ONE

# THE NORMAL BONE MARROW: EXAMINING AND REPORTING BONE MARROW SPECIMENS

*'The normal structure of the bone marrow is less well understood than any other tissue in the body'*
Dorothy M. Reed, 1902

## The distribution of haemopoietic marrow

During extra-uterine life haemopoiesis is normally confined to the bone marrow, which occupies interstices within bone. An understanding of normal bone structure is necessary for interpreting bone marrow specimens. Bones are composed of cortex and medulla. The cortex is a strong layer of compact bone; the medulla is a honeycomb of cancellous bone, the interstices of which form the medullary cavity and contain the bone marrow. Bone marrow is either red marrow, containing haemopoietic cells, or yellow marrow, which is largely adipose tissue. The distribution of haemopoietic marrow is dependent on age. In the neonate virtually the entire bone marrow cavity is fully occupied by proliferating haemopoietic cells; haemopoiesis occurs even in the phalanges. As the child ages, haemopoietic marrow contracts centripetally, being replaced by fatty marrow. By early adult life haemopoietic marrow is largely confined to the skull, vertebrae, ribs, clavicles, sternum, pelvis and the proximal half of the humeri and femora; however, there is considerable variation between individuals as to the distribution of haemopoietic marrow [1]. In response to demand, the volume of the marrow cavity occupied by haemopoietic tissue expands.

## The organization of the bone marrow

### Bone

Bone may be classified in two ways. Classification may be made on the basis of the macroscopic appearance into: (i) compact or dense bone with only small interstices that are not visible macroscopically; and (ii) cancellous (or trabecular) bone with large, readily visible interstices. Bone may also be classified histologically on the basis of whether there are well-organized osteons in which a central Haversian canal is surrounded by concentric lamellae composed of parallel bundles of fibrils (lamellar bone) (Fig. 1.1) or, alternatively, whether the fibrils of the bone are in disorderly bundles (woven or spongy bone) (Fig. 1.2).

The cortex and the medulla differ functionally as well as histologically. The cortex is a solid layer of compact bone that gives the bone its strength. It is composed largely of lamellar bone but also contains some woven bone. The lamellar bone of the cortex consists of either well-organized Haversian systems or angular fragments of lamellar bone, which occupy the spaces between the Haversian systems; in long bones there are also inner and outer circumferential lamellae. Extending inwards from the cortex is an anastomosing network of trabeculae, which partition the medullary space (Fig. 1.3). The medullary bone is trabecular or cancellous bone; it contains lamellae but the structure is less highly organized than that of the cortex. Most of the



*Bone Marrow Pathology*, Sixth Edition. Barbara J. Bain, David M. Clark, Bridget S. Wilkins, and Vishakha Sovani.

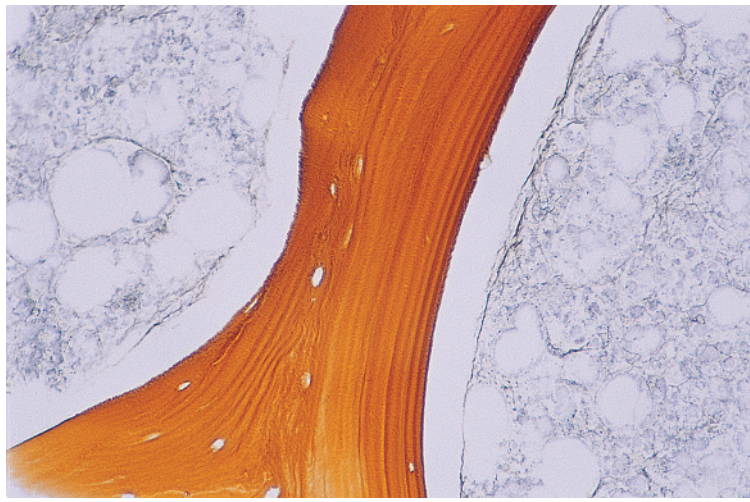


**Fig. 1.1** Bone marrow (BM) trephine biopsy section showing normal bone structure; trabeculae are composed of lamellar bone. Reticulin stain ×20 objective.

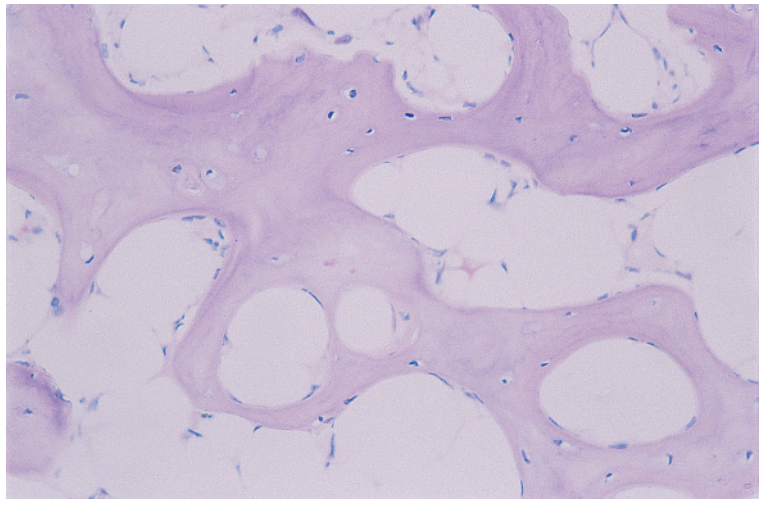


**Fig. 1.2** BM trephine biopsy section showing woven bone (pale pink; without lamellae) in a hypocellular but otherwise unremarkable bone marrow. Haematoxylin and eosin (H&E) ×20.

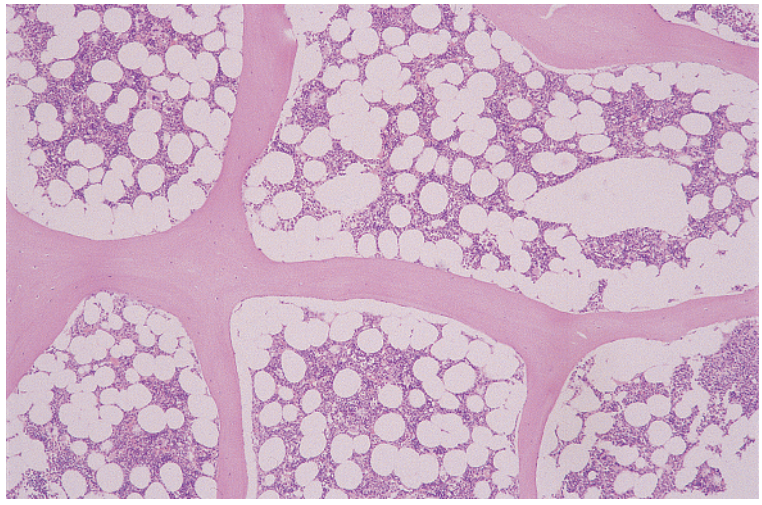


**Fig. 1.3** BM trephine biopsy section showing normal bone structure; there are anastomosing bony trabeculae. H&E ×5.

cortical bone is covered on the external surface by periosteum, which has an outer fibrous layer and an inner osteogenic layer. At articular surfaces, and more extensively in younger patients, cortical bone fuses with cartilage rather than being covered by periosteum. The bony trabeculae and the inner surface of the cortex are lined by endosteal cells; most of these are flattened endosteal cells that can be histologically inapparent but there are some actively osteogenic cells (osteoblasts) and occasional osteoclasts, both more numerous in children. Osteocytes are found within lacunae in bony trabeculae and in cortical bone. Although osteoblasts and osteoclasts share the surface of the bone trabeculae, they originate from different stem cells. Osteoblasts, and therefore osteocytes, are of mesenchymal origin, being derived from the same stem cell as chondrocytes and probably also stromal fibroblasts. Osteoclasts, however, are derived from a haemopoietic stem cell, being formed by fusion of cells of the monocyte lineage.

The cells that give rise to bone-forming cells are designated osteoprogenitor cells; they are flattened, spindle-shaped cells that are capable of developing into either osteoblasts or chondrocytes, depending on micro-environmental factors. Osteoblasts synthesize glycosaminoglycans of the bone matrix and also the collagenous fibres that are embedded in the matrix, thus forming osteoid or non-calcified bone; subsequently mineralization occurs. Bone undergoes constant remodelling. In adult life, remodelling of the bone takes place particularly in the subcortical regions. Osteoblasts add a new layer of bone to trabeculae (apposition) while osteoclasts resorb other areas of the bone; up to 25% of the trabecular surface may be covered by osteoid. The osteoclasts, which are resorbing bone, lie in shallow hollows, known as Howship lacunae, created by the process of resorption, while osteoblasts are seen in rows on the surface of trabecular bone or on the surface of a layer of osteoid. As new bone is laid down, osteoblasts become enclosed in bone and are converted into osteocytes. The bone that replaces osteoid is woven bone; this, in turn, is remodelled to form lamellar bone. Osteocytes within the lacunae of woven bone have more prominent, round nuclei than those in lamellar bone, which often appear flattened or may be inapparent. The difference between woven and lamellar bone can be easily appreciated by microscopy using polarized light. The organized structure of lamellar bone, with bundles of parallel fibrils running in different directions in successive lamellae, gives rise to alternating light and dark layers when viewed under polarized light. This structure is also easily seen in Giemsa- and reticulin-stained sections.

Trephine biopsy specimens from children may contain cartilage as well as bone, and endochondrial bone formation may be observed (Figs 1.4 and 1.5). Transition from resting cartilage to proliferating and hypertrophic cartilage can be observed, followed by a zone of calcifying cartilage, invading vessels and bone. Mature cartilage can also be seen in trephine biopsy specimens from adults (Fig. 1.6).

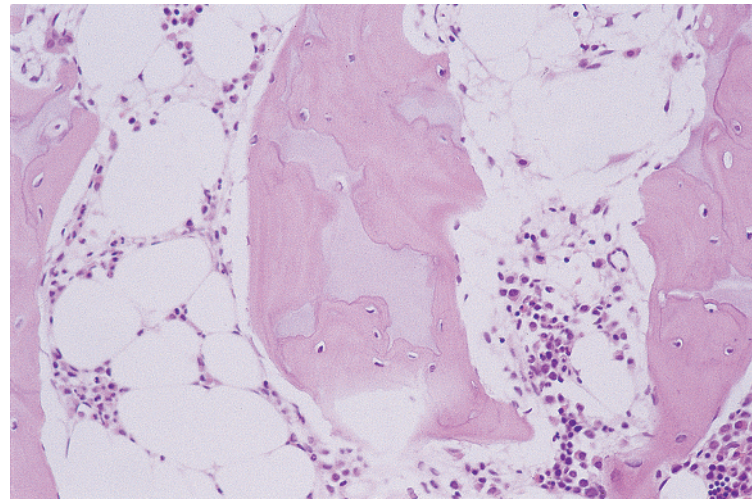


**Fig. 1.4** BM trephine biopsy section from a child showing endochondrial ossification in an island of cartilage. H&E ×20.

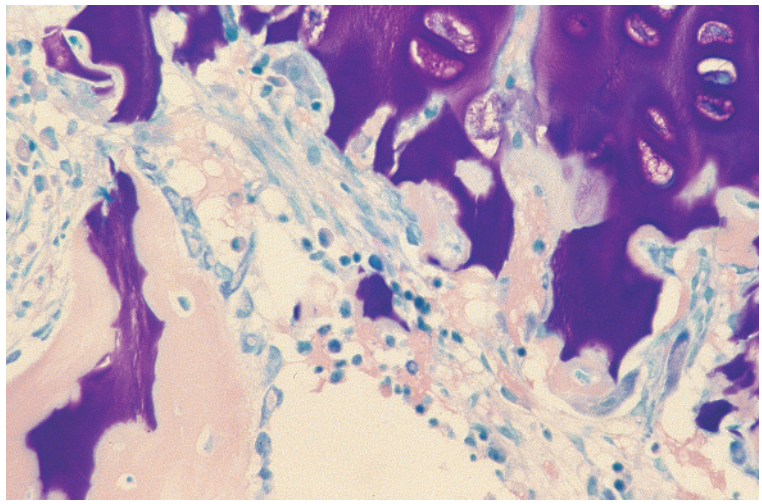


**Fig. 1.5** BM trephine biopsy section from a child showing endochondrial ossification; a bony spicule with a core of cartilage is lined by osteoblasts. Giemsa stain ×40.

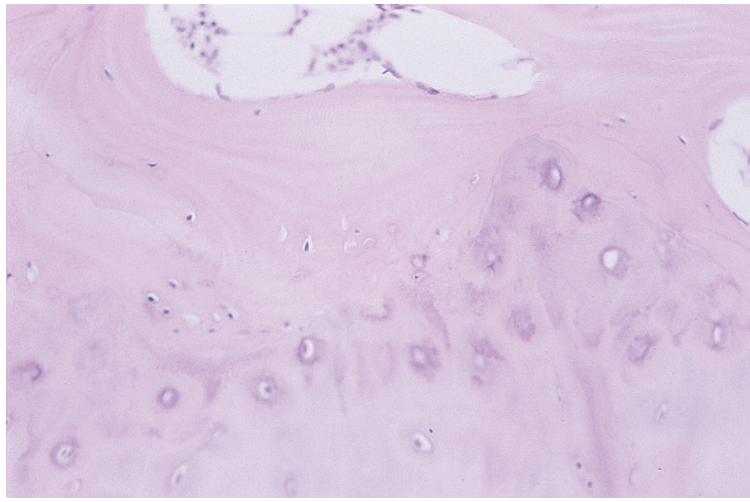


**Fig. 1.6** BM trephine biopsy section from an adult showing cartilage adjacent to the cortex. By contrast with childhood appearances, a well-defined layer of cortical bone separates this cartilage from the bone marrow. Cartilage cells are dispersed singly or in small groups and are not aligned into columns, as they are in childhood. H&E ×20.

## Other connective tissue elements

Haemopoietic cells of the bone marrow are embedded in a connective tissue stroma, which occupies the intertrabecular spaces of the medulla. This stroma is composed of fat cells and a meshwork of blood vessels, branching fibroblasts, macrophages, a few myelinated and non-myelinated nerve fibres and a small amount of reticulin. Stromal cells include cells that have been designated reticulum or reticular cells. This term probably includes two cell types of different origin. Phagocytic reticulum cells are macrophages and originate from a haemopoietic progenitor. Non-phagocytic reticulum or reticular cells are closely related to fibroblasts, adventitial cells of sinusoids (see later in this chapter) and probably also osteoblasts and chondrocytes. They differ from phagocytic reticulum cells in that the majority are positive for alkaline phosphatase. There is a close interaction between haemopoietic cells and their micro-environment, with each modifying the other.

The blood supply of the marrow is derived in part from a central nutrient artery, which enters long bones at mid-shaft and bifurcates into two longitudinal central arteries [2]. Similar arteries penetrate flat and cuboidal bones. There is a supplementary blood supply from cortical capillaries, which penetrate the bone from the periosteum. Branches of the central artery give rise to arterioles and capillaries, which radiate towards the endosteum

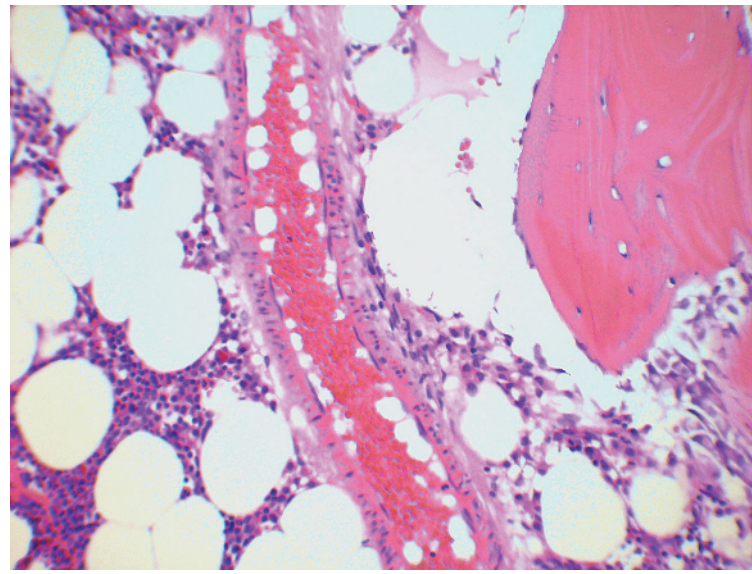


**Fig. 1.7** BM trephine biopsy section showing a longitudinal section of an arteriole. H&E ×20.

and mainly enter the bone, subsequently turning back to re-enter the marrow and open into a network of thin-walled sinusoids [2]. Only a minority of capillaries enter the sinusoids directly without first supplying bone. The sinusoids drain into a central venous sinusoid, which accompanies the nutrient artery. Sinusoids are large, thin-walled vessels through which newly formed haemopoietic cells enter the circulation. They are often collapsed in paraffin-embedded histological sections and are therefore not readily seen. In the presence of marrow sclerosis, these vessels are often held open and are then very obvious. The walls of sinusoids consist of endothelial cells, forming a complete cover with overlapping junctions, and an incomplete basement membrane. The outer surface is clothed by adventitial cells – large, broad cells that branch into the perivascular space and therefore provide scaffolding for the haemopoietic cells, macrophages and mast cells. Adventitial cells are thought to be derived from fibroblasts; they are associated with a network of delicate extracellular fibres, which can be demonstrated with a reticulin stain. Reticulin fibres are concentrated close to the periosteum as well as around blood vessels. It is likely that both adventitial cells and fibroblasts can synthesize reticulin [3], which is a form of collagen. Arterioles are easily recognized both in longitudinal section (Fig. 1.7) and in cross-section. Capillaries may also be visible. Collapsed sinusoids and capillaries are better visualized with the use of an immunohistochemical stain for an endothelial cell-associated antigen, such as CD31 or CD34*.

The marrow fat content varies inversely with the quantity of haemopoietic tissue. Fat content also increases as bone is lost with increasing age. Marrow fat is physiologically different from subcutaneous fat. The fat of yellow marrow is the last fat in the body to be lost in starvation. When haemopoietic tissue is lost very rapidly it is replaced by interstitial mucin (gelatinous transformation). Subsequently this mucin is replaced by fat cells. Rarely brown fat, distinguished by multivacuolated cells, is observed in the marrow [4].

## Haemopoietic and other cells

Haemopoietic cells lie in cords or wedges between the sinusoids. Normal haemopoiesis, with the exception of some thrombopoiesis at extramedullary sites, is confined to the interstitium. In pathological conditions haemopoiesis can occur within sinusoids. Mature haemopoietic cells enter the circulation by passing transcellularly, through sinusoidal endothelial cells [2]. The detailed disposition of haemopoietic cells will be discussed later.

* CD stands for cluster of differentiation.

Bone marrow also contains lymphoid cells, small numbers of plasma cells and mast cells (see later).

## Examination of the bone marrow

Bone marrow was first obtained from living patients for diagnostic purposes (for the diagnosis of leishmaniasis) during the first decade of the twentieth century; this was reported from Italy by M. Pianese and Giovanni Ghedini and from Germany by P. Wolff, following puncture of the femur and tibia respectively [5]. Ghedini reported aspiration and biopsy of the tibia for broader diagnostic purposes, also in this decade [6]. It was not until the introduction of sternal aspiration by Mikhael Arinkin in the late 1920s that this became an important diagnostic procedure; these initial sternal aspirates were obtained using a lumbar puncture needle. In the 1940s pelvic bones were used [6]. Specimens of bone marrow for cytological and histological examination may be obtained by aspiration biopsy, by core biopsy using a trephine needle or an electric drill, by open biopsy and at autopsy. The two most important techniques, which are complementary, are aspiration biopsy and trephine biopsy. A battery-powered device has been reported to give superior core biopsy specimens with less pain than a manual trephine biopsy [7,8]. In another study the quality of specimens was equivalent but pain was less [9]. In a very obese patient, it may be necessary to carry out the procedure under computed tomography control [10].

Bone marrow aspiration causes only mild discomfort to the patient. A trephine biopsy causes moderate discomfort and, in an apprehensive patient, sedation can be useful. Intravenous midazolam, 2–10 mg, is a commonly employed agent. Guidelines for safe sedation practice must be followed [11]. Local anaesthesia supplemented by inhaled nitrous oxide anaesthesia is also an option [12]. In children, aspiration and trephine biopsies are usually performed under general anaesthesia.

All bone marrow aspirates and needle biopsies require informed consent. Local policies should be followed as to whether written consent is required, but this is becoming more customary.

When flow cytometric immunophenotyping and molecular/cytogenetic analysis are available, it is prudent to take a suitable sample from all patients and retain it until the aspirate has been examined rapidly. Assessment of whether further analysis is needed is thus possible and the most appropriate investigations can be carried out.

## Bone marrow aspiration

Aspiration biopsy is most commonly carried out from the ilium, particularly from the posterior iliac crest. There is a greater risk of an adverse event with sternal aspiration. Aspiration from the medial surface of the tibia can yield useful diagnostic specimens up to the age of 18 months but is mainly used in neonates in whom other sites are less suitable. Aspiration from ribs and from the spinous processes of vertebrae is also possible but is now little practised. Sternal aspiration should be carried out from the first part of the body of the sternum, at the level of the second intercostal space. Aspiration from any lower in the sternum increases the risks of the procedure. Aspiration from the ilium can be from either the anterior or the posterior iliac crest. Aspiration from the anterior iliac crest is best carried out by a lateral approach, a few centimetres below and posterior to the anterior superior iliac spine. Approach through the crest of the ilium with the needle in the direction of the main axis of the bone is also possible but is more difficult because of the hardness of the bone. Aspirates from the posterior iliac crest are usually taken from the posterior superior iliac spine. When aspiration is carried out at the same time as a trephine biopsy it is easiest to perform the two procedures from adjacent sites, but being careful that the two needle tracks do not overlap. This necessitates the use of the ilium. If a trephine biopsy is not being carried out there is a choice between the sternum and the iliac crest. Either is suitable in adults and older children, although very great care must be exercised in carrying out sternal aspirations. In a study of 100 patients in whom both techniques were applied, sternal aspiration was found to be technically easier and to produce a suitable diagnostic specimen more frequently, although on average the procedure

was more painful, both with regard to bone penetration and to the actual aspiration [13]. Sternal aspiration is more dangerous at any age and is unsuitable for use in young children. Posterior iliac crest aspiration is suitable for children, infants and many neonates. Tibial aspiration is suitable for very small babies but has no advantages over iliac crest aspiration in older infants. The actual aspiration of bone marrow should be rapid; although this is somewhat more painful, it yields a more cellular and particulate sample [14].

Bone marrow specimens yielded by aspiration are suitable for the following: preparation of wedge-spread films and films of crushed marrow fragments; flow cytometric immunophenotyping; cytogenetic analysis; ultrastructural examination; culture for microorganisms; culture to study haemopoietic precursors; and the preparation of histological sections of fragments. The International Council for Standardization in Haematology (ICSH) recommends that both wedge-spread films and squash preparations be made [15]. Following drying and methanol fixation, such preparations are stained with a Romanowsky stain, either a May–Grünwald–Giemsa (MGG) or a Wright–Giemsa stain. Cytogenetic analysis is most often indicated in suspected haematological neoplasms but it also permits rapid diagnosis of suspected congenital karyotypic abnormalities such as trisomy 18; diagnosis is possible within a day, in comparison with the 3 days needed if peripheral blood lymphocytes are used.

Bone marrow aspiration may fail completely, this being referred to as a 'dry tap'. Although this can happen when bone marrow histology is normal, a dry tap usually indicates significant disease, most often metastatic cancer, chronic myeloid leukaemia, primary myelofibrosis or hairy cell leukaemia [16], with associated fibrosis. On other occasions only blood is obtained (a 'blood tap'); this is often also the result of bone marrow disease causing fibrosis. If aspirates are obtained by someone other than a haematologist, it is important that feedback is given as to the adequacy of sample and film.

