere with your assessment of the film or with the accuracy of the automated count. The most common problem is storage artefact (Fig. 1.20). This occurs when blood has been at room temperature for a day or more before reaching the laboratory. The red cells turn into echinocytes, i.e. their shape alters so that the surface is covered with numerous short, regular projections. This process is also known as crenation.

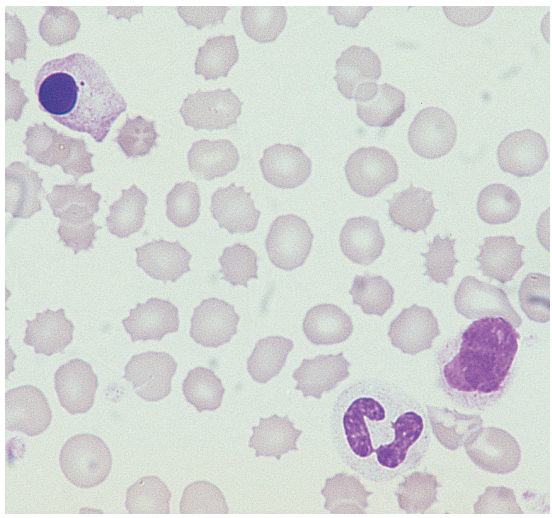

Fig. 1.20 Storage artefact. The red cells are crenated, a lymphocyte (right) has a fuzzy outline and one of the two neutrophils (left) has a nucleus which has become round, dense and homogeneous. (Compare the degenerating neutrophil with the nucleated red cells shown in Fig. 1.18.)

Some of the white cells develop fuzzy outlines or disintegrate entirely when the blood film is spread. The nuclei of neutrophils become dense, homogeneous and round and may break up into two or more round masses. It is important not to confuse these degenerating neutrophils with NRBC. They have a lower nucleo-cytoplasmic ratio and the cytoplasm is pink and slightly granular rather than reddish-brown. It is impossible to give any reliable opinion of films showing storage artefact. If the blood count is normal they can usually be ignored but if there is any reason to suspect a haematological abnormality a fresh blood sample must be obtained.

A common cause of inaccurate blood counts is partial clotting of the specimen or aggregation of the platelets. Platelets may aggregate because they have been activated (i.e. the process of blood clotting has started) or because there is an antibody present in the plasma which leads to platelet aggregation in blood that is anticoagulated with ethylenediaminetetra-acetic acid (EDTA). Aggregated platelets form masses between the red cells, that may

contain intact platelets (Fig. 1.21) or may be composed of totally degranulated platelets, which stain pale blue. Less often, partially clotted samples contain fibrin strands, which are seen as pale blue or almost non-staining linear structures running between and deforming red cells (Fig. 1.22). Another *in vitro* artefact, less common than platelet aggregation but which can also lead to falsely low platelet counts, is platelet satellitism (Fig. 1.23).

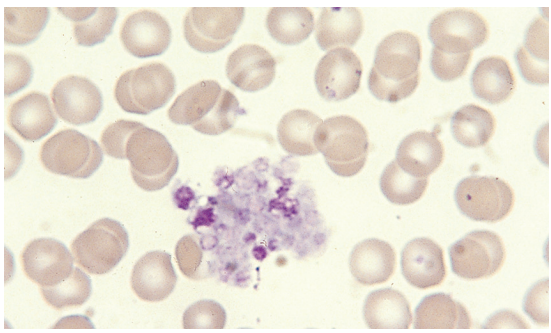

Fig. 1.21 A platelet aggregate containing a mixture of intact and degranulated platelets.

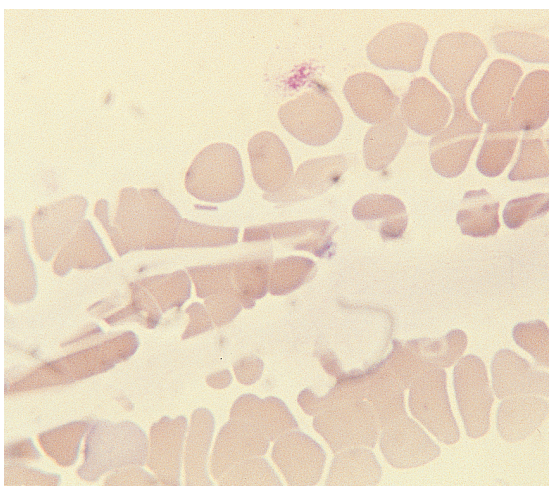

Fig. 1.22 Fibrin strands passing between and over red cells.

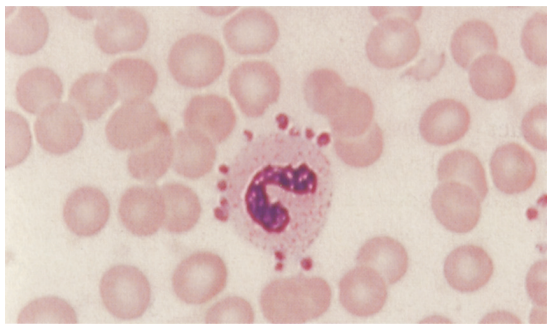

Fig. 1.23 Platelet satellitism.

Less common artefacts which should be recognized are those due to accidental freezing or overheating of the blood specimen before it reaches the laboratory and the presence of lipid (fat) in the plasma. All these abnormalities cause anomalous blood counts.

Interpreting blood films

When assessing blood films, always note the age, sex and ethnic origin of the patient and keep in mind what would be normal for that individual. Also consider the clinical details so that you can look carefully for any specific abnormalities which might be relevant, keeping in mind that the clinical details may provide you with an obvious explanation for an abnormality you have noted. For example, if the clinical details were 'alcohol excess' you would not be surprised to find that the patient had macrocytosis and you would go on to see if there were stomatocytes or any of the other abnormalities which could be caused by alcohol. Your report of these abnormalities would give the clinician very specific information which would help to confirm his/her clinical suspicion.