## Trephine biopsy of bone marrow

Trephine biopsy is most easily carried out on the iliac crest, either posteriorly or anteriorly, as described earlier. The posterior approach (Fig. 1.8) is now more generally preferred. It both gives longer specimens with a larger area for examination and is less painful for the patient [17]. Disposable needles are now almost always used, for example a Jamshidi or an Islam needle, the latter being designed to ensure retention of the core when the needle is withdrawn from the body. The Ranfac Snarecoil needle also has a capturing device [18]. There are also powered devices, one of which (OnControl, Vidacare Corporation) was found in a meta-analysis of five randomised controlled studies

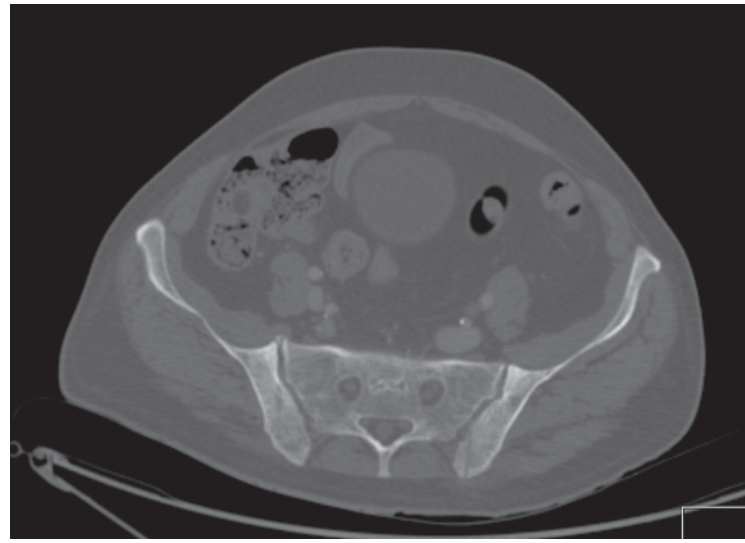


**Fig. 1.8** Computed tomography (CT) scan of the pelvis showing a trephine biopsy needle track through the posterior iliac crest. (With thanks to Dr Marc Heller, London.)

to produce a longer biopsy specimen with the procedure also being less painful [19]. However in a subsequent study, although the specimen was longer, the length of evaluable marrow was greater with a manual technique [20]. Currently available needles have been reviewed [21].

If a trephine biopsy and a bone marrow aspiration are both to be carried out, they can be performed through the same skin incision but with two areas of periosteum being infiltrated with local anaesthetic and with the needles being angled in different directions. A single-needle technique in which aspiration is followed by core biopsy should not be used as the quality of the core biopsy may be inadequate [22]. Most operators remove the trocar from the needle as soon as the needle has engaged with cortical bone so that the specimen includes cortical bone. An alternative technique is to remove the trocar only when the cortex has been penetrated so that cortical bone is not included in the specimen; this technique has been advised since the cortex is not generally informative and the modified technique lessens blunting of the needle [23]. In obese patients, ultrasound can be used to localize the posterior iliac crest [23]. Core biopsy specimens, obtained with a trephine needle, are suitable for histological sections, touch preparations (imprints) and electron microscopy. A touch preparation is particularly important when it is not possible to obtain an aspirate since it allows cytological details to be studied [24] and may provide a diagnosis some days in advance of the availability of histological sections. In addition, touch preparations may show more neoplastic cells than are detected in an aspirate; they may also demonstrate bone marrow infiltration when it is not detected in an aspirate, for example in hairy cell leukaemia, multiple myeloma or lymphoma [25]. Touch preparations may be made either by touching the core of bone on a slide or rolling the core gently between two slides. Biopsy specimens can be used for cytogenetic study but aspirates are much more suitable. Histological sections may be prepared from fixed biopsy specimens that have either been decalcified and paraffin-embedded or have been embedded in resin without prior decalcification. As for bone marrow aspirates, feedback to the person performing the biopsy is important; common problems include sampling entirely or largely of cortical bone and a subcortical biopsy specimen (taken parallel to the surface rather than at a right angle and composed largely of hypocellular marrow).

### *Processing of trephine biopsy specimens*

The two principal methods of preparation of fixed trephine biopsy specimens have advantages and disadvantages. Problems are created because of the difficulty of cutting tissue composed of hard bone and soft, easily torn bone marrow. Alternative approaches are to decalcify the specimen or to embed it in a substance that makes the bone marrow almost as hard as the bone. Decalcification can be achieved with weak organic acids, for example formic acid and acetic acid, or by chelation, for example with ethylene diamine tetra-acetic acid (EDTA). Decalcification and paraffin-embedding lead to considerable shrinkage and some loss of cellular detail. Because sections are thicker than those from resin-embedded specimens, cellular detail is harder to appreciate. Some cytochemical activity is lost; for example, chloroacetate esterase activity is lost when acid decalcification is used. Immunological techniques are more readily applicable to paraffin-embedded than to resin-embedded specimens. Resin-embedding techniques are more expensive and, for laboratories that are processing only small numbers of trephine biopsy specimens, are technically more difficult. There is minimal shrinkage, preservation of cellular detail is excellent and the thinness of the sections means that fine cytological detail can be readily appreciated. Some enzyme activities, for example chloroacetate esterase, are retained. Immunological techniques can be applied, but excessive background staining is often a problem, the repertoire of possible immunohistochemical stains is much less and specimens are also unsuitable for molecular techniques. Although excellent morphology is achieved with resin-embedded specimens it is now also possible to get very good results for both histology and immunohistochemistry with paraffin-embedding and this is the technique used in the great majority of laboratories. Methods that we have found satisfactory are given in the Appendix.

## Relative advantages of aspiration and core biopsy

Bone marrow aspiration and trephine biopsy each have advantages and limitations. The two procedures should therefore be regarded as complementary. Bone marrow aspirates are unequalled for demonstration of fine cytological detail. They permit a wider range of cytochemical stains and immunological markers than is possible with histological sections and are also ideal for cytogenetic and molecular genetic studies. Aspiration is particularly useful, and may well be performed alone, when investigating patients with suspected iron deficiency anaemia, anaemia of chronic disease, megaloblastic anaemia and acute leukaemia. Trephine biopsy is essential for diagnosis when a 'dry tap' or 'blood tap' occurs as a consequence of the marrow being fibrotic or very densely cellular. Only a biopsy allows a complete assessment of marrow architecture and of the pattern of distribution of any abnormal infiltrate. This technique is particularly useful in investigating suspected aplastic or hypoplastic anaemia, lymphoma, metastatic carcinoma, myeloproliferative neoplasms and diseases of the bones. It has also been found to be more often useful in investigating a fever of unknown origin [26]. We have also found trephine biopsy generally much more useful than bone marrow aspiration when investigating patients with the advanced stages of human immunodeficiency virus (HIV) infection in whom hypocellular, non-diagnostic aspirates are common. It should not be forgotten, however, that trephine biopsy undoubtedly causes more pain to the patient than does aspiration.

Complications of bone marrow aspiration and trephine biopsy are rare. Sternal aspiration is more hazardous than iliac crest aspiration and trephine biopsy. Although deaths are very rare, at least 21 have been reported and we are aware of four further fatalities, not reported in the scientific literature; deaths have been consequent mainly on laceration of vessels or laceration of the heart with pericardial tamponade. The risk may be greater when bones are abnormally soft, as in multiple myeloma [27]. Sternal aspiration may also be complicated by pneumothorax or pneumopericardium, and sternomanubrial separation has been observed in one patient.

Although haemorrhage is rare following iliac crest aspiration and uncommon following trephine biopsy it is, nevertheless, the most frequently observed serious complication, sometimes requiring blood transfusion and occasionally leading to, or contributing to, death [28,29]. Haemorrhage may be either intra-abdominal [30], retroperitoneal [28] (rarely with secondary haemothorax) [31] or into the buttock and thigh [28], in the latter two circumstances with the risk of nerve compression [28,32,33]. Pseudoaneurysm formation [34,35] and creation of an arteriovenous fistula with associated haemorrhage [36] have been reported and can require intervention; selective embolization can be useful to control bleeding in such cases. Risk factors are heparin or warfarin therapy, coagulation factor deficiencies, von Willebrand disease, disseminated intravascular coagulation, thrombocytopenia, functional platelet defects (either disease related – myeloid neoplasms or resulting from the presence of a paraprotein – or the result of aspirin or other anti-platelet agents) and a diagnosis of a myeloproliferative neoplasm. Haemorrhage is also occasionally a problem when a biopsy is carried out on bone with an abnormal vasculature, for example in Paget disease. Severe retroperitoneal haemorrhage has also been observed in patients with osteoporosis. Correction of any coagulation defect is advisable, when possible. Prolonged firm pressure is advised in patients with thrombocytopenia or functional platelet defects and, when clinically appropriate, pre-procedure platelet transfusion should be considered.

Damage to the lateral cutaneous nerve of the thigh occurs rarely and is suggestive of poor technique. In patients with osteosclerosis, needles may break. Infection leading to osteomyelitis, abscess formation and sometimes septicaemia is a rare complication [37–39]. Other rare complications include avulsion fracture at the biopsy site [40], pneumoretroperitoneum [41], implantation of malignant cells in the track of the biopsy needle in plasmacytoma and non-Hodgkin lymphoma [42–44], prolonged leak of serous fluid in a patient with nephrotic syndrome [45], bone marrow embolism [46], cerebrospinal fluid leak [47] and later development of exostosis [48]. A computed tomography study of 25 patients demonstrated that the sacroiliac joint was penetrated in three patients without any adverse consequences [49].

## Other techniques

It is occasionally necessary to obtain a bone marrow specimen by open biopsy under a general anaesthetic. This is usually only required when a specific lesion has been demonstrated at a relatively inaccessible site by radiology, magnetic resonance imaging or bone scanning.

At autopsy, specimens of bone marrow for histological examination are most readily obtained from the sternum and the vertebral bodies, although any bone containing red marrow can be used. Unless the autopsy is performed soon after death, the cytological detail is usually poor.

## Cellularity

Bone marrow cellularity can be assessed most accurately in histological sections (Fig. 1.9) although assessment can also be made from aspirated bone marrow fragments in wedge-spread films (Fig. 1.10) or from squash preparations. Squash preparations generally appear more cellular and show more megakaryocytes than wedge-spread films [50]. Specimens that are suitable for histological assessment of cellularity are: aspirated fragments; trephine or open biopsy specimens; and autopsy specimens. The cellularity of the bone marrow in health depends on the age of the subject and the site from which the marrow specimen was obtained. It is also influenced by technical factors, since decalcification and paraffin-embedding lead to some shrinkage of tissue in comparison with resin-embedded specimens; estimates of cellularity based on the former are approximately 5% lower than estimates based on the latter [51].

The cellularity of histological sections can be assessed most accurately by computerized image analysis or, alternatively, by point-counting using an eyepiece with a graticule; the process is known as histomorphometry. Results of the two procedures show a fairly close correlation [51,52]. Cellularity can also be assessed subjectively. Such estimates are less reproducible and may lead to some underestimation of cellularity but show a reasonable correlation with histomorphometric methods; in one study the mean cellularity was 78% by histomorphometry (point-counting) and 65% by visual estimation, with the correlation between the two methods being 0.78 [51]. Bone marrow cellularity is expressed as the percentage of a section that is occupied by haemopoietic tissue. However, the denominator may vary. The cellularity of sections of fragments is expressed in terms of haemopoietic tissue as a percentage of the total of haemopoietic and adipose tissue. In the case of a trephine biopsy, however, the cellularity may be expressed either as a percentage of the entire biopsy (including bone) [53] or as a percentage of the marrow cavity [51,54]. There are clear advantages in the latter approach, in which the area occupied by bone is excluded from the calculation, since the percentages obtained

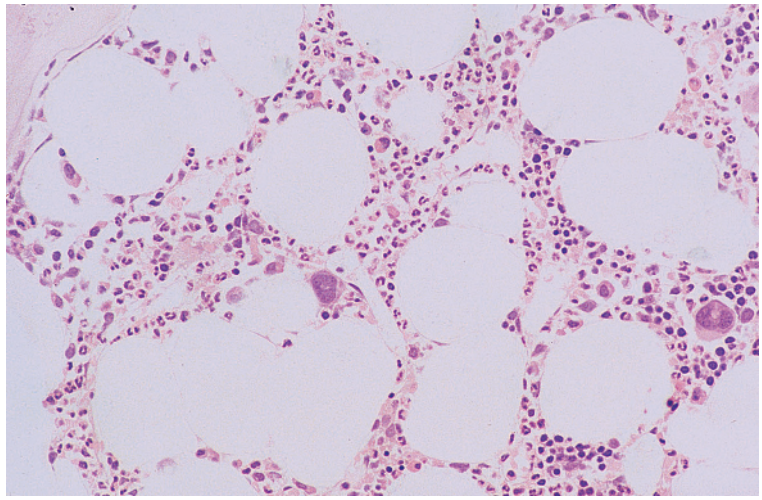


**Fig. 1.9** Section of normal BM: normal distribution of all three haemopoietic lineages; note the megakaryocyte adjacent to a sinusoid. Resin-embedded, H&E ×20.

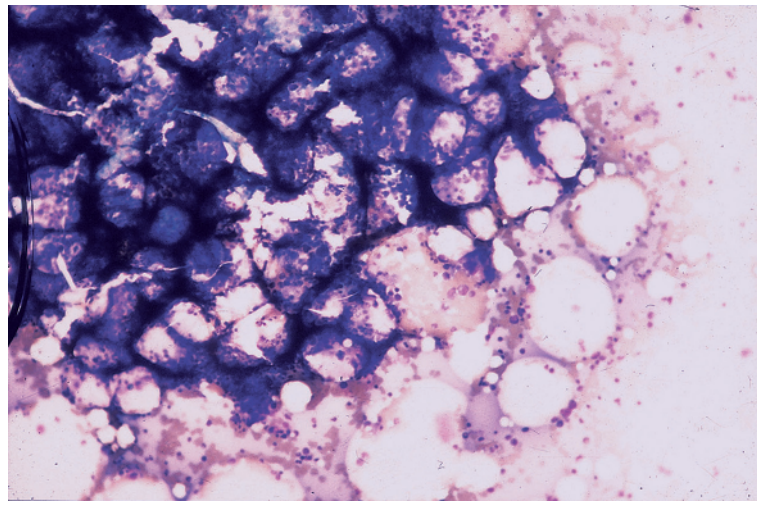


**Fig. 1.10** Aspirate of normal BM: fragment showing normal cellularity. May–Grünwald–Giemsa (MGG) ×40.

are then directly comparable with measurements made on histological sections of aspirated fragments or estimates made from fragments in bone marrow films. This is the usual method for assessing cellularity in trephine biopsy sections and is the approach used by all of the authors.

The bone marrow of neonates is extremely cellular, negligible fat cells being present. Cellularity decreases fairly steadily with age, with an accelerated rate of decline above the age of 70 years [53–57] (Figs 1.11 and 1.12). The decreasing percentage of the marrow cavity occupied by haemopoietic tissue is a consequence both of a true decline in the amount of haemopoietic tissue and of a loss of bone substance with age requiring adipose tissue to expand to fill the larger marrow cavity. In subjects with osteoporosis this effect can be so great that even young persons who are haematologically normal may have as little as 20% of their marrow cavity occupied by haemopoietic cells [55]. Average cellularity in the bone marrow of children, assessed on trephine biopsy or clot sections, is 80% at 2 years, 69% at 2–4 years, 59% at 5–9 years and around 60% thereafter [58]. In haematologically normal subjects without bone disease, typical reported rates of decline in average marrow cellularity (expressed as a percentage of haemopoietic cells plus adipose cells) are: from 64% in the second decade to 29% in the eighth decade in the iliac crest [54]; from 85% at age 20 years to 40% at age 60, also in the iliac crest [55]; and from 66% at age 20 to 30% at age 80 in the sternum [57].

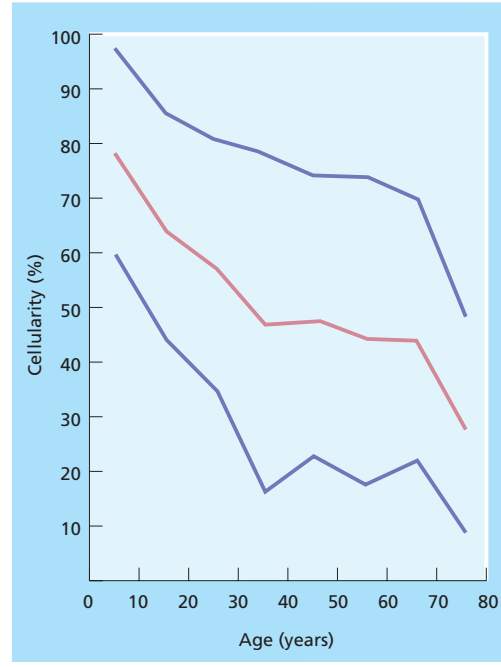



**Fig. 1.11** Mean and 95% range of cellularity at various ages of anterior iliac crest bone marrow which has been decalcified and paraffin-embedded. Cellularity is expressed as a percentage of the bone marrow cavity. (Calculated from Hartsock *et al.* [54]/ National Library of Medicine/Public domain.)

Bone marrow cellularity also depends on the site of biopsy. Study of the two tissues by the same techniques has shown that the cellularity of lumbar vertebrae is, on average, about 10% more than the cellularity of the iliac crest [16]. Vertebrae are also more

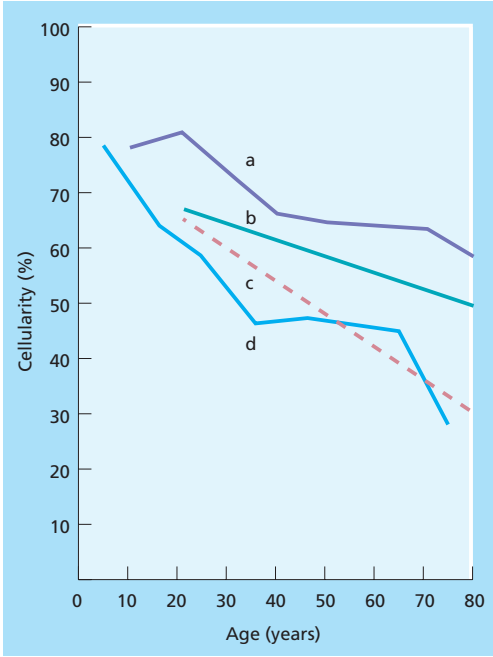



**Fig. 1.12** Mean value of bone marrow cellularity at various ages expressed as a percentage of bone marrow cavity: (a) iliac crest, autopsy, not decalcified (recalculated from Frisch *et al.* [53]); (b) iliac crest, autopsy, not decalcified [56]; (c) sternum, biopsy, not decalcified [57]; and (d) ilium, autopsy, decalcified [54].

cellular than the sternum. Because of the considerable dependence of the assessment of cellularity on methods of processing and counting, it is much more difficult to make generalizations when different tissues have not been assessed by the same techniques. Bennike *et al.* [13], in comparing the two sites in 100 subjects, considered the sternum to be on average somewhat more cellular than the iliac crest. However, comparison of the results of histomorphometric studies by different groups found that, comparing a single study of the sternum with four studies of the iliac crest, the sternum was generally *less* cellular [53–57]. It should be noted that the lowest estimates of iliac crest cellularity are from a study using decalcified, paraffin-embedded bone marrow specimens [54] while the highest estimates are from a study using non-decalcified, resin-embedded specimens [53]. Some studies have been conducted on biopsy specimens [57] and others on specimens obtained at autopsy [53,54,56]. Because of such technical considerations it is difficult to make any generalizations about normal bone marrow cellularity. However, it is possible to say that, except in extreme old age, cellularity of less than 20% is likely to be abnormal, as is cellularity of more than 80% in those above 20 years of age.

In making a subjective assessment of the cellularity of films prepared from aspirates, the cellularity of fragments is of more importance than the cellularity of trails, although occasionally the presence of quite cellular trails – despite hypocellular fragments – suggests that the marrow cellularity is adequate. An average fragment cellularity between 25% and 75% is usually taken to indicate normality, except at the extremes of age.

Because of the variability of cellularity from one intertrabecular space to the next, it is not possible to assess marrow cellularity if few fragments are aspirated or if a biopsy core is of inadequate size. In particular, a small biopsy sample containing only a small amount of subcortical marrow does not allow assessment of cellularity since this area is often of low cellularity, particularly in the elderly. A biopsy specimen containing at least five or six intertrabecular spaces is desirable, not only for an adequate assessment of cellularity but also to give a reasonable probability of detecting focal bone marrow lesions (Fig. 1.13). Ideally this requires a core of 2–3 cm in length. A core length of at least 0.5 cm has been advised in children but one study found 1.0 cm was necessary to avoid a high rate of non-interpretable specimens [59]. The British Committee for Standards in Haematology and Royal College of Pathologists guidelines recommend at least 16 mm [60].

## Haemopoietic and mesenchymal cells

A multipotent stem cell gives rise to all types of myeloid cell: erythrocytes and their precursors; granulocytes and their precursors; macrophages, monocytes and their precursors; mast cells; and megakaryocytes and their precursors (Fig. 1.14). It should be mentioned that the term 'myeloid' can be used with two rather different meanings. It is used to indicate all cells derived from the common myeloid stem cell and also to indicate only the granulocytic and monocytic lineages, as in the expression

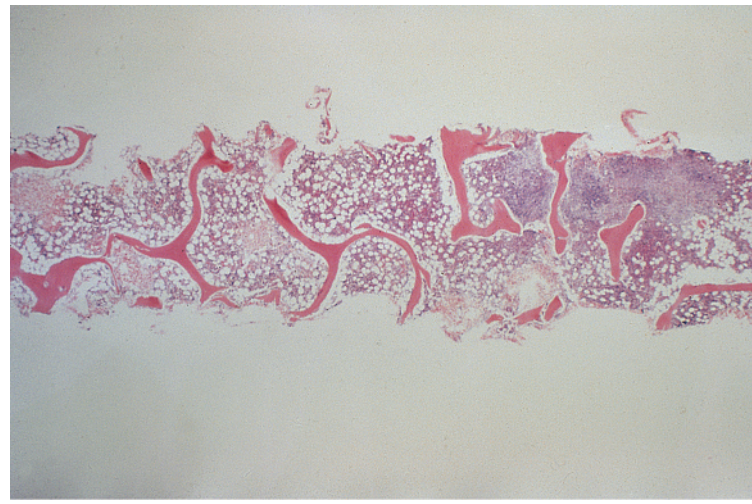


**Fig. 1.13** A section of a trephine biopsy specimen of adequate size from a patient with Hodgkin lymphoma showing only a small area of infiltration at one end of the specimen, illustrating how a small biopsy may miss focal lesions. H&E ×2.5. (With thanks to Dr Ken Maclennan.)

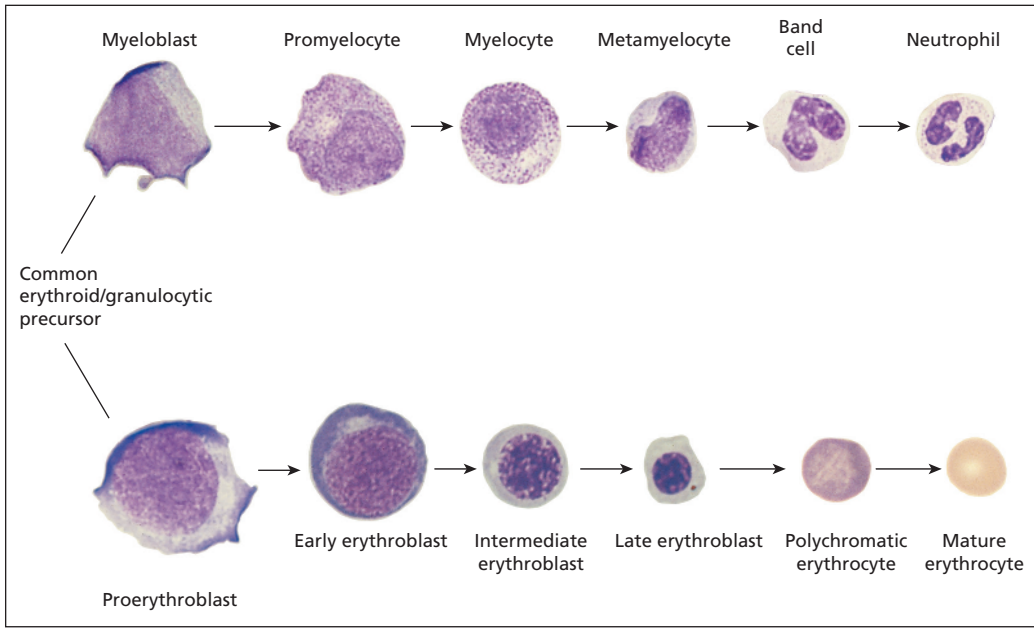



**Fig. 1.14** A semi-diagrammatic representation of granulopoiesis and erythropoiesis. Cell division occurs up to the myelocyte and intermediate erythroblast stages.

'myeloid : erythroid ratio'. It is usually evident from the context which sense is intended but it is important to avoid ambiguity in using this term. The common myeloid stem cell and stem cells committed to the specific myeloid lineages cannot be identified morphologically but it is likely that they are cells of similar size and appearance to a lymphocyte. The various myeloid lineages differ both morphologically and in their disposition in the bone marrow. The normal bone marrow contains, in addition to myeloid cells, smaller numbers of lymphoid cells (including plasma cells) and the stromal cells, which have been discussed earlier.

## Erythropoiesis

### *Cytology*

Precursors of erythrocytes are designated erythroblasts. The term normoblast can also be used but has a narrower meaning; 'erythroblast' includes all recognizable erythroid precursors whereas 'normoblast' is applicable only

when erythropoiesis is normoblastic. There are at least five generations of erythroblasts between the morphologically unrecognizable megakaryocyte–erythroid stem cell and the erythrocyte. Erythroblasts develop in close proximity to a macrophage, the cytoplasmic processes of which extend between and around individual erythroblasts. Several generations of erythroblasts are associated with one macrophage, the whole cluster of cells being known as an erythroblastic island [61]. Intact erythroblastic islands are sometimes seen in bone marrow films (Fig. 1.15). Erythroblasts are conventionally divided, on morphological grounds, into four categories – proerythroblasts and early, intermediate and late erythroblasts. An alternative terminology is: proerythroblast, basophilic erythroblast, early polychromatophilic erythroblast and late polychromatophilic erythroblast. The term orthochromatic erythroblast is best avoided since the most mature erythroblasts are only orthochromatic (that is acidophilic, with the same staining characteristics as mature red cells) when erythropoiesis is abnormal.

Proerythroblasts (Fig. 1.16) are large round cells with a diameter of 12–20 μm and a large round nucleus. The cytoplasm is deeply basophilic with a pale perinuclear zone, attributable to the Golgi apparatus, sometimes being apparent. The nucleus has a finely granular or stippled appearance and contains several nucleoli.

Early erythroblasts (Fig. 1.17) are smaller than proerythroblasts and more numerous.

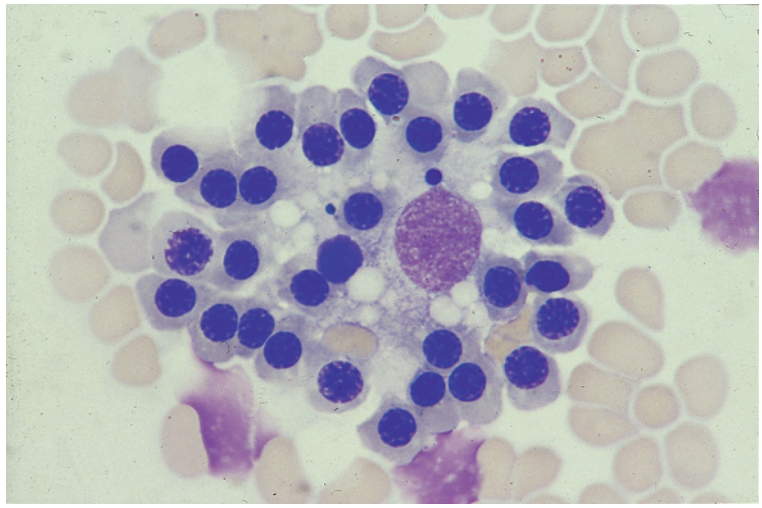


**Fig. 1.15** BM aspirate: an erythroid island. MGG ×100.

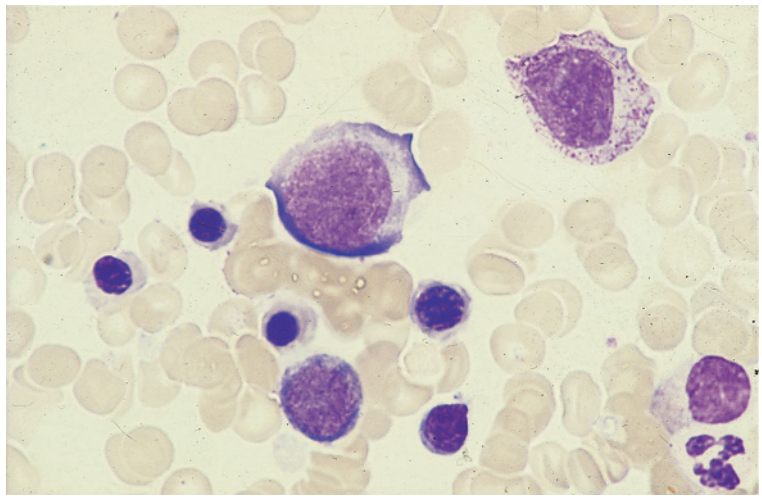


**Fig. 1.16** Aspirate of normal BM: a proerythroblast, an intermediate erythroblast, four late erythroblasts, a myelocyte, large and small lymphocytes and a neutrophil. MGG ×100.

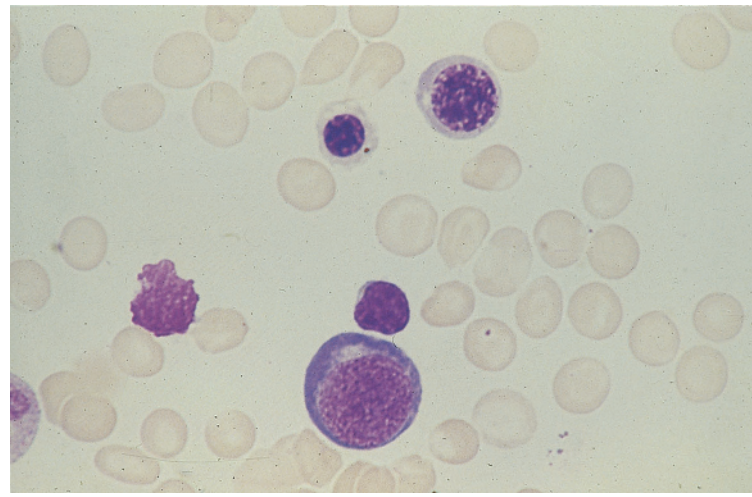


**Fig. 1.17** Aspirate of normal BM: early, intermediate and late erythroblasts and a lymphocyte. MGG ×100.

The nucleocytoplasmic ratio is somewhat lower. They have strongly basophilic cytoplasm and a granular or stippled chromatin pattern without visible nucleoli. A perinuclear halo, which is less strongly basophilic than the rest of the cytoplasm, may be apparent.

Intermediate erythroblasts (Figs 1.16 and 1.17) are smaller again, with a lower nucleocytoplasmic ratio than that of the early erythroblast, less basophilic cytoplasm and moderate clumping of the chromatin. They are more numerous than early erythroblasts.

Late erythroblasts (Figs 1.16 and 1.17) are smaller and more numerous than intermediate erythroblasts. They are only slightly larger than mature red cells. Their nucleocytoplasmic ratio is lower than that of the intermediate erythroblast and the chromatin is more clumped. The cytoplasm is only weakly basophilic and in addition has a pink tinge due to the increased amount of haemoglobin. Because of the resultant pinky-blue colour the cell is described as polychromatophilic.

Late erythroblasts extrude their nuclei to form polychromatophilic erythrocytes, which are slightly larger than mature erythrocytes. These cells can be identified by a specific stain as reticulocytes; when haemopoiesis is normal they spend about 2 days of their 3-day life span in the bone marrow.

Small numbers of normal erythroblasts show atypical morphological features such as irregular nuclei, binuclearity and cytoplasmic bridging between adjacent erythroblasts [62].

***Histology***

Erythroblastic islands (Figs 1.18 and 1.19) are recognizable as distinctive clusters of cells in which one or more concentric circles of erythroblasts closely surround a macrophage. The erythroblasts that are closer to the macrophage are less mature than the peripheral ones. The central macrophage sends out extensive slender processes, which envelop each erythroblast. The macrophage phagocytoses defective erythroblasts and extruded nuclei; nuclear and cellular debris may therefore be recognized in the cytoplasm and a Perls stain (see page 67) may demonstrate the presence of haemosiderin. Erythropoiesis occurs relatively close to marrow sinusoids although it is probable that, as in the rat [63], only a minority of erythroblastic islands actually abut on sinusoids.

Early erythroblasts (Fig. 1.20) are large cells; they have relatively little cytoplasm and large nuclei with dispersed chromatin and multiple small, irregular or linear nucleoli often abutting on the nuclear membrane. The nuclei are rounder than those of myeloblasts but, in contrast to the nuclei of early erythroid cells in bone marrow aspirates of healthy subjects, in histological sections some appear ovoid or slightly irregular. More mature erythroid cells have condensed nuclear chromatin and cytoplasm that is less basophilic. The chromatin in the erythroblast nuclei is evenly distributed and, as chromatin condensation occurs, an even, regular pattern is retained.

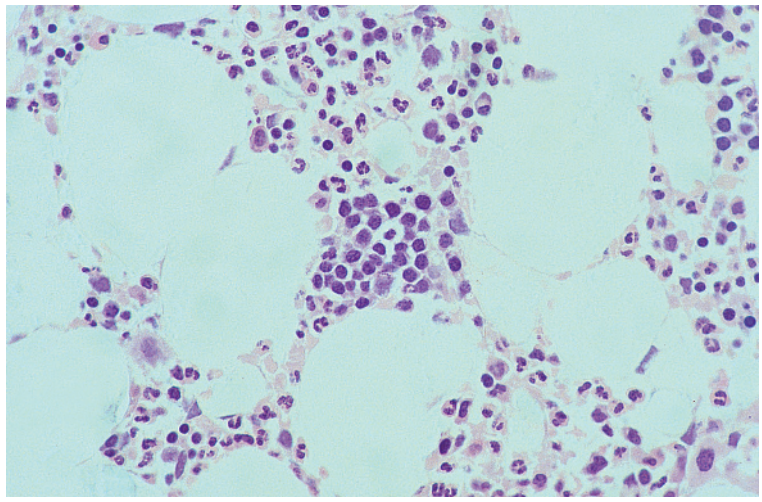


**Fig. 1.18** Section of normal BM: an erythroid island (centre). Resin-embedded, H&E ×40.

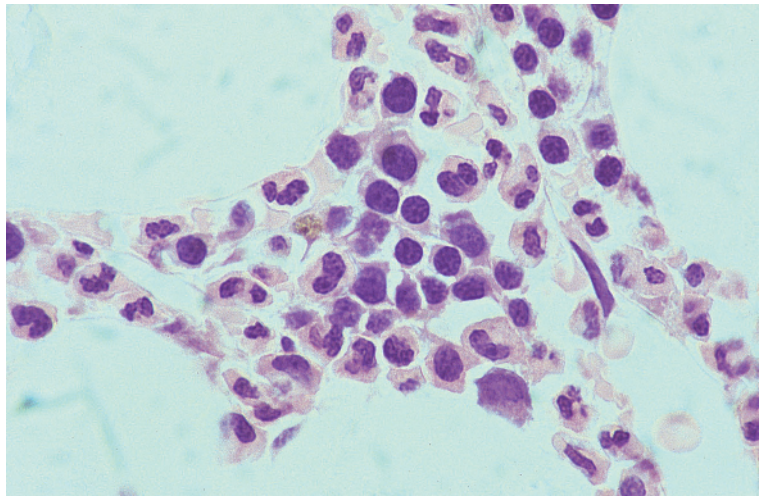


**Fig. 1.19** Section of normal BM: an erythroid island containing intermediate and late erythroblasts and a haemosiderin-laden macrophage; a Golgi zone can be seen in some of the intermediate erythroblasts. Resin-embedded, H&E ×100.

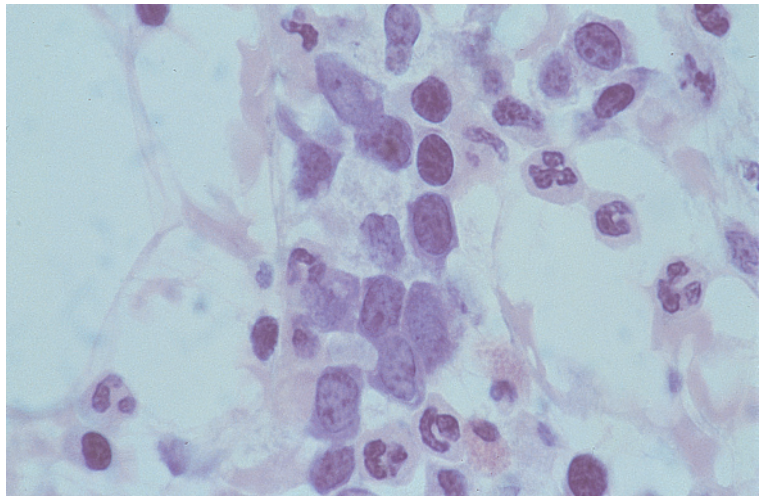


**Fig. 1.20** Section of normal BM: an erythroid island containing early and intermediate erythroblasts. Resin-embedded, Giemsa ×100.

There are four features that are useful in distinguishing erythroid precursors in the marrow from other cells: (i) in normal bone marrow they occur in distinctive erythroblastic islands containing several generations of cells of varying size and maturity; (ii) erythroblasts adhere tightly to one another; (iii) their nuclei are round; and (iv) in late erythroblasts the chromatin is condensed in a regular manner whereas nuclei of small lymphocytes show coarse clumping. With a Giemsa stain (Fig. 1.21), the intense cytoplasmic basophilia with a small, negatively staining Golgi zone adjacent to the nucleus is also distinctive. In paraffin-embedded specimens (Fig. 1.22), artefactual shrinking of cytoplasm of later erythroblasts can be useful in distinguishing them from lymphocytes. Shrinkage artefact is absent in resin-embedded sections, in which the identification of erythroid cells is aided by their syncytial appearance (Fig. 1.23).

When the bone marrow is regenerating rapidly, erythroid islands may be composed of cells all of which are at the same stage of maturation. This results in some islands consisting only of immature elements. A similar pattern is sometimes seen when erythropoiesis is abnormal, for example in myelodysplasia, in which the intramedullary death of erythroblasts is a major mechanism.

The identification of abnormal erythroblasts can be more difficult than the identification of their normal equivalents, for example, if well-organized erythroblastic islands are not

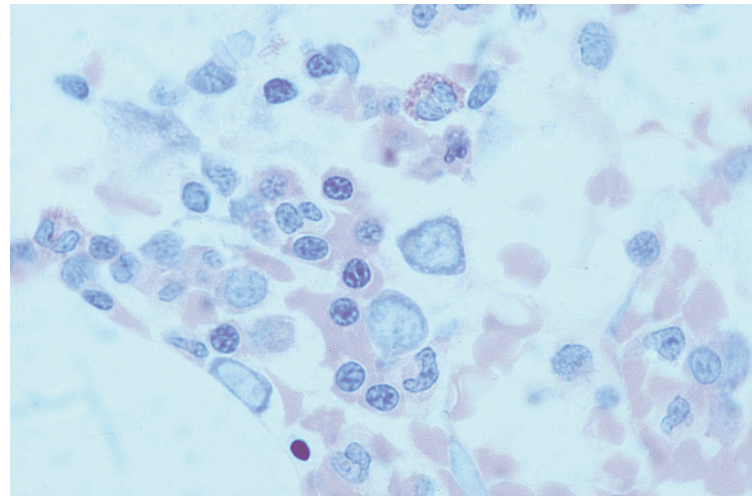


**Fig. 1.21** Section of normal BM: erythroid island containing three early, one intermediate and numerous late erythroblasts; note the cytoplasmic basophilia of early erythroblasts. Resin-embedded, Giemsa ×100.

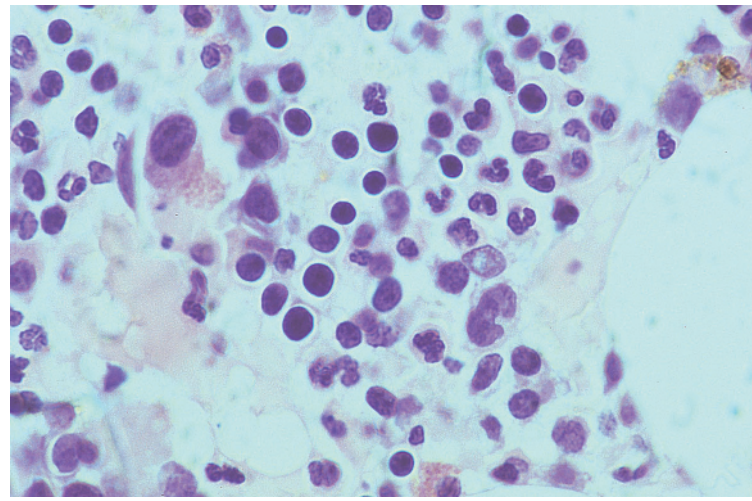


**Fig. 1.22** Section of normal BM: erythroid island showing intermediate and late erythroblasts with haloes surrounding the nuclei. H&E ×100.

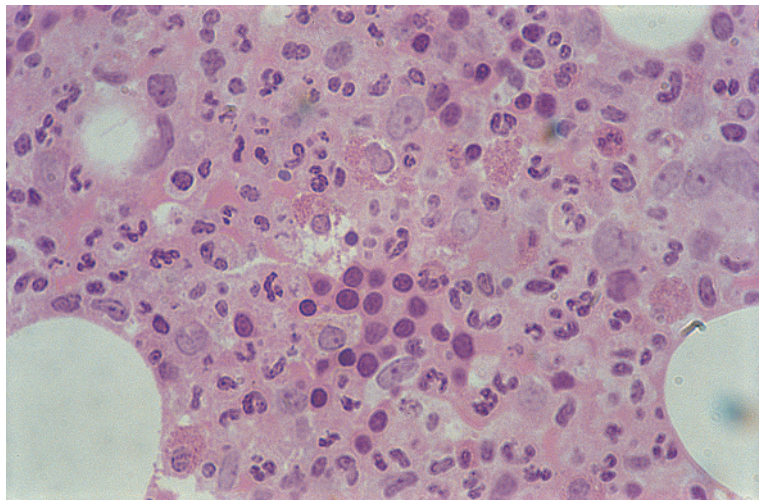


**Fig. 1.23** Syncytial appearance of erythroblasts in an erythroid island in sections from a trephine biopsy specimen. Resin-embedded, H&E ×60.

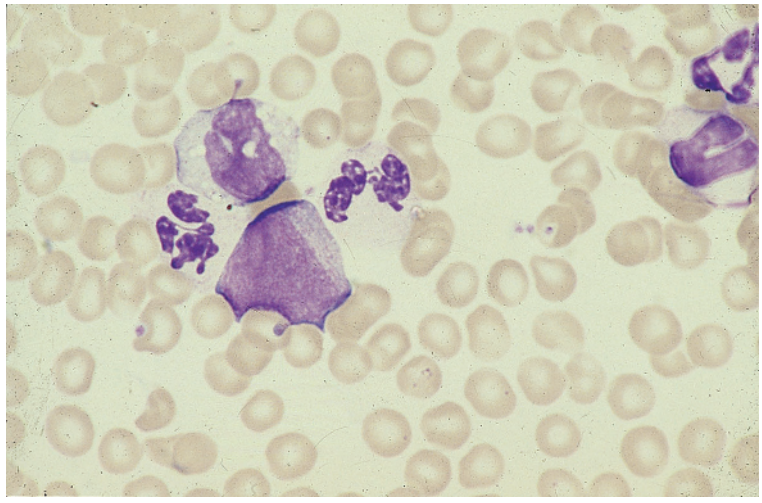


**Fig. 1.24** Aspirate of normal BM: a myeloblast, three neutrophils and two monocytes; the myeloblast has a high nucleocytoplasmic ratio, a diffuse chromatin pattern and a nucleolus. MGG ×100.

present or if they contain only immature cells. When there is any difficulty in recognizing erythroid precursors their identity can be confirmed by cytochemical or immunohistochemical staining (see page 82).

## Granulopoiesis

### *Cytology*

There are at least four generations of cells between the morphologically unrecognizable committed granulocyte–monocyte precursor and the mature granulocyte, but cell division does not necessarily occur at the same point as maturation from one stage to another. It is probable that the relevant stem cell can be either an erythroid–myeloid progenitor or a lymphoid–myeloid progenitor [64]. The first recognizable granulopoietic cell is the myeloblast (Figs 1.24 and 1.25). It is similar in size to the proerythroblast, about 12–20 μm. It is more irregular in shape than a proerythroblast and its cytoplasm is moderately rather than strongly basophilic. The chromatin pattern is diffuse and there are several nucleoli. Myeloblasts are generally defined as being cells that lack granules but, in the context of the abnormal myelopoiesis of acute myeloid leukaemia and the myelodysplastic syndromes, primitive cells with granules may also be accepted as myeloblasts. Myeloblasts are capable of cell division and mature to promyelocytes.

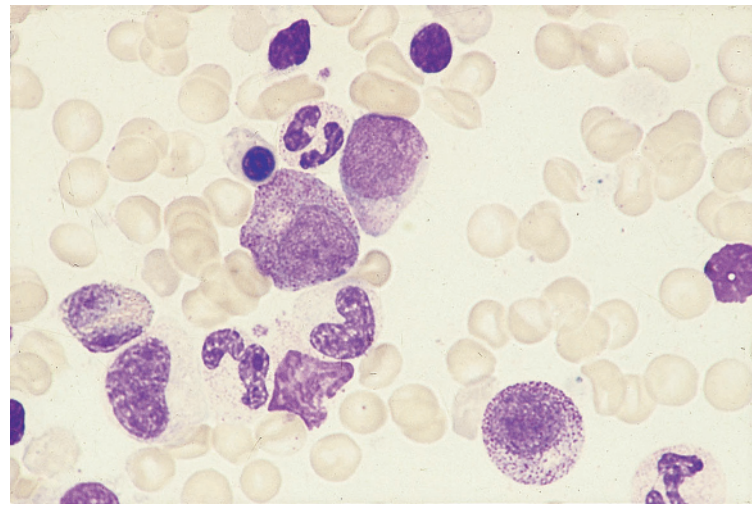


**Fig. 1.25** Aspirate of normal BM: a myeloblast and a promyelocyte (centre), a myelocyte (lower right), a metamyelocyte, band forms, a neutrophil and a late erythroblast; the promyelocyte is larger than the myeloblast and is showing some chromatin condensation but with persisting nucleoli, well-developed cytoplasmic granulation and a Golgi zone. MGG ×100.

Promyelocytes (Fig. 1.25) have a nucleolated, slightly indented nucleus, a Golgi zone and primary or azurophilic granules, which are reddish-purple with a Romanowsky stain. Promyelocytes are larger than myeloblasts, usually 15–25 μm, and their cytoplasm is often more strongly basophilic. By light microscopy, promyelocytes of the three granulocytic lineages cannot easily be distinguished, but by ultrastructural examination the distinction can be made. Promyelocytes are capable of cell division and mature to myelocytes.

Myelocytes (Fig. 1.25) are smaller than promyelocytes and are quite variable in size – from 10 to 20 μm. Their nuclei show partial chromatin condensation and lack nucleoli. Their cytoplasm is less basophilic than that of promyelocytes and specific neutrophilic, eosinophilic and basophilic granules can now be discerned, staining lilac, orange-red and purple, respectively. Eosinophil myelocytes may also contain some granules that take up basic dyes and stain purple; these differ ultrastructurally from the granules of the basophil lineage and are best designated pro-eosinophilic granules. There are probably normally at least two generations of myelocytes so that at least some cells of this category are capable of cell division. Late myelocytes mature to metamyelocytes, which are 10–12 μm in diameter and have a markedly indented or U-shaped nucleus (Fig. 1.25). The metamyelocyte is not capable of cell division but matures to a band form with a ribbon-shaped nucleus. The band cell, in turn, matures to a polymorphonuclear granulocyte with a segmented nucleus and specific neutrophilic, eosinophilic or basophilic granules. The bone marrow is a major reservoir for mature neutrophils.

***Histology***

Myeloblasts (Fig. 1.26) are the earliest granulocyte precursors identifiable histologically; they are present in small numbers and are most frequently found adjacent to the bone marrow trabecular surfaces or to arterioles. They are fairly large cells with round to oval nuclei and one to five relatively small nucleoli. There is no chromatin clumping. They have relatively little cytoplasm. They are readily distinguished from lymphoid cells by the absence of chromatin clumping and the presence of nucleoli. Myeloblasts are far outnumbered in normal marrows by promyelocytes (Figs 1.26 and 1.27) and myelocytes (Fig. 1.27), which are recognized by their granularity. Primary and neutrophilic granules may be seen as faintly eosinophilic granules in good quality haematoxylin and eosin (H&E)-stained sections but they are best seen with a Giemsa stain. Granules of cells of eosinophil lineage are large, refractile and more strongly eosinophilic. They are therefore easily recognized on both H&E and Giemsa stains. Basophil granules are water-soluble and, since trephine biopsy specimens are fixed in aqueous fixatives, basophils are

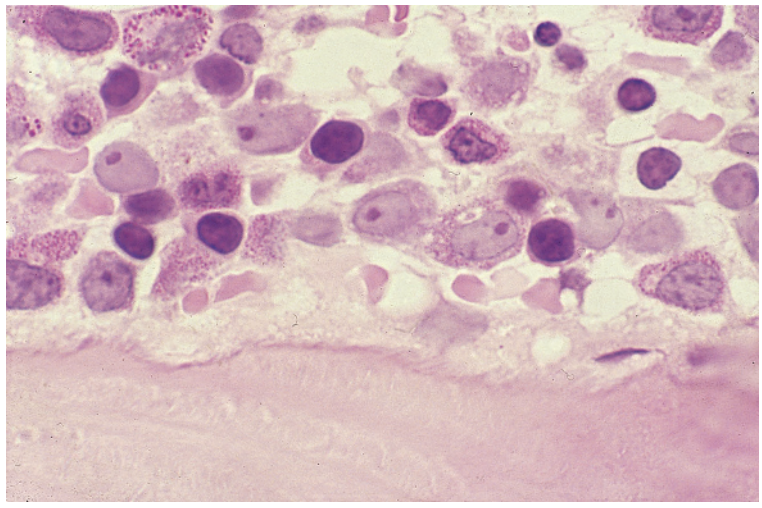


**Fig. 1.26** Section of normal BM: myeloblasts and promyelocytes adjacent to a bony trabecula. Resin-embedded, H&E ×100.

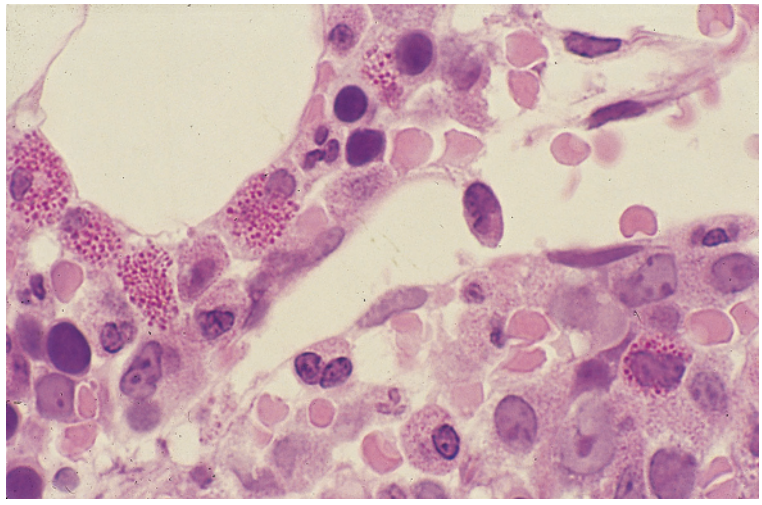


**Fig. 1.27** Section of normal BM: promyelocytes, myelocytes and maturing neutrophils and eosinophils adjacent to a sinusoid. Resin-embedded, H&E ×100.

not recognizable in histological sections. As maturation occurs, granulocytic precursors are found progressively more deeply in the haemopoietic cords but away from the sinusoids. When they reach the metamyelocyte stage, they appear to move towards the sinusoids and, at the polymorphonuclear granulocyte stage, cross the wall to enter the circulation.

In undecalcified resin-embedded sections, and in sections from specimens decalcified using EDTA, the chloroacetate esterase stain is a reliable marker of neutrophil haemopoiesis from the promyelocyte stage onwards. Overnight incubation of acid-decalcified sections in a buffer at pH 6.8 partly restores chloroacetate esterase activity. Alternatively, the identity of cells of the granulocytic lineage can be confirmed by immunohistochemistry.

## Monocytopoiesis

### *Cytology*

Monocytes are derived from a morphologically unrecognizable common granulocytic–monocytic precursor, probably in fact a lymphoid–myeloid progenitor [64]. The earliest morphologically recognizable precursor is a monoblast, a cell that is larger than a myeloblast with abundant cytoplasm showing a variable degree of basophilia and with a large, round nucleus. Monoblasts are capable of division and mature into promonocytes,

which are similar in size to promyelocytes; they have nucleoli, some degree of nuclear lobation and azurophilic cytoplasmic granules. Promonocytes mature into monocytes, which migrate rapidly into the peripheral blood. Monocytes are 12–20 μm in diameter. They have a lobated nucleus and abundant cytoplasm, which is weakly basophilic. The cytoplasm may contain small numbers of fine azurophilic granules and often has a ground-glass appearance, in contrast to the clear cytoplasm of a lymphocyte.

Monocytes mature into macrophages (Fig. 1.28) in the bone marrow as well as in other tissues. These are large cells, 20–30 μm in diameter, of irregular shape, with a low nucleocytoplasmic ratio and voluminous weakly basophilic cytoplasm. When relatively immature, they may have an oval nucleus with a fairly diffuse chromatin pattern. When mature, the nucleus is smaller and more condensed and the cytoplasm may contain lipid droplets, recognizable degenerating cells and amorphous debris; an iron stain commonly shows the presence of haemosiderin. Bone marrow macrophages may develop into various storage cells, which will be discussed in later chapters.

Both monocytes and their precursors are quite infrequent among marrow cells partly because monocytes, in contrast to mature neutrophils, are released rapidly into the peripheral blood rather than being stored in the bone marrow. Macrophages (histiocytes), however, are readily apparent.

***Histology***

Monocytes are recognized in histological sections of the marrow as cells that are larger than neutrophils with lobated nuclei; monocyte precursors are not usually recognizable. In haematologically normal subjects, only small numbers of randomly distributed monocytes are present and they are difficult to identify.

Macrophages (Fig. 1.29) are identified as irregularly scattered, relatively large cells with a small nucleus and abundant cytoplasm. In thin sections, only the cytoplasm may be visible, the nucleus being out of the plane of the section. Phagocytosed debris may be prominent in the cytoplasm. Some are associated with erythroblasts (forming erythroblastic islands), plasma cells or lymphoid nodules. Immunohistochemistry of trephine biopsy sections highlights a prominent network of dendritic macrophages dispersed through the stroma (Fig. 1.30).

## Stromal dendritic cells

Follicular dendritic cells (FDCs) expressing CD21 and CD23, typical of lymph nodes, are not seen in normal bone marrow. However, other stromal dendritic cells of mesenchymal origin are abundant. These probably represent multiple functional subsets and originate from CD34-positive mesenchymal stem cells that may in turn be derived from

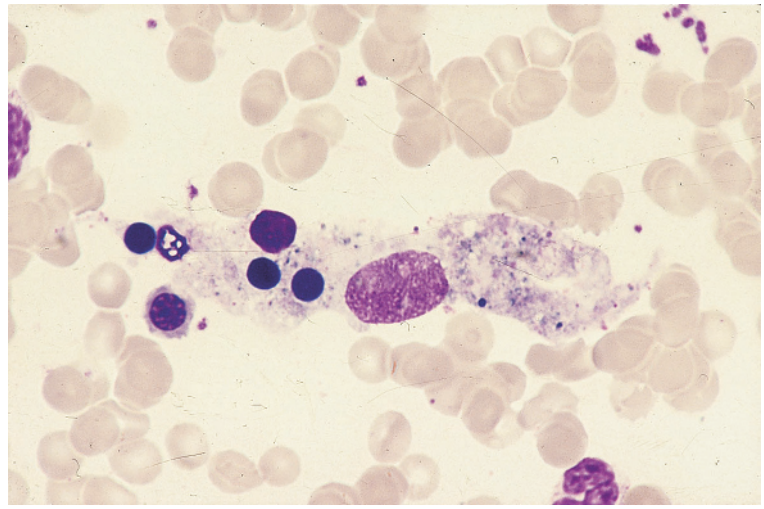


**Fig. 1.28** Aspirate of normal BM: a macrophage containing granular and refractile debris and several normoblast nuclei. MGG ×100.

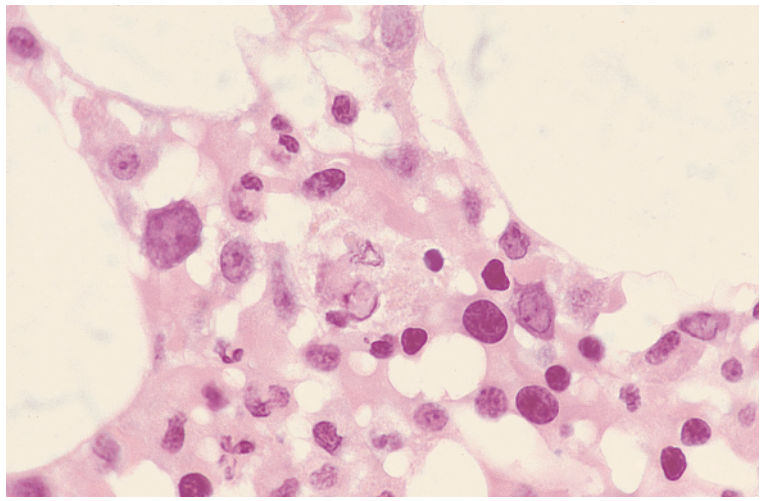


**Fig. 1.29** Section of normal BM: a macrophage containing cellular debris. Resin-embedded, H&E ×100.

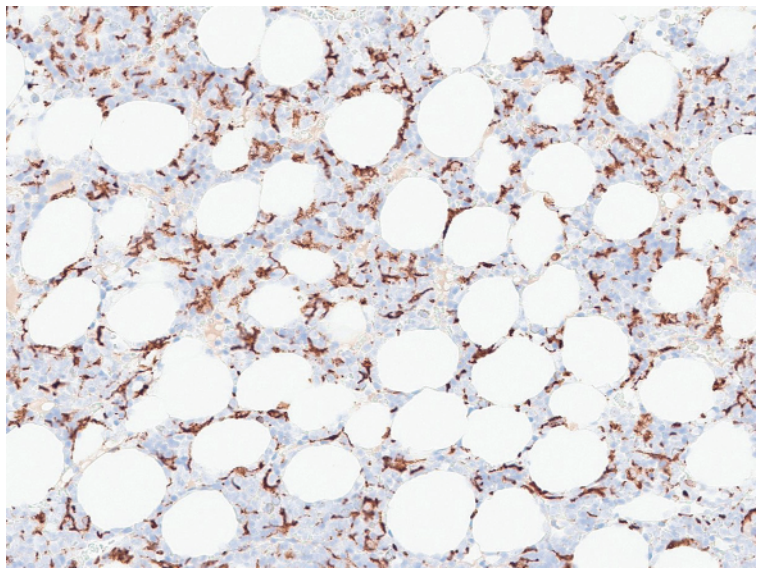


**Fig. 1.30** Section of trephine biopsy specimen showing a network of dendritic macrophages. Immunoperoxidase with CD68R monoclonal antibody ×20.

'haemangioblasts', known to be present during embryonic development, that are capable of differentiating into both myeloid and stromal lineages. Mesenchymal stem cells [65,66] are capable of adipocytic, osteogenic and chrondrogenic differentiation. The predominant mature stromal dendritic cell type in postnatal bone marrow resembles myofibroblastic stellate stromal cells found at a variety of other sites including liver, dermis, endometrium and prostate. In bone marrow, these cells have previously been called 'adventitial reticular cells'. They have regulatory roles in haemopoietic differentiation and in immune cell interactions, and are presumed to be the origin of fibrosis occurring in inflammatory myelopathies and myeloproliferative neoplasms. However, their role in fibrosis occurring in reaction to metastatic solid tumours, lymphomas and some granulomatous disease processes is unclear. It is also unknown whether cells forming true FDC meshworks in reactive and neoplastic lymphoid nodules in bone marrow arise from these stromal dendritic cells or their precursors or, alternatively whether that arise from cells that have migrated into the marrow.

*Cytology*

Mature stromal dendritic cells are not found in peripheral blood. A small proportion of circulating CD34-positive precursor cells are of stromal rather than haemopoietic origin, particularly following mobilisation of marrow stem cells. In bone marrow samples enriched for CD34-positive precursors and subsequently immunostained for CD271, mesenchymal stem cells are round with distinctive ruffled cytoplasm. Mature stromal dendritic cells in aspirate films typically remain localized to particles, where they are obscured by adipocytes and haemopoietic cells. Occasionally they may be identifiable as bipolar or tripolar cells, with longer cytoplasmic processes than the rare endothelial cells that may also be found.

*Histology*

Stromal dendritic cells form a meshwork throughout the bone marrow stroma with accentuated density at trabecular margins and around larger blood vessels. They are typically invisible without immunohistochemical demonstration as they intercalate between adipocytes and their long, interconnecting dendritic processes are too fine to visualise readily. They are also mimicked by a completely separate population of highly dendritic resident histiocytes.

*Immunohistochemistry*

Stromal dendritic cells share with FDCs the expression of human leucocyte antigen (HLA)-DR, CD11c and CD271 (low affinity nerve growth factor receptor; L-NGFR) (Fig. 1.31) but they are negative for CD21 and CD23. They are variably positive for CD10, CD13, factor XIIIA and smooth muscle actin. In routine diagnostic practice, CD271 is the best marker of these cells. They do not express antigens associated with myelomonocytic lineages and hence can be distinguished from dendritic histiocytes that express CD68R.

## Megakaryopoiesis and thrombopoiesis

*Cytology*

It is probable that megakaryocytes can arise either from haemopoietic stem cells via a common megakaryocyte–erythroid progenitor cell or from a multipotent haemopoietic stem cell that is committed to the megakaryocyte lineage [64]. The latter are small, proliferative cells with diploid nuclei, not generally recognizable in normal bone marrow. In normal marrow, the earliest morphologically recognizable cell in the megakaryocyte lineage is the megakaryocyte itself although, when haemopoiesis is abnormal, megakaryoblasts of

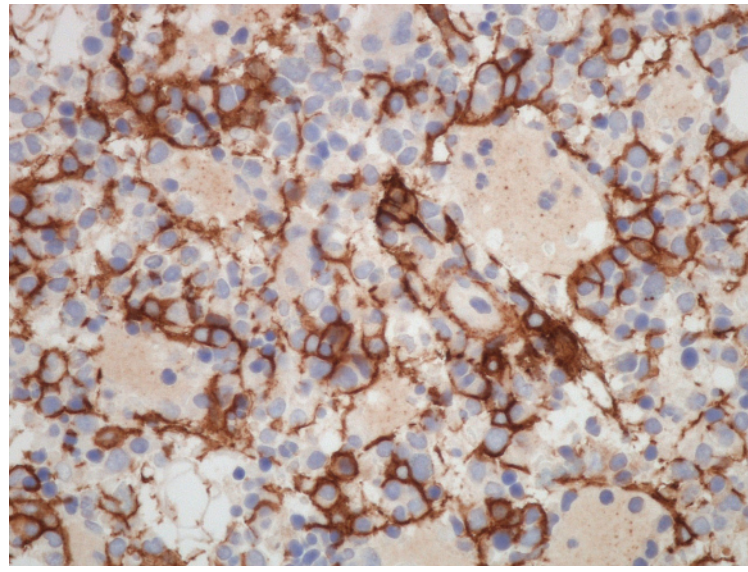


**Fig. 1.31** Section of trephine biopsy specimen showing a network of stromal dendritic cells. Immunoperoxidase with CD271 monoclonal antibody ×40.

similar size and morphology to myeloblasts can sometimes be recognized. Megakaryocytes undergo endoreduplication as they mature, resulting in large cells (30–160 μm) with a marked degree of heterogeneity in both nuclear deoxyribonucleic acid (DNA) content (ploidy) and nuclear size. Endoreduplication is encountered only rarely in any other mammalian cell. It is promoted by upregulation of cyclin D3 and is believed to contribute to the high productive capacity of megakaryocytes for platelet components [67]. Megakaryocytes can be classified by their ploidy level. In normal marrow they range from 4 N (tetraploid) to 32 N with the dominant ploidy category being 16 N. Megakaryocytes can also be classified on the basis of their nuclear and, more particularly, their cytoplasmic characteristics into three stages of maturation [68]. Group I megakaryocytes (Fig. 1.32) have strongly basophilic cytoplasm and a very high nucleocytoplasmic ratio. Group II megakaryocytes have a lower nucleocytoplasmic ratio and cytoplasm that is less basophilic; the cytoplasm contains some azurophilic granules. Group III megakaryocytes (Fig. 1.33) have plentiful weakly basophilic cytoplasm containing abundant azurophilic granules; the cytoplasm at the cell margins is agranular. Group III megakaryocytes are mature cells, capable of producing platelets and no longer synthesizing DNA. There is some correlation between the three stages of maturation and ploidy level. All stages of maturation include megakaryocytes that are

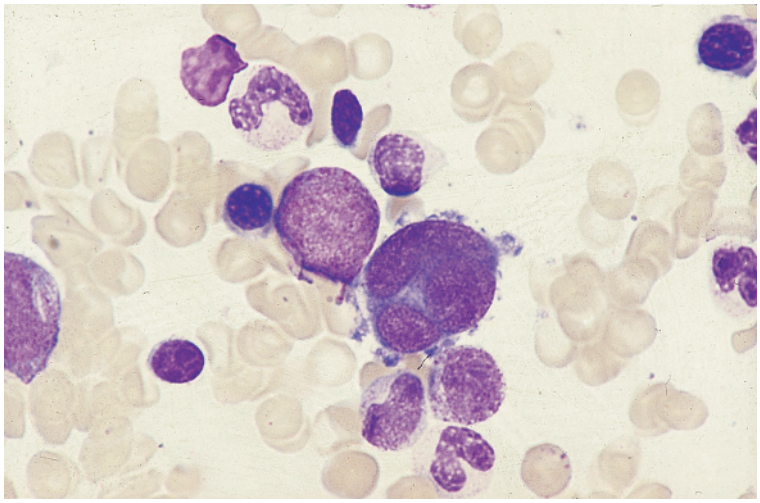


**Fig. 1.32** Aspirate of normal BM: an immature megakaryocyte with a polyploid nucleus showing little chromatin condensation; the cytoplasm is scanty and basophilic. MGG ×100.

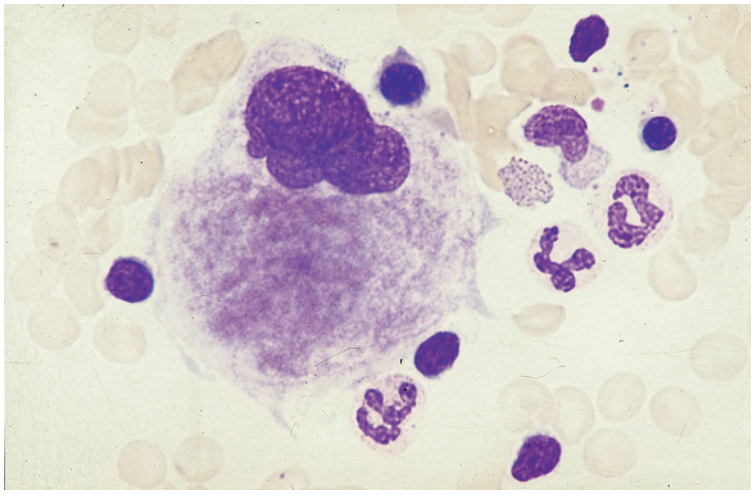


**Fig. 1.33** Aspirate of normal BM: a mature megakaryocyte with a lobated nucleus and voluminous granular cytoplasm. MGG ×100.

8 N, 16 N and 32 N, but 4 N megakaryocytes are confined to group I and 32 N megakaryocytes are more numerous in group III. The nuclei of the great majority of normal polyploid megakaryocytes form irregular lobes joined by strands of chromatin. A minority have either a non-lobated nucleus or more than one nucleus. Megakaryocytes of the fetus and the neonate are smaller and have a lower ploidy level than megakaryocytes of adults but their cytoplasm is mature; it may take several years before the size and ploidy level are the same as those seen in adults [64]. Platelet production involves aggregation of components within the cell cytoplasm, segregation within a demarcation membrane system and organization into proplatelets. The latter are then shed directly into bone marrow sinusoids in a highly coordinated process of cytoplasmic fragmentation. The final stage in megakaryocyte maturation is an apparently bare nucleus (actually with a thin cytoplasmic rim), the great bulk of the cytoplasm having been shed into sinusoids as platelets (Fig. 1.34).

Megakaryocyte proliferation and platelet production are primarily regulated by interactions between thrombopoietin (TPO) and its cell surface receptor, MPL [69]. An increased demand for platelets, for example due to peripheral destruction, leads to an increase in ploidy level and cell size, apparent in a bone marrow film as an increased volume of cytoplasm and a large, usually well-lobated nucleus. It should be noted that whether or not megakaryocytes appear to be producing platelets shows little correlation with the number of platelets being produced. In patients with thrombocytosis, particularly with essential thrombocythaemia, there are often many 'budding' megakaryocytes but in autoimmune thrombocytopenia, in which platelet production is also greatly increased, 'budding' megakaryocytes are quite uncommon.

It is necessary to assess megakaryocyte numbers as well as morphology. In films of an aspirate this can only be a subjective assessment – that megakaryocytes are decreased, normal or increased. A more accurate assessment can be made from histological sections of aspirated fragments or from sections of trephine biopsy specimens. Somewhat fewer megakaryocytes are seen in sections of aspirated fragments than in trephine biopsy sections, possibly because these large cells are not as readily aspirated as smaller marrow cells.

Megakaryocytes may 'engulf' other haemopoietic cells (lymphocytes, erythrocytes, erythroblasts and granulocytes and their precursors), a process known as emperipolesis (Fig. 1.35). The phenomenon was described and the name coined by Humble *et al*. in 1956, the word emperipolesis meaning 'inside round about wandering' [70]. This process differs from phagocytosis in that the engulfed cells have entered dilated cavities in the demarcation membrane system rather than being in phagocytic vacuoles; on examination of bone marrow films the cells within the megakaryocyte are observed to be intact and morphologically normal.

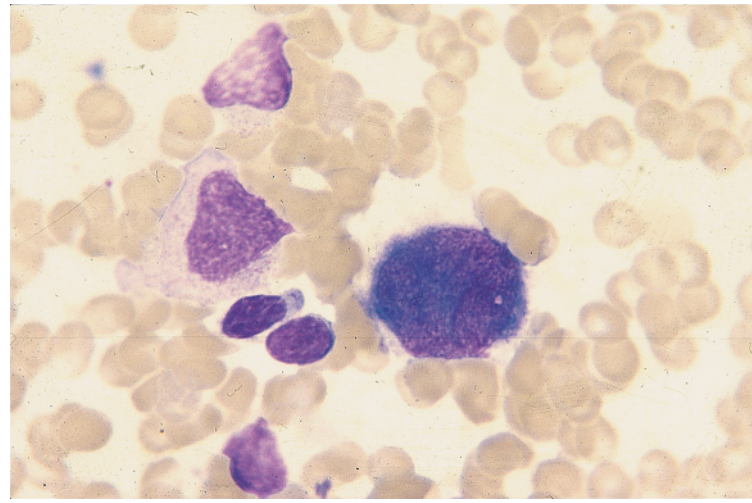


**Fig. 1.34** Aspirate of normal BM: a late megakaryocyte that has shed most of its cytoplasm as platelets. MGG ×100.

Identification of megakaryocytes is aided by cytochemistry and immunohistochemistry (see pages 72 and 83).

### *Histology*

Megakaryocytes are by far the largest of normal bone marrow cells, their size being related to their ploidy. They have plentiful cytoplasm and usually a lobated nucleus. The chromatin pattern is finely granular and evenly dispersed. With a Giemsa stain, demarcation of platelets within the cytoplasm is apparent.

Megakaryocytes are most frequently found associated with sinusoids, at some distance from bony trabeculae (Figs 1.9, 1.36 and 1.37). They are found in a paratrabecular position only when haemopoiesis is abnormal. Serial sections show that, in normal marrow, all megakaryocytes abut on sinusoids [71]. Megakaryocytes lie directly outside the sinusoid and discharge platelets by protruding cytoplasmic processes through endothelial cells; such processes break up into platelets. 'Bare nuclei' which have shed almost all their cytoplasm in this manner can be recognized in histological sections (Fig. 1.38) as well as in bone marrow films. Intact megakaryocytes and bare nuclei can also enter the circulation and are seen within vessels in histological sections of lung, spleen, liver and other organs. Multiple mitotic figures are sometimes observed in megakaryocytes (Fig. 1.39). Emperipolesis is readily observed in histological sections (Fig. 1.40).

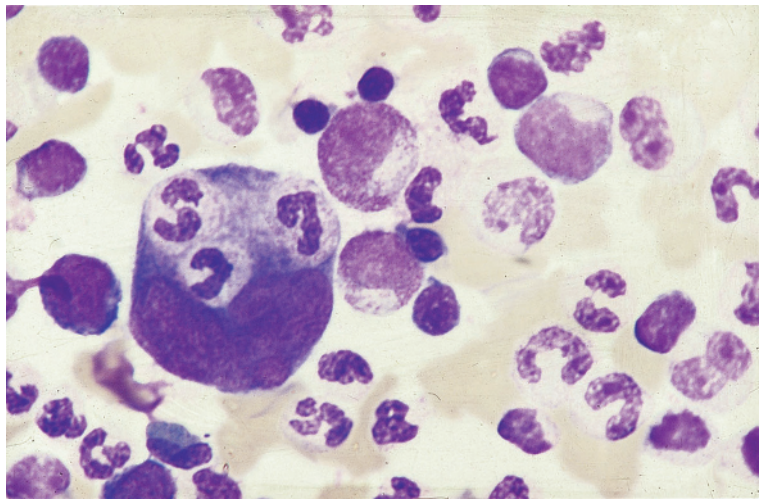


**Fig. 1.35** Aspirate of non-infiltrated BM from a patient with Hodgkin lymphoma: a mature megakaryocyte exhibiting emperipolesis. MGG ×100.

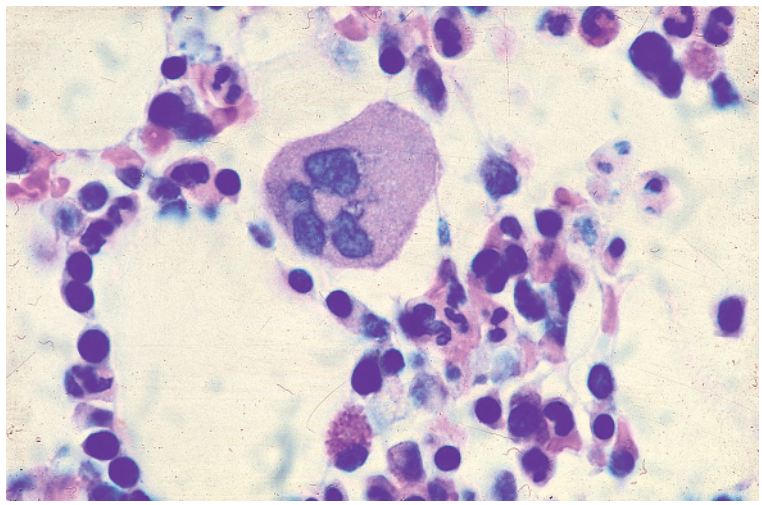


**Fig. 1.36** Section of normal BM showing cells of all haemopoietic lineages including a normal megakaryocyte with finely granular cytoplasm. Giemsa ×100.

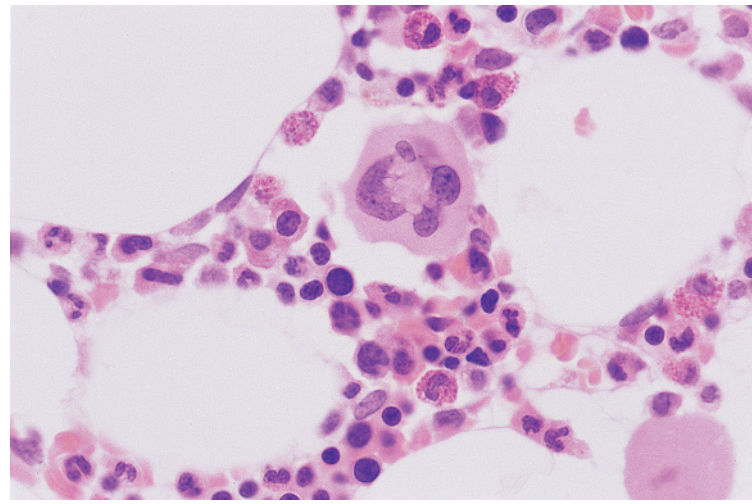


**Fig. 1.37** Section of normal BM showing a normal megakaryocyte and other normal haemopoietic cells. H&E ×100.

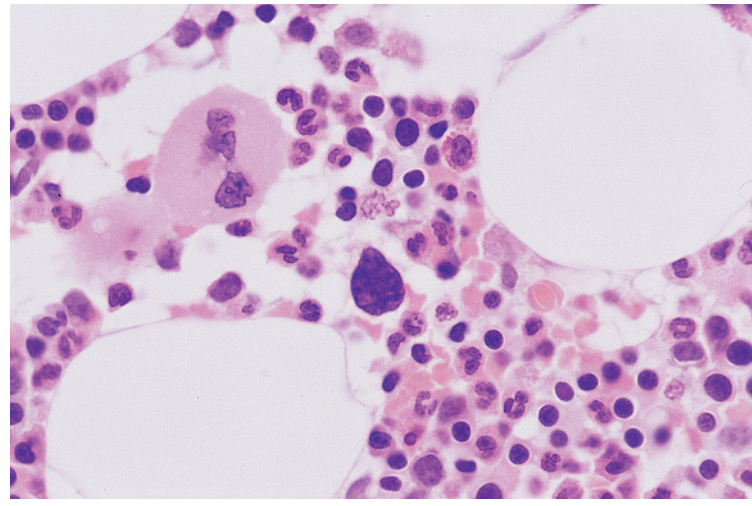


**Fig. 1.38** Section of normal BM showing a normal mature megakaryocyte and a 'bare' megakaryocyte nucleus. H&E ×100.

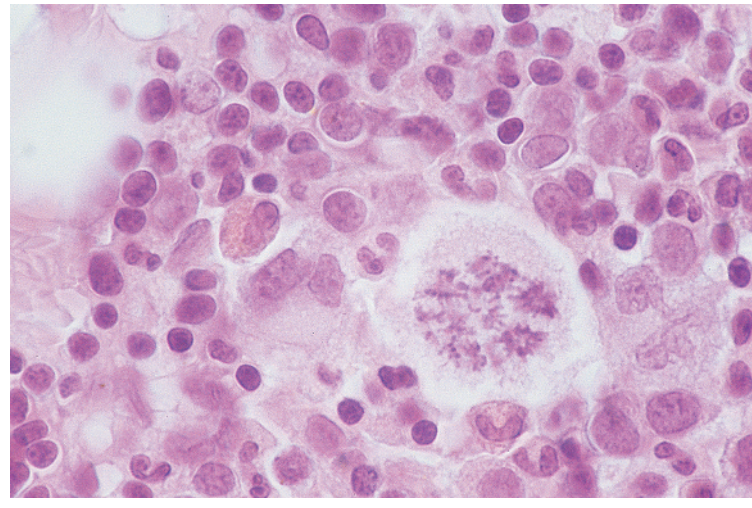


**Fig. 1.39** Section of a trephine biopsy specimen from a patient with polycythaemia vera showing two megakaryocytes, one of which shows multiple mitotic figures; note shrinkage haloes around the intermediate and late erythroblasts. H&E ×100.

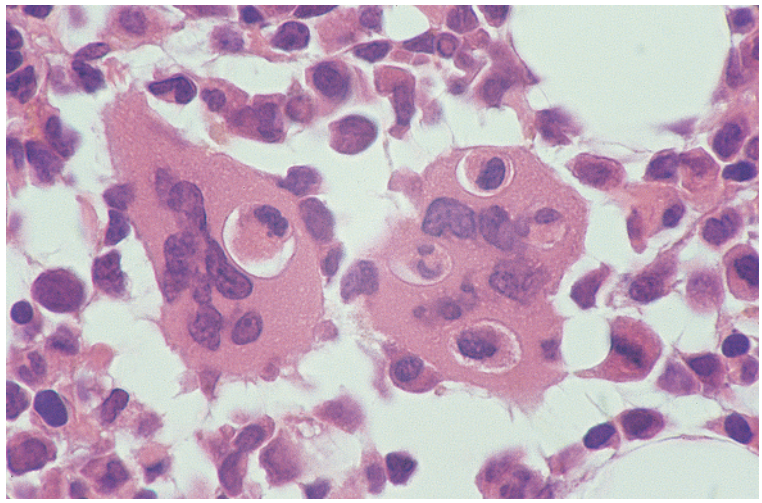


**Fig. 1.40** Section of a trephine biopsy specimen from a patient with acquired immune deficiency syndrome (AIDS): two megakaryocytes show prominent emperipolesis; in normal marrow emperipolesis is less striking. H&E ×100.

In assessing the morphology of megakaryocytes, it is important to remember that the megakaryocyte is a very large cell and only a cross-section of it is being examined. It is therefore not possible to determine the size or degree of nuclear lobation of single megakaryocytes. However, by examining a large number of cells it is possible to form a judgement as to the average size of the megakaryocytes, the size distribution, the average degree of lobation, and whether there are abnormal features such as micromegakaryocytes or an increased number of non-lobated megakaryocytes (see pages 242 and 243). Megakaryocytes of haematologically normal neonates and infants, up to the age of 10 months, are smaller and more homogeneous in size than those of older children and adults [72].

When haemopoiesis is normal, megakaryocytes do not form clusters of more than two or three cells. Larger clusters of megakaryocytes are seen in regenerating marrow, following chemotherapy and bone marrow transplantation, and also in various pathological states; this feature is diagnostically useful.

Megakaryocytes can be quantified by counting their number per unit area, or a subjective impression can be formed as to whether they are present in decreased, normal or increased numbers. Depending on the processing and staining techniques employed, estimates of mean megakaryocyte number in normal marrow vary from 7 to 15 per $mm^2$ [73]. If an immunohistochemical technique is used, estimates are considerably higher, with the mean normal value being 25 per $mm^2$; this is probably because more small megakaryocytes and megakaryocyte precursors are recognized [73].

## Mast cells

### *Cytology*

Mast cells (Fig. 1.41) are derived from the multipotent myeloid stem cell. In bone marrow films they appear as oval or elongated cells varying in size from 5 to 25 μm. The nucleus is central, relatively small and either round or oval. The cytoplasm is packed with granules that stain deep purple with Romanowsky stains. Mast cells can be distinguished from basophils by the different nuclear characteristics (non-lobated nucleus with less chromatin clumping) and by the fact that the granules do not obscure the nucleus. Crushed bone marrow particles can be useful for visualizing mast cells since they are often closely related to the stroma.

### *Histology*

Mast cells are rare in normal marrow. They are difficult to recognize in H&E-stained histological sections because the granules do not stain distinctively. They are readily recognizable in a Giemsa stain (Fig. 1.42) in which the granules stain metachromatically; their vivid purple colour make them conspicuous. Mast cell granules

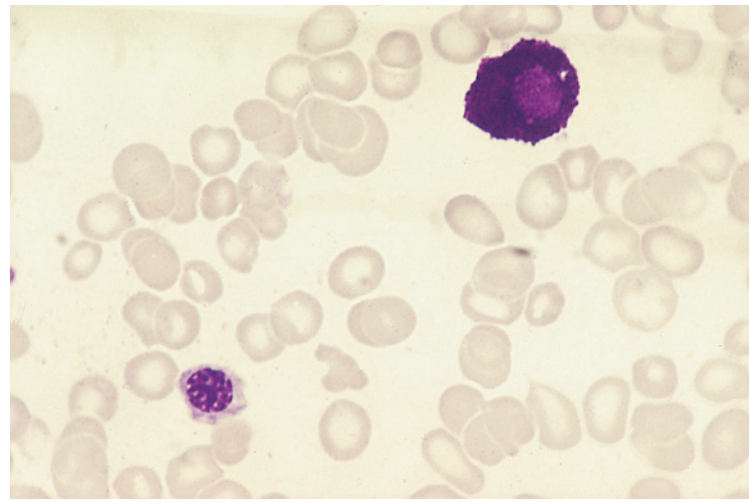


**Fig. 1.41** Aspirate of normal BM: a mast cell and a normoblast; the mast cell has a round nucleus and cytoplasm packed with deeply basophilic granules. MGG ×100.

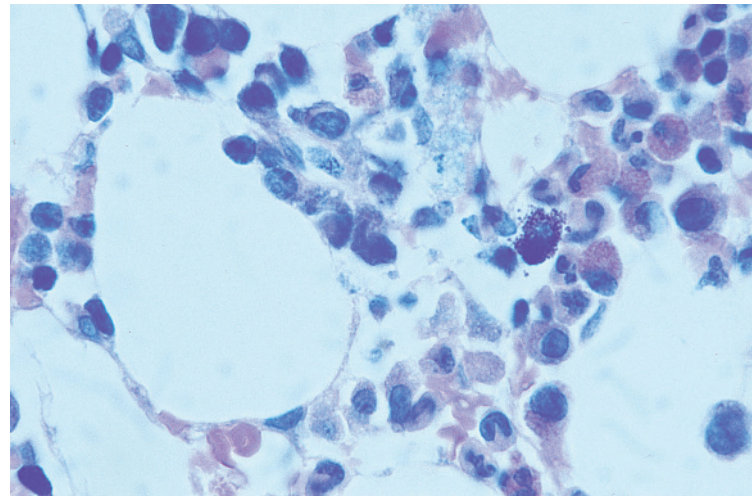


**Fig. 1.42** Section of a trephine biopsy specimen from a patient with renal failure: a mast cell and maturing granulocytes. Resin-embedded, Giemsa ×100.

also give positive reactions for chloroacetate esterase, are periodic acid–Schiff (PAS) positive and stain metachromatically with toluidine blue. They show tartrate-resistant acid phosphatase activity in EDTA- but not acid-decalcified specimens. Mast cells are distributed irregularly in the medullary cavity but are most numerous near the endosteum, in the periosteum, in association with the adventitia of small blood vessels and at the periphery of lymphoid nodules or aggregates [74]. They appear as elliptical or elongated cells with an average diameter of 12 μm. Their cytoplasmic projections stretch out between haemopoietic cells. Their distribution and cytological features can be demonstrated by immunohistochemistry (Figs 1.43 and 1.44).

## Ostoblasts and osteoclasts

Osteoblasts and osteoclasts differ in their origin but have complementary functions. Osteoblasts have a common origin with other mesenchymal cells and are responsible for bone deposition. Osteoclasts are formed by fusion of cells of monocyte lineage and are responsible for dissolution of bone.

### *Cytology*

Osteoblasts (Fig. 1.45) are mononuclear cells with a diameter of 20–50 μm. They have an eccentric nucleus, moderately basophilic cytoplasm and a Golgi zone that is not in

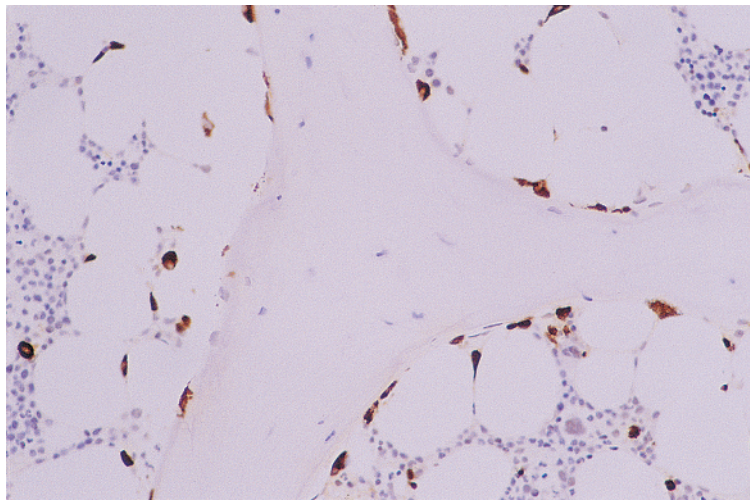


**Fig. 1.43** Section of a trephine biopsy specimen showing a reactive increase in interstitial and paratrabecular mast cells. Immunoperoxidase, mast cell tryptase (monoclonal antibody AA1) ×20.

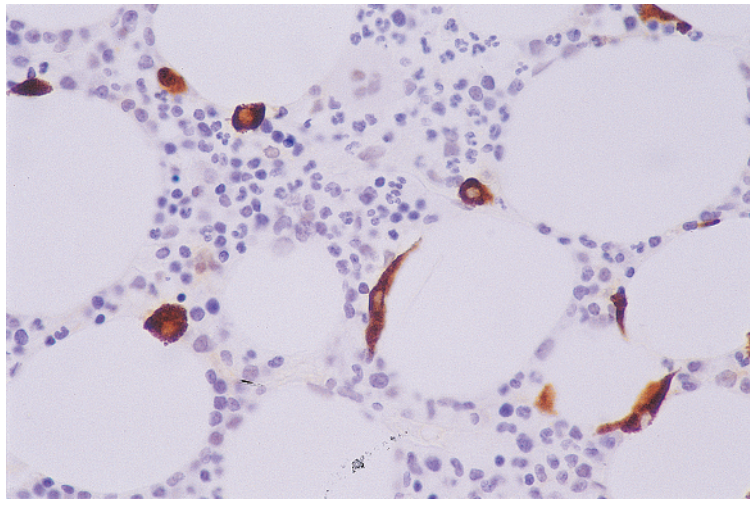


**Fig. 1.44** Sections of a trephine biopsy specimen showing a reactive mast cell increase; note that some mast cells are round and some are spindle-shaped. Immunoperoxidase, mast cell tryptase (monoclonal antibody AA1) ×40.

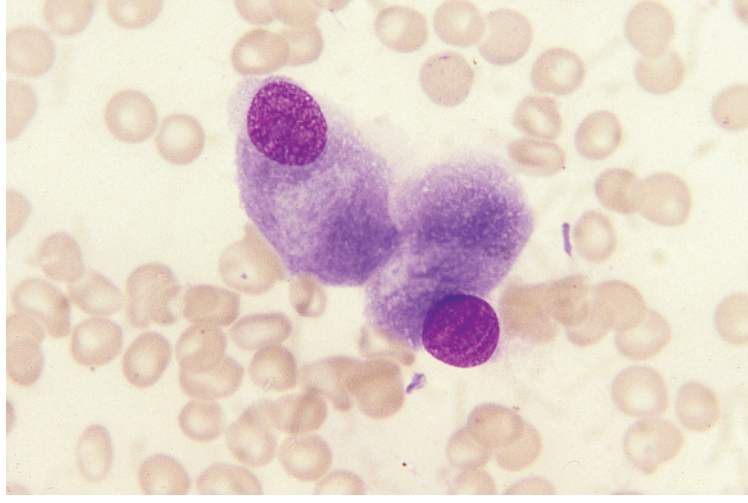


**Fig. 1.45** Aspirate of normal BM: two osteoblasts; note the eccentric nucleus and basophilic cytoplasm. These cells can be distinguished from plasma cells by their larger size and the position of the Golgi zone, which is not immediately adjacent to the nucleus. MGG ×100.

apposition to the nuclear membrane. The nucleus shows some chromatin condensation and may contain a small nucleolus. Osteoblasts can be distinguished from plasma cells, to which they bear a superficial resemblance, by the lesser degree of chromatin condensation and the separation of the Golgi zone from the nucleus. Osteoblasts are uncommon in bone marrow aspirates of healthy adults but, when present, often appear in small clumps. They are much more numerous in the bone marrow of children and adolescents.

Osteoclasts (Fig. 1.46) are multinucleated giant cells with a diameter of 30–100 μm or more. Their nuclei tend to be clearly separate, uniform in appearance and slightly oval with a single lilac-staining nucleolus. The voluminous cytoplasm contains numerous azurophilic granules, which are coarser than those of megakaryocytes. The cytoplasm is more basophilic in less mature cells. Osteoclasts are not commonly seen in marrow aspirates of healthy adults but are much more often seen in aspirates from children.

***Histology***

Osteocytes, osteoblasts and osteoclasts in histological sections are identified by their position and their morphological features. Osteocytes (Fig. 1.47) lie within bone lacunae. Osteoblasts (Figs 1.48 and 1.49) appear in rows

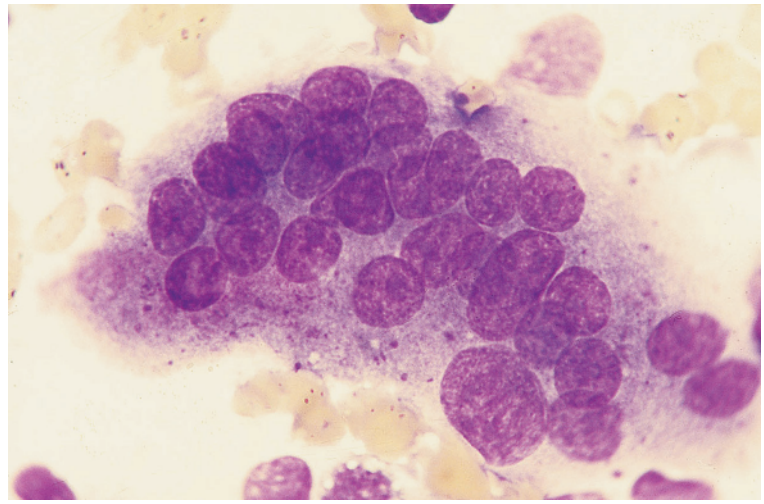


**Fig. 1.46** Aspirate of normal BM: an osteoclast; note the highly granular cytoplasm and the multiple nuclei, which are uniform in size and have indistinct, medium-sized, single nucleoli. MGG ×100.

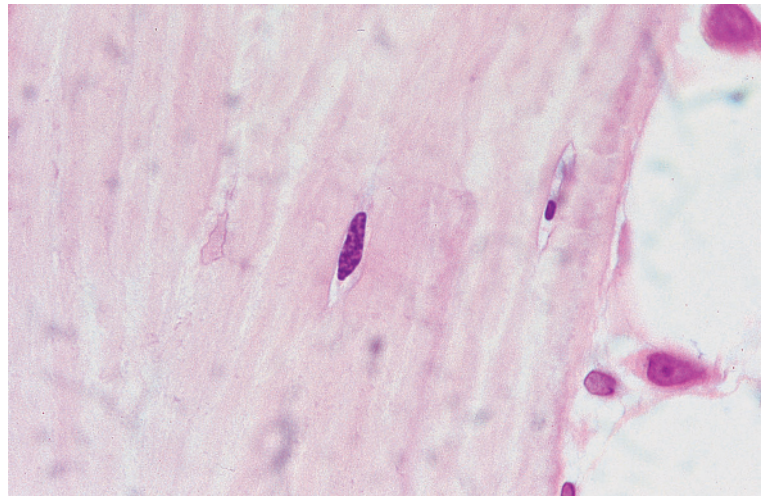


**Fig. 1.47** Section of normal BM: a bone spicule containing an osteocyte; note the myeloid cell in the adjacent marrow. Resin-embedded, H&E ×100.

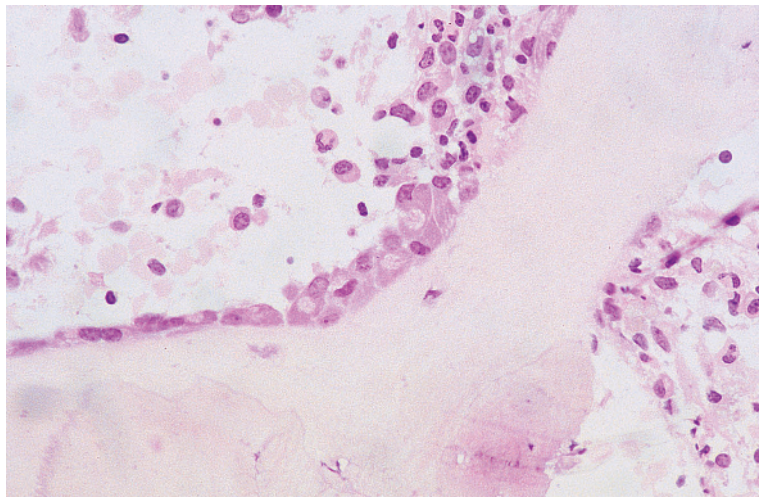


**Fig. 1.48** Section of BM from a patient with Fanconi anaemia: the trabecula is lined by osteoblasts; note the distinct Golgi zones which do not abut on the nuclear membrane. Resin-embedded, H&E ×40.

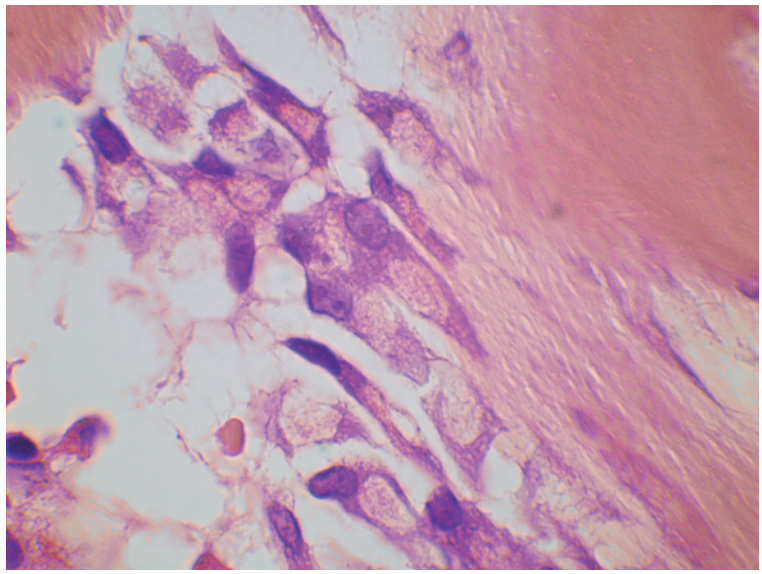


**Fig. 1.49** Trephine biopsy sections showing normal osteoblasts in the bone marrow of a child. Golgi zones are very clearly shown; the nuclei are oval and some contain a small nucleolus. H&E ×100.

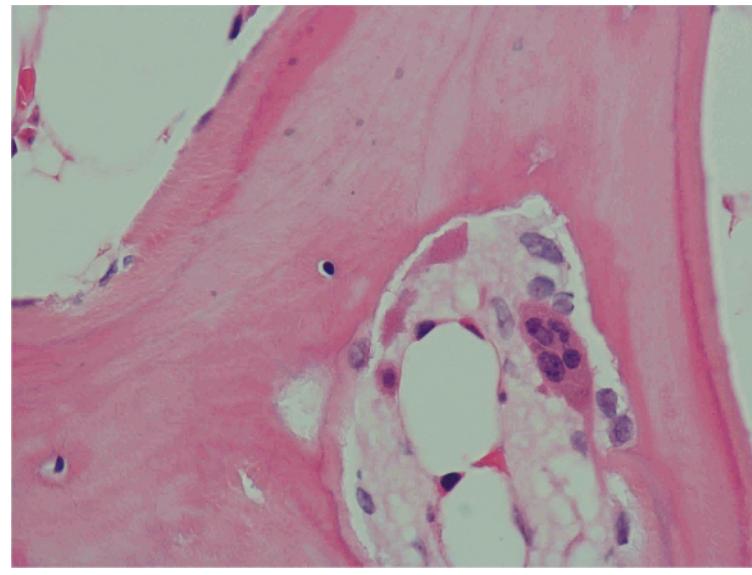
along a bone spicule or a layer of osteoid and their eccentric nuclei and prominent Golgi zones are apparent. A decline in number per unit area of bone occurs during the second and third decades [75]. Osteoclasts (Fig. 1.50) are likely to be found on the other side of a spicule from osteoblasts or some distance away. They are identified as multinucleated cells lying in hollows known as Howship lacunae. A decline in numbers occurs during the first and second decades [75]. They show tartrate-resistant acid phosphatase activity in EDTA- but not acid-decalcified specimens.

## Fat cells

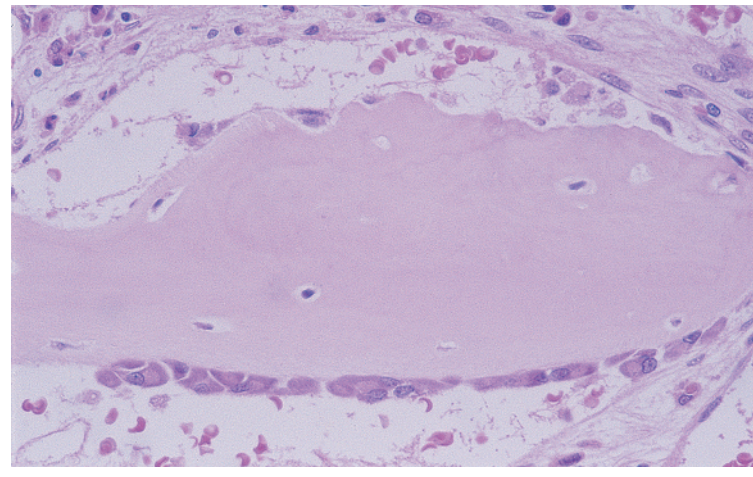
Fat cells are almost always recognizable in bone marrow specimens, exceptions being found in very young infants and when the bone marrow is markedly hypercellular.

### *Cytology*

Stromal fat cells are present mainly in aspirated fragments. Since the fat dissolves during processing the cytoplasm appears completely empty. In isolated fat cells (Fig. 1.51), an oval



(a)



(b)

**Fig. 1.50** (a) Section of BM from a patient with renal osteodystrophy: an osteoclast with four nuclei. H&E ×100. (b) Section of BM showing a bone spicule; one side is lined with osteoblasts while the other shows Howship lacunae, two of which contain osteoclasts. H&E ×40.

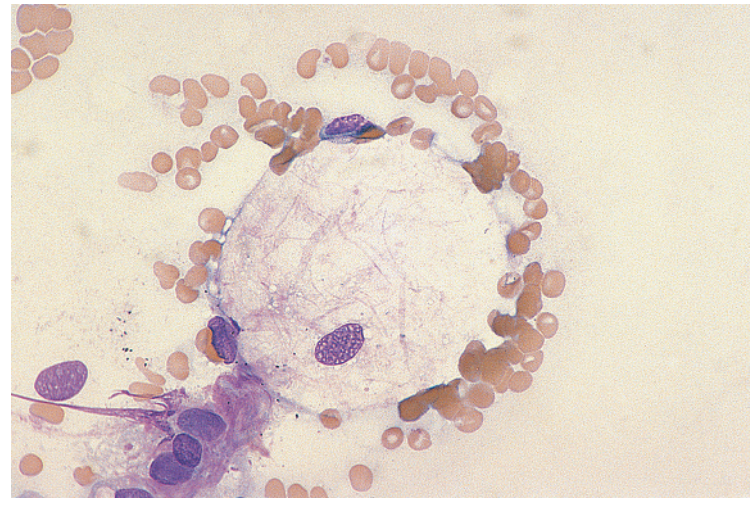


**Fig. 1.51** BM aspirate: a fat cell. MGG ×50.

nucleus, either peripheral or central, is present within apparently empty cytoplasm.

***Histology***

In sections of bone marrow, the fat cells appear in clusters, separated by haemopoietic tissue. They are often particularly prominent adjacent to trabeculae. Fat cells appear as empty spaces with an oval nucleus at one edge of the cell.

## Lymphopoiesis

### Lymphocytes

Both B and T lymphocytes share a common origin with myeloid cells, all of these lineages being derived from a pluripotent stem cell. The bone marrow contains mature cells and precursor cells of both T- and B-lymphoid lineages. T cells are more numerous among mature cells whereas among precursor cells those of B lineage are more frequent.

***Cytology***

Bone marrow lymphocytes are small cells with a high nucleocytoplasmic ratio and scanty, weakly basophilic cytoplasm. The nuclei show some chromatin condensation but the chromatin often appears more diffuse than that of peripheral blood lymphocytes. Lymphocytes are not very numerous in the marrow in the first few days of life but otherwise during infancy they constitute a third to a half of bone marrow nucleated cells [76]. Numbers decline during childhood and in adults they do not generally comprise more than 15–25% of nucleated cells, unless the marrow aspirate has been considerably diluted with peripheral blood. If there is no haemodilution they usually account for approximately 10% of nucleated cells. The majority of lymphocytes in normal bone marrow are CD8-positive T lymphocytes.

The bone marrow of healthy children may show significant numbers of immature cells with a cytological resemblance to leukaemic lymphoblasts, referred to as haematogones (see pages 368–369); these are B-lymphocyte precursors. They are present in smaller numbers in adults.

***Histology***

Normal marrow contains scattered interstitial lymphocytes and, sometimes, small lymphoid nodules or follicles. Estimates of lymphocyte numbers based on histological sections are considerably lower than those based on aspirates. In one study approximately 10% of bone marrow cells were lymphocytes, with the ratio of T to B cells being 6 : 1 [77]. In another investigation of a small number of subjects, not all of whom were strictly normal, the T : B cell ratio was 4–5 : 1 [78]. In a third study, median numbers were of the order of 2%, representing approximately equal numbers of B and T cells; the range of B cells, identified by CD20 reactivity, was 0% to 5.97%, while the range of T cells, identified by CD45R0 reactivity, was 0% to 6.7% [79]. In contrast to the peripheral blood, CD8-positive T lymphocytes are more numerous in the marrow than CD4-positive cells. Lymphocytes appear to concentrate around arterioles near the centre of the haemopoietic cords. Lymphoid follicles of normal marrow have small blood vessels at their centre and may contain a few macrophages, peripheral mast cells or plasma cells. Lymphoid follicles are discussed further on pages 152–154.

### Plasma cells

***Cytology***

Plasma cells (Fig. 1.52) are infrequent in normal bone marrow, in which they rarely constitute more than 1% of nucleated cells. In healthy children they are even less frequent [80]. They are distinctive cells with a diameter of 15–20 μm and an eccentric nucleus, moderately basophilic cytoplasm and a prominent paranuclear Golgi zone. The cytoplasm may contain occasional vacuoles and sometimes appears pink with an MGG stain, consequent on the presence of carbohydrate. The nuclear chromatin shows prominent coarse clumps, although the clock-face chromatin pattern that is often discernible in histological sections is usually less apparent in films. Occasional normal plasma cells have two or more nuclei. Plasma cells may occur in small clumps and may be detected within aspirated marrow fragments and around capillaries.

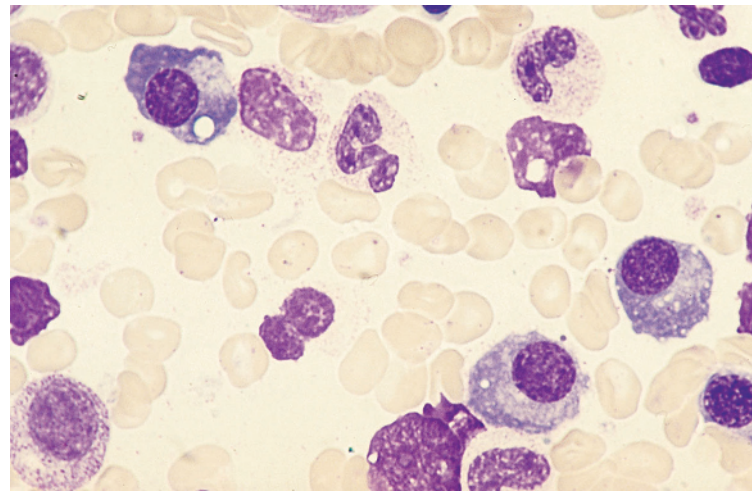


**Fig. 1.52** Aspirate of BM from a patient with an inflammatory condition: three plasma cells; note the basophilic cytoplasm, eccentric nucleus and Golgi zone adjacent to the nucleus. MGG ×100.

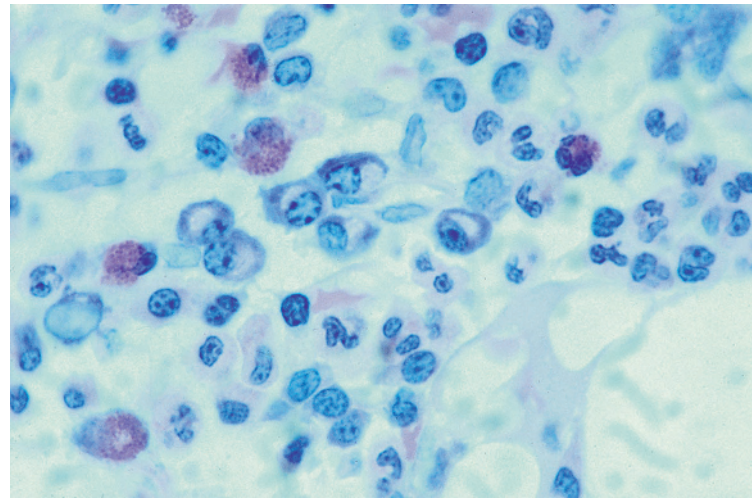


**Fig. 1.53** Section of BM from a patient with Hodgkin lymphoma (without marrow infiltration): pericapillary plasma cells, neutrophils, eosinophils and erythroblasts. Resin-embedded, Giemsa ×100.

*Histology*

Normal marrow contains scattered interstitial plasma cells but plasma cells may also be associated with macrophages and are preferentially located around capillaries (Fig. 1.53). Typical mature plasma cells in histological sections are readily identified by their eccentric nuclei and prominent Golgi zones. The chromatin is coarsely clumped and often distributed at the periphery of the nucleus with clear spaces between the chromatin clumps, giving the appearance of a cartwheel or clock-face. In Giemsa-stained sections the cytoplasm, with the exception of the Golgi zone, is deeply basophilic.

## Storage and erythroblast iron

*Cytology*

A Perls stain permits the evaluation of macrophage and erythroblast iron (see page 67). It should be noted that a significant proportion of healthy, haematologically normal women do not have any storage iron in bone marrow macrophages. This was observed in two of 17 in a UK population, with another four individuals having only a trace of iron [62], and in nine of 17 subjects in a Finnish population [81]. Erythroblasts of iron-replete subjects contain a very variable number of fine Perls-positive granules that represent haemosiderin [62].

Unless iron supplementation is given, iron stores fall during pregnancy and storage iron may be absent [82].

***Histology***

A Perls stain of trephine biopsy sections often shows the presence of iron and increased iron stores may be recognised. However it should be noted that iron may have been leached from paraffin-embedded decalcified specimens so that assessment of iron stores is not reliable. In addition, normal siderotic granules cannot be recognized in such sections. Sections of bone marrow fragments that have not been decalcified can, however, be assessed reliably.

## The cellular composition of bone marrow

***Cytology***

The cellular composition of aspirated bone marrow is determined by the volume of the aspirate since the larger the volume aspirated the more sinusoidal blood is sucked into the aspirate. Dilution of marrow with blood leads to a higher percentage of lymphocytes and mature granulocytes and a lower percentage of granulocyte and erythroid precursors. Dresch *et al*. [83] found, for example, that as the volume of aspirate from the sternum increased from 0.5 to 4.5 ml, the total concentration of nucleated cells fell to about one sixth; the percentage of granulocyte precursors (myeloblasts to metamyelocytes) declined from approximately 55% to approximately 30%, while the percentage of mature neutrophils showed a more than twofold rise. Ideally, a cell count should be performed on films prepared from the first one or two drops of aspirated marrow. If large volumes are required for further tests a second syringe can be applied to the needle after the syringe containing the first few drops has been removed. The differential count is then representative of the cellular composition of the bone marrow.

Determining the cellular composition of marrow requires that large numbers of cells be counted so that a reasonable degree of precision is achieved. This is particularly important when the cell of interest is one that is normally infrequent, such as the myeloblast or the plasma cell. A 500 cell count provides a reasonable compromise between what is desirable and what is practicable. The cell count should be performed in the trails behind fragments so that the cells counted represent cells that have come from fragments rather than contaminating peripheral blood cells. Alternatively, the cell count can be performed on squashed bone marrow fragments. Because some cells, for example plasma cells and lymphocytes, are distributed unevenly through the marrow it is important to count the trails behind several fragments or several squashed fragments. Because cells of different lineages may not be released from the fragments into the trails to the same extent there may not be good correlation between differential counts on wedge-spread films and squash preparations. It is likely that counts on the latter are more valid. However, counts are usually performed on wedge-spread films and hence published reference ranges are based on such counts.

It is customary and useful to determine the myeloid : erythroid (M : E) ratio of aspirated marrow since consideration of this value, together with an assessment of the overall cellularity, allows an assessment of whether erythropoiesis and granulopoiesis are reduced, normal or increased. It is simplest to include in the myeloid component all granulocytes and their precursors and any monocytes and their precursors. However, some haematologists exclude mature neutrophils and others include neutrophils but exclude eosinophils, basophils and monocytes. These inclusions and exclusions will make a slight difference to what is regarded as a normal M : E ratio but their effects are heavily outweighed by differences caused by different aspiration volumes. The larger the volume of the aspirate the higher the M : E ratio, particularly if mature neutrophils are included in the count.

The bone marrow at birth has major erythroid and myeloid components with few lymphocytes and very few plasma cells [76,80,84,85] (Fig. 1.54). The percentage of erythroid cells declines steeply in the first weeks [84,85]. The percentage of lymphocytes increases during the first month and

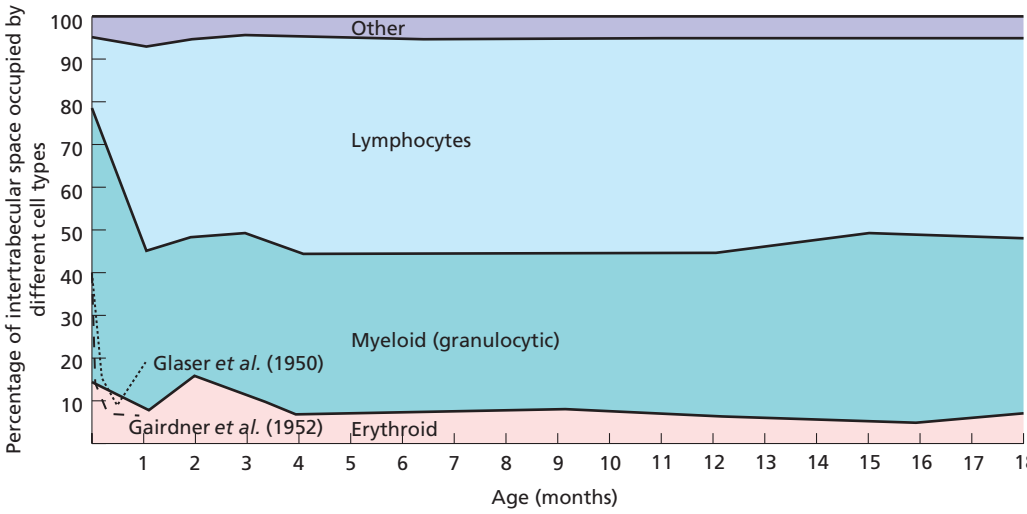



**Fig. 1.54** The percentage of the intertrabecular space occupied by cells of different lineages during the first 18 months of life, derived from Rosse *et al.* [76]; the higher initial erythroid percentage and sharp fall in erythroblast number observed by Glaser *et al.* [84] and Gairdner *et al.* [85] are shown as dotted and dashed lines.

remains at a high level until 18 months of age [76]. In children over the age of 2 years, the proportions of different cell types do not differ greatly from those in normal adult bone marrow. However, children may have increased numbers of immature lymphoid cells (see pages 368–369). Typical values determined for the cellular composition of normal marrow at various ages are shown in Tables 1.1 and 1.2 [62,76,84–90]. Bain [62] found a significantly higher proportion of granulocytes in the bone marrow of women than of men. This was not observed in a smaller cohort studied by den Ottolander [91]. Reference ranges derived from squash preparations differ from those derived from wedge-spread films [50]. Neutrophils and monocytes are more numerous in films while granulocyte precursors and erythroblasts are less numerous; plasma cells and mature eosinophils are more numerous in squash preparations while lymphocytes do not differ between the two preparations [50]. The M : E ratio is significantly lower, with a mean of 1.6 compared with a mean of 2.7, and with a narrower reference range [50].

The bone marrow of healthy volunteers shows a low proportion of cells with features that could be regarded as dysplastic, such as erythroid cells showing cytoplasmic bridging or megakaryocytes with non-lobated nuclei [92]. Fernández-Ferrero and Ramos, studying haematologically normal surgical patients, found the frequency of these minor dysplastic features to increase with age [93].

### *Histology*

It is possible to perform differential counts and estimate an M : E ratio from resin-embedded bone marrow biopsy sections [94,95] although this is not necessary in practice. Such counts have the potential to be more accurate than those obtained from aspirates since there is no dilution with sinusoidal blood. It is also possible that larger cells or cells adjacent to trabeculae might be less likely to be aspirated. However, an element of inaccuracy is introduced by the fact that larger cells appear at more levels of the biopsy and so are more likely to be counted in any given section. Lack of dilution by blood means that the estimated M : E ratio is likely to be lower than that determined from an aspirate. This is borne out by the results of a study of 13 healthy subjects which found a mean M : E ratio of 1.52 with a range of 1.36–1.61 [94]. The M : E ratio can also be estimated from a paraffin-embedded section.

**Table 1.1** Mean values (observed range) for bone marrow cells in healthy infants and children.

| | Birth [44] (*n* = 57) | 0–24 hours [85] (*n* = 19) | 8–10 days [84] (*n* = 23) | 3 months [85] (*n* = 12) |
|---|---|---|---|---|
| M : E ratio | 4.4 | 1.2 | 1.35 | 2.4 |
| Myeloblasts (%) | 0.3* | 1.0 (0.5–2) | 1.0 (0–3) | 1.5 (0–4) |
| Promyelocytes (%) | 0.8 | 1.5 (0.5–5) | 2.0 (0.5–7) | 2.0 (1.5–5) |
| Myelocytes (%) | 4 | 4.0 (1–9) | 4.0 (1–11) | 5.0 (0.5–16) |
| Metamyelocytes (%) | 19 | 14.0 (4.5–25) | 18.0 (7–35) | 11.0 (3–33) |
| Bands (%) | 29 | 22.0 (10–40) | 20.0 (11–45) | 15.0 (2–24) |
| Neutrophils (%) | 7 | | | |
| Eosinophil series (%) | 2.7 | 3.5 (1–8)† | 3.0 (0–6)† | 2.5 (0–6)† |
| Basophil series (%) | 0.12 | – (0–1.5)† | – (0–1)† | – (0–0.5) |
| Monocytes (%) | 0.9 | – (0–2.5)† | 1.0 (0–3) | 0.5 (0–1) |
| Erythroid (%) | 14.5 | 39.5 (23.5–70)† | 7.5 (0–20.5)† | 16.0 (3.5–33.5)† |
| Lymphocytes (%) | 15 | 12.0 (4–22) | 37.0 (20–62) | 47.0 (31–81) |
| Plasma cells (%) | 0 | 0 | 0 | 0 |

* 'Unknown blasts'.
† Approximate (sum of ranges for different categories).
E, erythroid; M, myeloid.

**Table 1.2** Mean values (95% ranges) for bone marrow cells in sternal or iliac crest aspirates of healthy adult Caucasians.

| | 20–29 years [87] | 20–30 years [88] | 20–30 years [88] |
|---|---|---|---|
| | Males and females (*n* = 28), sternum | Males (*n* = 52), sternum | Females (*n* = 40), sternum |
| Volume aspirated (ml) | ≤0.5 | 0.2 | 0.2 |
| M : E ratio | 3.34 | – | – |
| Myeloblasts (%) | 1.21 (0.75–1.67) | 1.32 (0.2–2.5) | 1.2 (0.1–2.3) |
| Promyelocytes (%) | 2.49 (0.99–3.99) | 1.35 (0–2.9) | 1.65 (0.5–2.8) |
| Myelocytes (%) | 17.36 (11.54–23.18) | 15.0 (7.5–22.5) | 16.6 (11.4–21.8) |
| Metamyelocytes (%) | 16.92 (11.4–22.44) | 15.7 (9.2–22) | 15.8 (11.0–20.6) |
| Band cells (%) | 8.70 (3.58–13.82) | 10.5 (3–17.9) | 8.3 (4–12.4) |
| Neutrophils (%) | 13.42 (4.32–22.52) | 20.9 (9.9–31.8) | 21.7 (11.3–32) |
| Eosinophils (%) | 2.93 (0.28–5.69)* | 2.8 (0.1–5.6)* | 3 (0–7.2)* |
| Basophils (%) | 0.28 (0–0.69)* | 0.14 (0–0.38) | 0.16 (0–0.46) |
| Monocytes (%) | 1.04 (0.36–1.72) | 2.3 (0.5–4) | 1.61 (0.2–3) |
| Erythroblasts (%) | 19.26 (9.12–29.4)† | 12.9 (4.1–21.7) | 11.5 (5.1–17.9) |
| Lymphocytes (%) | 14.60 (6.66–22.54) | 16.8 (7.2–26.3) | 18.1 (10.5–25.7) |
| Plasma cells (%) | 0.46 (0–0.96) | 0.39 (0–1.1) | 0.42 (0–0.9) |

* Including eosinophil and basophil myelocytes and metamyelocytes.
† Approximate (sum of ranges for different categories of erythroblast).
‡ Promyelocytes were categorized either with myeloblasts or with myelocytes.
§ Neutrophils plus precursors : erythroblasts.
‖ Including basophil precursors and mast cells.
¶ Neutrophil plus eosinophil myelocytes: mean and range: 8.9 (2.14–15.3); band cells included in neutrophil category; macrophages: mean and range: 0.4 (0–1.3).
E, erythroid; M, myeloid.

**Table 1.1** (continued)

| 3 months [76] (*n* = 24) | 1 year [38] (*n* = 12) | 18 months [38] (*n* = 19) | 2–6 years [46] (*n* = 12) | 2–9 years [47] (*n* = 13) |
|---|---|---|---|---|
| 4.9 | 4.8 | 5 | 5.8 (2–13) | 5.3 |
| 0.6* | 0.5* | 0.4* | 1.0 | 1.3 (0.7–1.8) |
| 0.8 | 0.7 | 0.6 | 0.5 | 2.8 (0.8–4.8) |
| 2 | 2 | 2.5 | 17 | 26.7 (18–35)† |
| 12 | 11 | 12 | 20 | 22.0 (15.7–29) |
| 15 | 14 | 14 | 11 | 4.5 (0.9–8) |
| 3.5 | 6 | 6 | 10 | 8.3 (2.6–14) |
| 2.5 | 2 | 3 | 6 | 1.2 (0–2.5) |
| 0.1 | 0.1 | 0.1 | – | 0 |
| 0.7 | 1.5 | 2 | 0.4 | 0 |
| 12 | 8 | 8 | 13 | 12.5 (9.5–22.3)† |
| 44 | 49 | 46 | 21 | 18.2 (8.5–28) |
| 0 | 0.03 | 0.06 | – | 0.13 (0.05–0.41) |

**Table 1.2** (continued)

| 17–45 years [80] | Age not stated [50] | 21–56 years [29] | Age not stated [51] |
|---|---|---|---|
| Males (*n* = 42) and females (*n* = 8), sternum | Males (*n* = 12), sternum | Males (*n* = 30) and females (*n* = 20), iliac crest | Males (*n* = 53) and females (*n* = 14), site not stated |
| 3 | – | 0.1–0.2 | – |
| 6.9 | 2.3 (1.1–3.5)§ | 2.4 (1.4–3.6) | 2.2 (0.8–3.6) |
| 1.3 (0–3) | 0.9 (0.1–1.7) | 1.4 (0–3) | 0.4 (0–1.3) |
| –‡ | 3.3 (1.9–4.7) | 7.8 (3.2–12.4) | 13.7 (8–19.4) |
| 8.9 (3–15) | 12.7 (8.5–16.9) | 7.6 (1.9–13.3)¶ | |
| 8.8 (4–15) | 15.9 (7.1–24.7) | 4.1 (2.3–5.9) | |
| 23.9 (12.5–33.5) | 12.4 (9.4–15.4) | – | 35.5 (22.2–48.8) |
| 18.5 (9–31.5) | 7.4 (3.8–11) | 34.2 (23.4–45)¶ | |
| 1.9 (0–5.5) | 3.1 (1.1–5.2)* | 2.2 (0.3–4.2) | 1.7 (0.2–3.3) |
| 0.2 (0–1) | <0.1 (0–0.2)‖ | 0.1 (0–0.4) | 0.2 (0–0.6) |
| 2.4 (0–6) | 0.3 (0–0.6) | 1.3 (0–2.6) | 2.5 (0.5–4.6) |
| 9.5 (2.5–17.5) | 25.6 (15–36.2) | 25.9 (13.6–38.2) | 23.6 (14.7–32.6) |
| 16.2 (7.5–26.5) | 16.2 (8.6–23.8) | 13.1 (6–20) | 16.1 (6.0–26.2) |
| 0.3 (0–1.5) | 1.3 (0–3.5) | 0.6 (0–1.2) | 1.9 (0–3.8) |

## Interpretation of bone marrow aspirates and trephine biopsies

Examination of a bone marrow aspirate in isolation permits cytomorphological features to be ascertained but does not permit full interpretation of the findings. The haematologist/haematopathologist must also know the age and sex of the patient, the full blood count and relevant clinical details, and must have examined a peripheral blood film. Similarly, examination of trephine biopsy sections in isolation permits detection of histomorphological abnormalities but not a full assessment of a case. The pathologist should beware of the risks of either over-interpreting biopsy findings or failing to offer an adequate interpretation because of lack of consideration of clinical and haematological features and aspirate findings. It is desirable that trephine biopsy specimens are either reported by a haematopathologist able also to interpret bone marrow aspirates or that the histopathologist and haematologist examine aspirate films and biopsy sections together. It should be appreciated that a trephine biopsy is just one part of a jigsaw puzzle and it may not always be possible to make a definitive diagnosis. Sometimes it is desirable to seek a second opinion. When this is so, it is essential that the haematopathologist is sent full clinical and haematological information. Films of the peripheral blood and the bone marrow aspirate should accompany trephine biopsy specimens being sent for a second opinion, with all material being carefully labelled and dated.

## Examination of bone marrow aspirate films

A minimum of three or four films should always be stained and examined. These should include a Perls-stained film for all initial diagnostic aspirates. If there is a likelihood of infiltration of the marrow and the first films do not show any abnormality, it is important to stain and examine a larger number. In addition to wedge-spread films, squashed bone marrow fragments should be examined in all patients. Squashed fragments may reveal abnormal cells that are trapped in fragments and absent from wedge-spread films. For example, a diagnosis of systemic mastocytosis may be suggested by a squash preparation but not by a wedge-spread film. The percentage of blast cells may be higher in a squash preparation leading to a diagnosis of acute myeloid leukaemia rather than myelodysplastic syndrome, or a diagnosis of high grade rather than low grade myelodysplastic syndrome. Similarly, the percentage of plasma cells in multiple myeloma is significantly higher in squash preparations than in wedge-spread films [96].

Bone marrow films should first be examined under low power (×10 objective) in order to assess cellularity and megakaryocyte numbers and to scan the entire film for any abnormal infiltrate. The film should then be examined with a ×40 or ×50 objective, which will allow appreciation of most morphological features. At this stage, all cell populations should be specifically and systematically examined from the point of view of both numbers and morphology – the erythroid lineage, granulocytic lineages including eosinophils and basophils, megakaryocytes, lymphocytes and plasma cells. Consideration should be given to whether there is an increased number of mast cells, macrophages, osteoblasts or osteoclasts and whether any non-haemopoietic cells are present. Fragments should be examined not only to assess cellularity but also to determine if any cells have been preferentially retained in the fragments, such as mast cells or myeloma cells. Increased storage iron can also sometimes be detected in fragments stained by MGG, haemosiderin having a greenish tinge (Fig. 1.55). Only when a thorough assessment of several films has been carried out with a ×40 or ×50 objective should the film be examined under high power (×100) with oil immersion in order to assess fine cytological detail. A differential count of cells in the trails behind several fragments is best carried out under high power but only after assessment of whether there is any increase of minority populations, for example blast cells or plasma cells, confined to one film or to the cell trail behind one fragment. It can be useful to have an oil immersion ×50 or ×60 objective as well as a ×100 as it is then easy to switch between lenses for an overall and more detailed view of the same area. Perls-stained films should similarly be examined under low power to assess storage iron, with a ×40 or ×50 objective to detect abnormally prominent sideroticgranulation and with a ×100 objective to

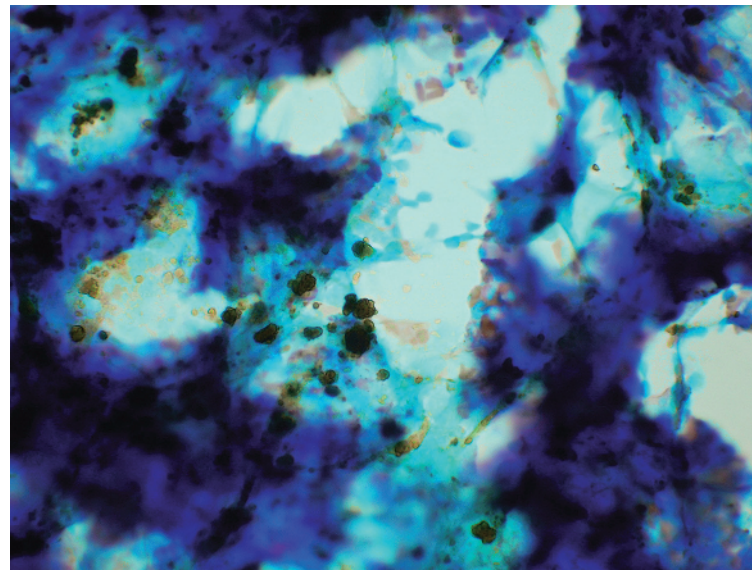


**Fig. 1.55** BM aspirate showing increased haemosiderin. MGG ×50.

assess whether siderotic granulation is reduced, normal or increased.

Films of squashed bone marrow fragments should similarly be examined in a systematic manner.

## Reporting a bone marrow aspirate

The report of a bone marrow aspirate should commence with the clinical details given to the haematologist and a record of the full blood count and peripheral blood film appearances at the time of bone marrow aspiration. There should then be a statement as to the site of aspiration, the texture of the bone and the ease of aspiration. The aspirate report should include an assessment of overall cellularity, an M : E ratio and a description of each lineage. Storage iron in fragments and siderotic granules in cells should be described. If a trephine biopsy was performed this should be stated and, if a trephine biopsy would normally have been expected but was not performed or was unsuccessful, this should also be included in the report. Any supplementary tests for which samples were taken, for example immunophenotyping, cytogenetic analysis or molecular genetic analysis, should be listed so that the clinician is aware that other results are to be expected. Finally, a brief summary of significant findings should be made and an interpretation offered, bearing in mind that this might be the only part of the report read by some clinicians. Reports should distinguish between factual statements and opinions. The description of the aspirate should be purely factual whereas it is useful for the final summary to include an explanation of the significance of the findings and, when relevant, suggestions for further tests. The level of certainty of any opinion should be expressed by careful use of terms such as ‘diagnostic of’, ‘suggestive of’ or ‘consistent with’. If an attempted aspirate fails or there is a ‘dry tap’ a report should be issued stating this. The report should be signed or computer-authorized by the haematologist or haematopathologist responsible for it.

## Examination of trephine biopsy sections

The interpretation of trephine biopsy sections is often viewed as one of the more difficult areas of histopathology. This is probably because the organized structure of haemopoietic tissue is not as readily apparent as that of many other tissues. However, as the preceding part of this chapter illustrates, the bone marrow is actually highly organized, with the various elements maturing in different microanatomical sites. Failure to recognize this and failure to identify individual categories of cell

may lead to a lack of systematic analysis, with diagnoses being made only by a process of pattern recognition. Conversely, the haematologist, although experienced in cytology, may be unfamiliar with the interpretation of tissue sections, in which architectural features are often of prime importance.

A systematic approach, which is essential for accurate diagnosis, requires a working knowledge of the normal micro-anatomy and the pathological changes that can occur, coupled with a methodical examination of the various component parts. Initially, the whole section should be examined at low power, preferably using a ×4 objective. This allows a general impression of the biopsy specimen to be gained, including overall cellularity and megakaryocyte number and distribution. Abnormalities of the bone are often apparent at this magnification. It should also be noted if the biopsy specimen is too small, or is composed largely of cortical bone and subcortical marrow, or shows crush artefact or other artefactual distortion of the architecture. Focal lesions, such as granulomas or infiltrates of metastatic tumour or lymphoma, are often better appreciated on low power examination. Inclusion of extracortical components in trephine sample collection should not be encouraged but, when present, these components should be included in the overall histological assessment. For example, they may be a site of amyloid deposition in patients with plasma cell neoplasms or suspected amyloid elsewhere (Fig. 1.56).

Following low power examination, the bone, haemopoietic elements and marrow stromal elements should be studied using medium power (×10 or ×20 objective) and a high power dry objective (×40 or ×60); examination under oil immersion (×100 objective) is not necessary as a routine but may be useful to study fine cytological detail. The bone should be examined for trabecular thickness, number of osteoblasts and osteoclasts, and presence and number of Howship lacunae; undecalcified resin-embedded sections should be assessed for the quantity of osteoid (see Chapter 11). With a little experience, visual estimations of the marrow cellularity, of the relative amounts of granulocytic and erythroid elements, and of any deviations from normal can easily be made. The next step is to examine the various haemopoietic elements for the following features:

**1** *Erythroid series*: the proportion of erythroid cells and relative proportions of cells at different stages of maturation; presence, appearance and location of erythroblastic islands; morphology of erythroblasts including any evidence of dyserythropoiesis.

**2** *Granulocytic series*: the proportion of granulocytic cells (eosinophil and neutrophil lineages); morphology and relative proportions of immature and mature granulocytic precursors; position of immature precursors (promyelocytes and myeloblasts); morphology of granulocytic cells.

**3** *Megakaryocytes*: number of megakaryocytes; megakaryocyte morphology (cell size and nuclear features) and localization; presence or absence of megakaryocyte clusters.

**4** *Lymphoid cells*: number, localization and morphology of lymphocytes; presence, position and morphology of lymphoid aggregates; number, localization and morphology of plasma cells.

**5** *Macrophages and mast cells*: number of macrophages; presence of haemophagocytosis; intracellular microorganisms (usually fungi or protozoa); evidence of lysosomal storage disorders such as Gaucher disease; granulomas; mast cell numbers, morphology and localization.

It is easy to neglect the stromal elements, yet these are disturbed in a variety of conditions. Important changes that should be noted include: oedema; gelatinous change; necrosis; fibrosis; ectasia of sinusoids; vasculitis; amyloid deposition; and bone abnormalities.

A stain for reticulin should be examined in every case. In some laboratories an iron stain is also examined in all cases but this is not the policy in all of the authors' laboratories. Choice of further histochemical or immunohistochemical stains is dependent on clinical features and histological findings.

Obviously, in many cases, as when there is heavy infiltration by leukaemic cells or metastatic carcinoma, the above scheme is modified.

### *Problems and pitfalls*

Some fine details are not readily apparent in H&E-stained trephine biopsy sections, for example reduced haemoglobinization of erythroid cells, hypogranular neutrophils and

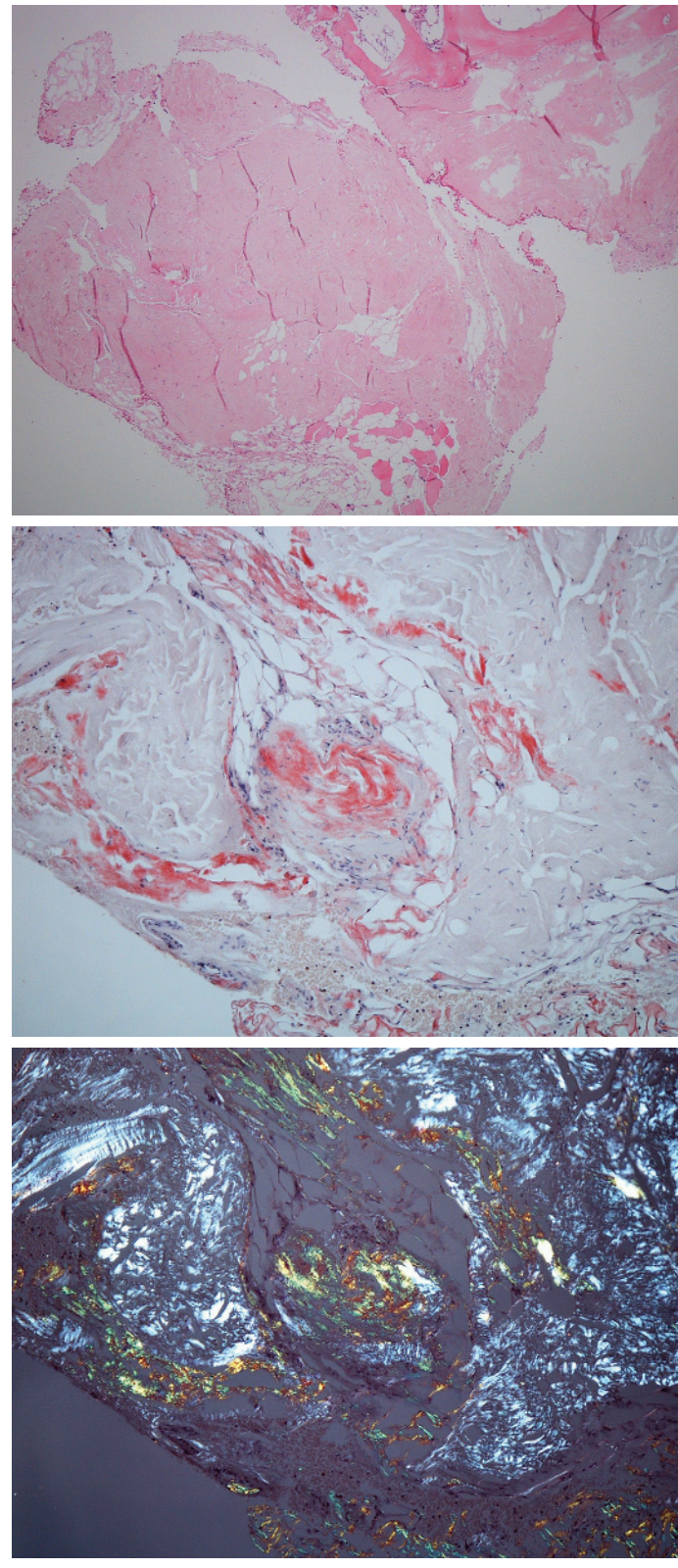



**Fig. 1.56** BM trephine biopsy section from a patient in whom amyloidosis was suspected showing light-chain-associated amyloid in extracortical fascia; this was barely detectable on H&E staining but was confirmed by Congo red staining. (a) H&E ×4. (b) Congo red ×10. (c) Congo red, polarized light ×10.

Pelgeroid neutrophils. Basophils cannot be distinguished from neutrophils.

## Reporting trephine biopsy sections

The trephine biopsy report [97] should include a statement as to the length of the biopsy specimen and its integrity. The report of the microscopic appearance should describe the cellularity and any abnormalities in bone, stroma or haemopoietic tissue. The reticulin stain and, if performed, the iron stain should be described. In sections from a resin-embedded specimen, iron stores should be graded; with a decalcified biopsy specimen an assessment should be made as to whether stainable iron is absent, present or increased but further grading should not be attempted (see pages 67–69). Any other histochemical or immunohistochemical stains should be described. In describing the results of immunohistochemistry, antibodies with a CD designation should be identified by the appropriate CD number.

Following a description of biopsy histology, a conclusion should be given in which all relevant findings are summarized and interpreted (as for the bone marrow aspirate, bearing in mind that many clinical staff will read only the summary). The level of certainty of any opinion should be expressed by careful use of terms such as 'diagnostic of', 'suggestive of' or 'consistent with'. If the report is provisional, either because further investigations are pending or because a second opinion is being sought, this should be stated clearly in the concluding summary. The report must be signed or computer-authorized by the responsible pathologist or haematologist.

## Guidelines, integrated reporting and audit

Guidelines for best practice in performing, processing and reporting bone marrow aspirates and trephine biopsies have been published [98,99]. If sedation is employed, guidelines for safe practice should be followed [11].

Optimal practice dictates that, for haematological neoplasms, an integrated final report should be assembled. This should include the results of all tests performed on a bone marrow aspirate and trephine biopsy specimen, interpreted in the context of full clinical and haematological information. When appropriate, results of peripheral blood analyses (e.g. clonality studies, immunophenotyping, molecular analysis, human T-cell lymphotropic virus 1 (HTLV-1) serology) should also be included. For the National Health Service in England and Wales, this advice is included in a guideline from the National Institute for Health and Care Excellence (NICE), *Haematological cancers: improving outcomes* [100]. Ideally, information technology systems should facilitate the production of integrated reports. When necessary, a provisional report should be issued with a final report being produced when the results of all supplementary investigations are available. The provisional report should make clear that a definitive report will follow. The final report should include a clear statement of the definitive diagnosis along with the degree of confidence and any suggestions for further investigation.

Periodic audit of clinical and laboratory procedures is advised and national schemes to document morbidity and mortality are recommended.

## Digital pathology

Digital pathology systems have been used for diagnostic reporting of surgical pathology specimens for over a decade. Widespread adoption was slow initially, but use has increased rapidly more recently, driven by improvements in the technology and the need to work remotely during the COVID-19 (corona virus disease 2019) pandemic. Three of us have now been using a digital pathology system to report all bone marrow trephine specimens for several years.

In haematology departments, digital imaging systems have been used mainly to view and analyse blood films but this technology is now starting to be applied to bone marrow aspirate films. There are technical constraints for digital image quality for such films, particularly from marrow aspirates, which are inherently uneven in the vertical ('Z') plane in comparison with the generally consistent thickness of a histology section.

Digital pathology systems allow pathologists to view high resolution whole slide digital images (WSI) of tissue sections at computer workstations rather than viewing the glass slides at a microscope.

A fully integrated digital pathology system consists of the following elements:

**1** High throughput automated slide scanners to capture WSI, usually at objective ×40.

**2** Computer servers to store the WSI data files, along with archive servers for long-term storage of images.

**3** Image management software (IMS) to integrate the WSI with the laboratory information system to link to case metadata (case number, patient demographics, date and type of biopsy, etc.) and slide-level metadata (slide number and type of stain). The IMS streams data from the WSI data file to allow panning across the slide and zooming through magnifications, giving a similar experience to that of viewing a glass slide using a conventional microscope. In addition, WSI can be directly compared side by side and easily annotated and selected images can be photographed as screen-shots. IMS can also integrate with other artificial intelligence (AI) image analysis tools to detect, classify and measure features of interest. Most IMS can be accessed by major internet browsers such as Google Chrome and Microsoft Edge.

**4** Digital pathology workstations are personal computers with large high definition or ultra-high definition (UHD) monitors used to access the IMS and view the WSI via internet browser software. Attention is needed to the environment in which these are placed (lighting, ventilation, ergonomics, ambient sounds, etc.), particularly when they are to be used in the home or in other non-clinical office environments.

**5** Computer networks with sufficient bandwidth capacity to allow uploading of high volumes of WSI data files from scanners to storage servers and streaming WSI data from the servers to the viewing software on workstations.

It is essential to have a scientific workforce with capacity and training specific for scanning and integrating WSI and patient data into the IMS and the laboratory information management system (LIMS). Personnel also need to have specific training to be competent and safe working (remotely, if appropriate) using WSI within the full scope of their digital diagnostic practice. In the UK the Royal College of Pathologists has issued guidance on the training and validation of pathologists for reporting cases using digital pathology systems [101,102].

A digital pathology workstream is dependent on all of the above elements operating together as a seamless system to deliver an effective diagnostic service.

Advantages of digital pathology include:

- The ability to view cases remotely and share cases for consultation without requiring physical access to the glass slides.
- Potentially greater efficiency when reviewing cases, particularly complex cases with multiple slides and immunohistochemical stains.
- Easier side by side comparison of the H&E stains and immunohistochemical stains performed on different sections from a single biopsy specimen (Fig. 1.57).
- Annotation of individual image files for quick reference to features of interest for multidisciplinary team discussion.
- Cases can be made available for teaching and training more easily than when using glass slides.
- Over time, the acquisition of a local archive of diagnostic WSI makes comparison of sequential samples from individual patients much quicker and easier than relying on retrieval of glass slides from storage.
- Automated image analysis tools can be developed by AI machine learning systems using large datasets of WSI.

Disadvantages of digital pathology include:

- Cost – systems are expensive and require fast, high bandwidth hospital intranet systems to transfer data.
- An additional scanning workstream is needed in the laboratory, requiring adequate staff training and capacity, and lengthening the overall specimen processing time. This can be ameliorated by lean (quality and efficiency-focused) working practices.
- Making the transition from reporting using glass slides can be difficult for some pathologists, requiring effective change management leadership and support. Self-validation for digital image reporting requires a significant commitment to learning, and tolerance of a potentially uncomfortable phase of 'conscious incompetence' as a highly skilled professional.
- Most pathologists will have reduced reporting capacity and productivity when making

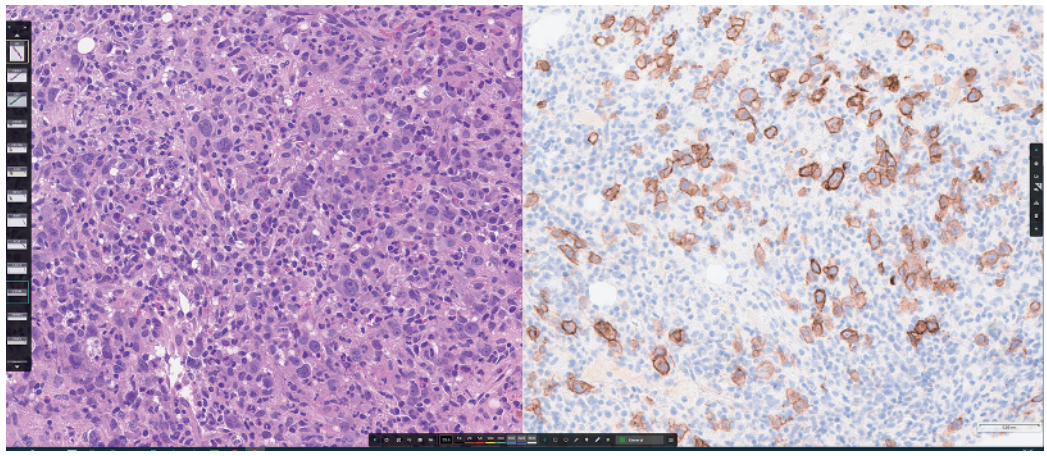


**Fig. 1.57** Screen-shot of digital images (Halo AP, Indica Labs) from a patient with classic Hodgkin lymphoma showing side by side comparison of an H&E-stained section (left) and immunoperoxidase for CD30 (right).

the transition from glass slides to digital reporting, particularly (but not only) during the validation period. It can take many months to achieve an equivalent level of fluency to that experienced using a conventional microscope.

- Long periods of time spent working at a UHD computer monitor create substantially more eye fatigue than using a conventional microscope.
- Scanned images allow less 'tolerance' of technical imperfections in sections than conventional microscopy (see later).
- Scanning technology is unable to recreate the parameters of polarized illumination. Consequently, the presence of amyloid suspected from digital images cannot be confirmed without viewing Congo red staining using conventional microscopy and polarizing light filters.

One feature of digital pathology that can at first seem problematic when reporting is that high power images (×40 and above) can appear 'flat' if the image has been scanned at a single Z-plane within the tissue section and some cells are fractionally out of focus. When using a conventional microscope it is possible to continually adjust the fine focus when viewing the section to overcome this, something that is not possible when viewing digital images scanned at a single Z-plane. It is possible to scan images at more than one Z-plane but this considerably increases the scanning time, the size of the WSI data files and the data storage costs. A possible approach is to apply selective Z-stacking (scanning at multiple Z-planes) to the H&E-stained sections only. Other individual slides are re-scanned with Z-stacking upon request, if needed. However, in our combined experience the flat image effect is not a major problem in routine reporting practice. The most important factor in delivering high quality WSI for digital reporting is having high quality, thin and well stained sections to start with, without creases or lifting artefact. Even more than for conventional microscopy, excellent quality of section preparation by laboratory scientists is essential for digital pathology reporting.

## Artificial intelligence and automated image analysis

Several commercially available AI image analysis tools can be integrated with digital pathology IMS and have achieved regulatory approval within the European Union [103,104] and US [105]. Examples include algorithms to detect, grade and measure foci of cancer within prostate and breast biopsy specimens [106,107] to be used as decision support tools or quality assurance tools by reporting pathologists. Other tools can score hormone receptor status and percentage of cells expressing Ki-67 from immunohistochemical-stained sections of breast tumours [108].

Currently there are no commercially available AI image analysis applications to enhance bone marrow biopsy specimen interpretation. However, there are several studies showing ways in which diagnostic accuracy could be improved by the application of AI image analysis to bone marrow sections.

Automated image analysis has been used to quantify plasma cell infiltration in bone marrow trephine biopsy sections stained immunohistochemically for CD138 and IRF4/MUM1 to assist in the assessment of plasma cell neoplasms (plasma cell myeloma and monoclonal gammopathy of uncertain significance) [109,110]. The automated digital process analysed, on average, 100 084 cells in each bone marrow trephine section WSI, whereas the manual method counted 500 cells in representative areas of each section using conventional light microscopy. The automated digital enumeration method gave significantly lower counts than the manual method, attributable to unconscious bias with manual counting [109].

Another group [111] used machine learning to develop an automated approach to phenotyping megakaryocytes in bone marrow trephine biopsy WSI to assist in the assessment of cases of essential thrombocythaemia (ET), polycythaemia vera (PV), primary myelofibrosis (PMF) and reactive conditions. Using a supervised machine learning approach megakaryocytes were separated into nine distinct morphological subgroups, some of which were not easily distinguishable by expert haematopathologists. This approach allows assessment of all megakaryocytes in each WSI, generating a number of metrics, including density of megakaryocytes, mean cell radius and measures of clustering. The resulting metrics are displayed in a radar plot giving a visual representation of the data for each WSI. Applying machine learning to the results from each sample it was possible to distinguish between reactive cases and myeloproliferative neoplasms, and between cases of ET, PV and PMF with high levels of sensitivity and specificity. This AI-driven approach to morphological analysis has potential to identify important morphological features not apparent to pathologists, quantify them across an entire section in an unbiased way, and present the results in a way that can indicate the probability of the underlying diagnosis. The same group applied a similar machine learning approach to the analysis of reticulin fibrosis in WSI from bone marrow sections in myeloproliferative neoplasms; they found that it could identify cases with a presumptive diagnosis of ET who were at high risk of progression to post-ET myelofibrosis because it detected microfoci of fibrosis that were not identified using conventional microscopic examination [112]. Combining the machine learning approach to reticulin fibre analysis with automated megakaryocyte analysis improved the discrimination between cases of ET and prefibrotic myelofibrosis. The authors stress that the results from this approach need to be interpreted along with other features, such as bone marrow cellularity and collagen fibrosis, and suggest that in the future machine learning may be able to objectively assess other cytomorphological and stromal features and integrate them into diagnostic algorithms.

It is likely that as the computing power and sophistication of machine learning improves, and larger pools of digital images linked to case metadata to train algorithms become available, AI-driven image analysis will become a routine part of the assessment of bone marrow trephine biopsy specimens in the relatively near future.

## Artefacts

Various artefacts in bone marrow aspirates and trephine biopsy specimens need to be recognized so that they are not misinterpreted as evidence of disease [113,114]. Artefacts are of three main types: (i) introduced by the biopsy process or by processing in the laboratory; (ii) consequent on extraneous material or tissue being included in the biopsy; and (iii) resulting from previous tissue damage at the biopsy site. The latter group are not, strictly speaking, artefacts since what is observed are real changes in the tissues. Nevertheless, they are potentially misleading in the same way as artefacts and will therefore be considered here.

### *Cytology*

If bone marrow is anticoagulated in EDTA and if delay then occurs in making films, storage artefacts can develop and can simulate dyserythropoiesis [115,116]. Such features may include nuclear lobation and fragmentation and cytoplasmic vacuolation. Excess EDTA

also has deleterious effects on cytology, including hypolobation of neutrophils and shrinkage of neutrophils and megakaryocytes [116]; it should be avoided by using a paediatric EDTA tube or EDTA diluted in isotonic saline. Ideally, films should be made from bone marrow that has not been anticoagulated. Processing artefacts can be induced in bone marrow aspirates by inadequate drying of the film, poor fixation or prolonged storage of the film prior to fixation and staining. If slides are fixed before they have dried adequately there is an appearance suggesting that nuclear contents are leaking into the cytoplasm and cellular outline is indistinct (Fig. 1.58). Water uptake into methanol used in fixation causes refractile 'inclusions' in red cells and poor definition of cellular details. Delayed fixation and staining of archival bone marrow slides usually leads to a strong blue or turquoise tint to the film; this can be avoided by fixing slides prior to storage although this limits their subsequent uses.

If an aspirate is partly clotted, small bone marrow clots may be mistaken for bone marrow particles, leading to a mistaken attempt to assess cellularity or the presence or absence of storage iron in the clot. The presence of fibrin strands and the lack of any organized structure of the apparent particle is a clue to its true nature. In patients with ET, solid clumps of large numbers of platelets can also be mistaken for bone marrow fragments.

Extraneous non-haemopoietic cells that may appear in bone marrow aspirates include endothelial cells (Fig. 1.59) and epithelial cells (Fig. 1.60). Endothelial cells may appear in

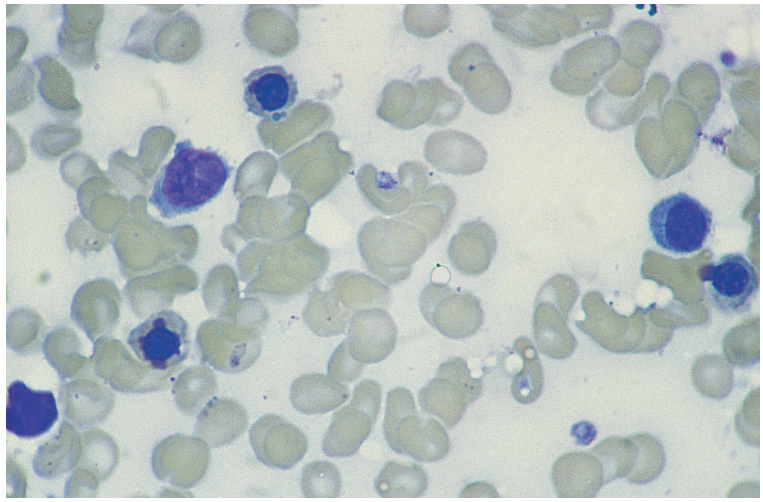


**Fig. 1.58** BM aspirate showing the effect of fixing and staining the film before adequate drying has occurred; the erythroblast nuclear content appears to have leaked into the cytoplasm. MGG ×100.

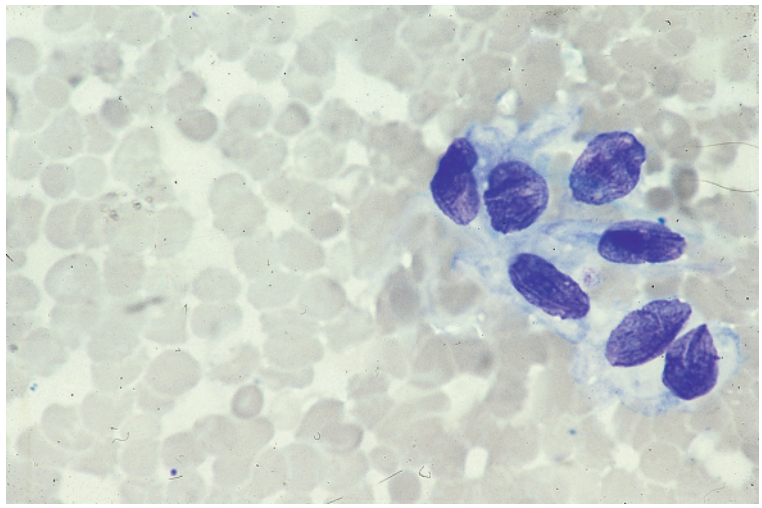


**Fig. 1.59** Vena cava scraping showing endothelial cells; similar cells are occasionally observed in BM aspirates. MGG ×100. (By courtesy of the late Dr Marjorie Walker.)

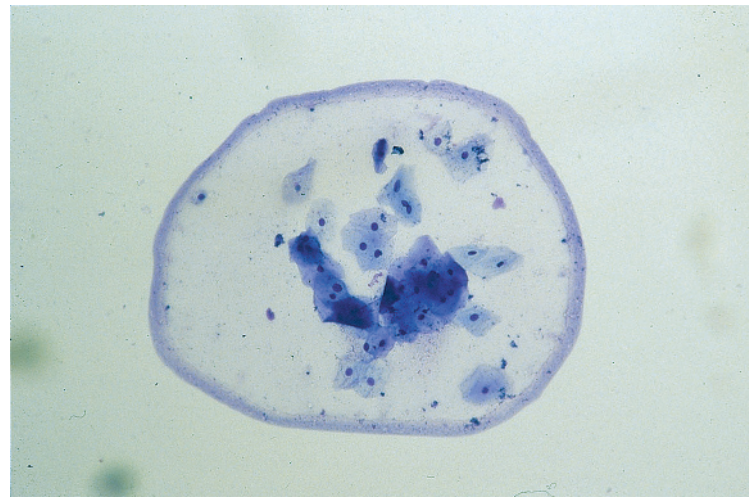


**Fig. 1.60** BM aspirate: epithelial cells. MGG ×10.

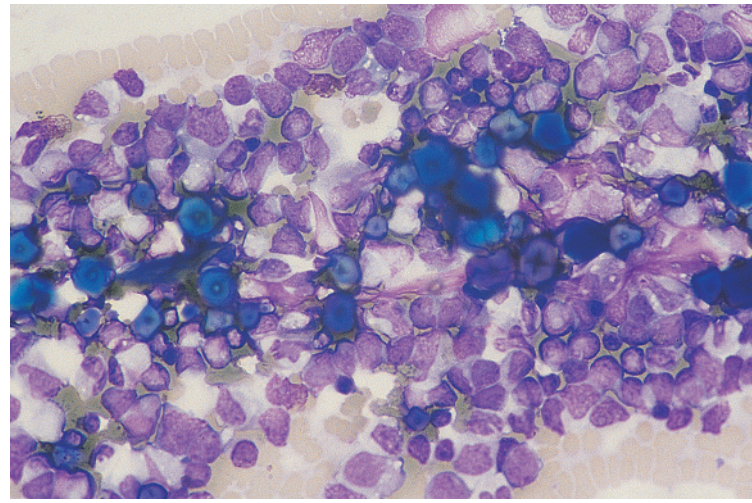


**Fig. 1.61** Crystals of glove powder in a BM aspirate. MGG ×40.

masses and be pleomorphic. It is important that they are not confused with tumour cells. They have weakly basophilic cytoplasm and oval nuclei, which appear grooved. Epithelial cells, both nucleated and anuclear, are more readily recognized by their voluminous, opaque, powder-blue cytoplasm. Extraneous material that may appear in bone marrow aspirate films includes crystals of glove powder. These are blue with an MGG stain (Fig. 1.61) and red with a PAS stain.

Abnormalities in bone marrow aspirates may result from a previous biopsy performed at the same site a short time before. Increased numbers of macrophages, including foamy macrophages, can be found. The scars of previous biopsies are usually apparent and repeat biopsies should be carried out from the other side of the pelvis or a centimetre or so away from any recent biopsy on the same side. It should also be noted that, if the pelvis has previously been irradiated, biopsies will show bone marrow hypoplasia or aplasia which is not indicative of the appearance of the bone marrow at other sites. Biopsy of previously irradiated bone marrow should therefore generally be avoided.

If imprints are made from trephine biopsy specimens containing cartilage, for example in children, there may be deposition of purple granular material in the imprint [117].

***Histology***

A trephine biopsy specimen that is too short or a biopsy that is performed at the wrong angle may mean that the specimen includes only subcortical marrow, which is often markedly hypocellular. This can create a mistaken impression of aplasia. Performing a biopsy and processing the specimen can induce crumbling of bone to amorphous material (Fig. 1.62), or bone marrow tissue may be absent from the intertrabecular spaces (Fig. 1.63). The latter artefact may be related to the use of blunt needles since it does not appear to be a problem with disposable needles. Torsion artefact (Fig. 1.64) is common. The elongated nuclei that are produced by twisting should not be confused with the nuclei of fibroblasts. Usually, twisted bone marrow is not interpretable but sometimes it is possible to recognize neoplastic cells (e.g. myeloma cells or carcinoma cells) despite the artefact.

Artefacts can be introduced during fixation. If formol-saline is used as a fixative, it is necessary to allow at least 18 hours for fixation. If more rapid fixation is required then a protein precipitant formulation should be used. Poor fixation leads to glassy nuclei in which detailed structure cannot be recognized (Fig. 1.65). Poor fixation is aggravated by the use of strong decalcifying agents. Often it is impossible to give any reliable interpretation of a poorly fixed marrow. Another fixation-related problem is deposition of formalin pigment. Formalin pigment is blackish-brown and should be

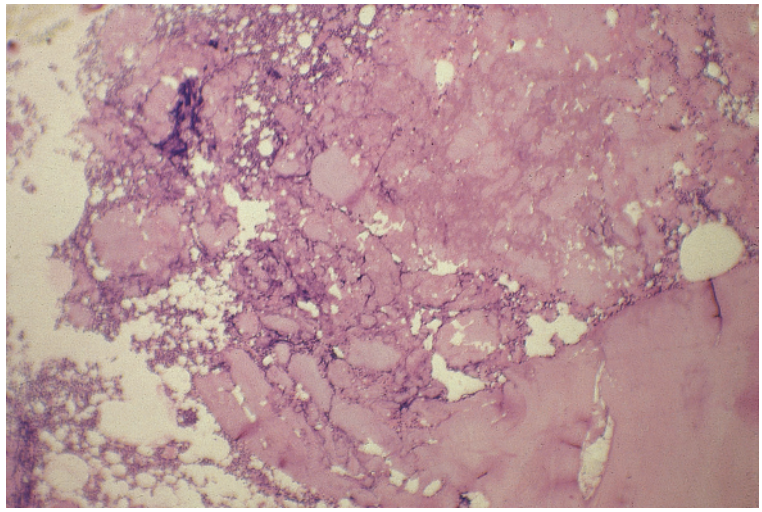


**Fig. 1.62** BM trephine biopsy section, crushed bone. H&E ×20.

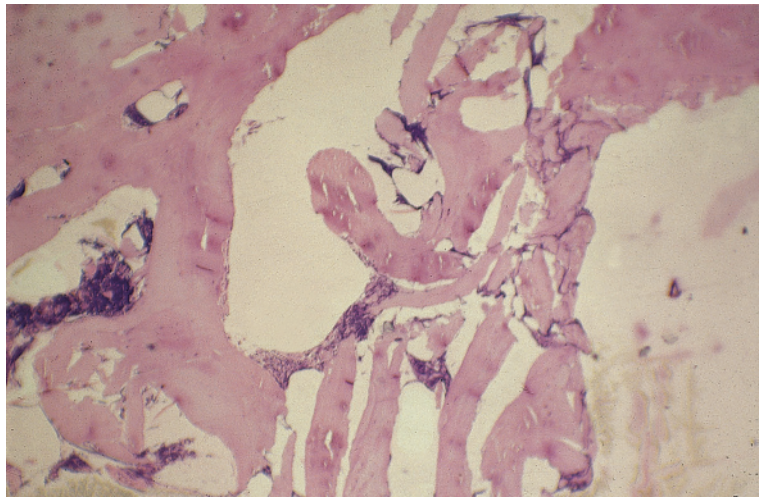


**Fig. 1.63** BM trephine biopsy section showing apparently empty intertrabecular spaces consequent on squeezing of the biopsy specimen. H&E ×10.

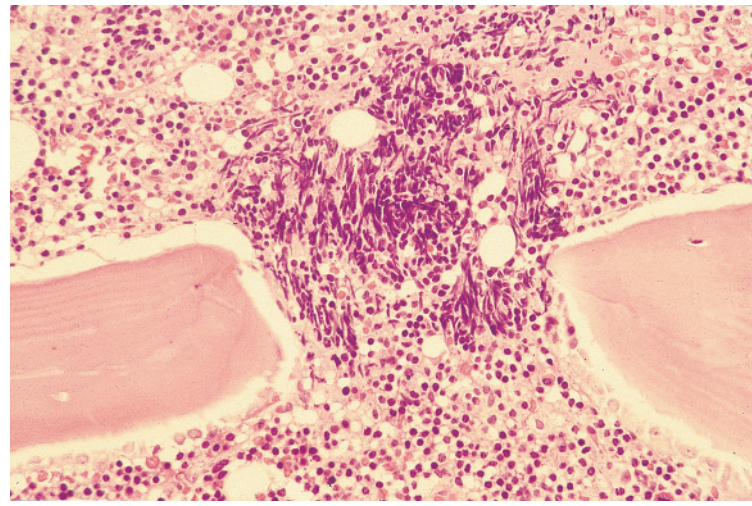


**Fig. 1.64** BM trephine biopsy section from a patient with chronic lymphocytic leukaemia showing torsion artefact. H&E ×20.

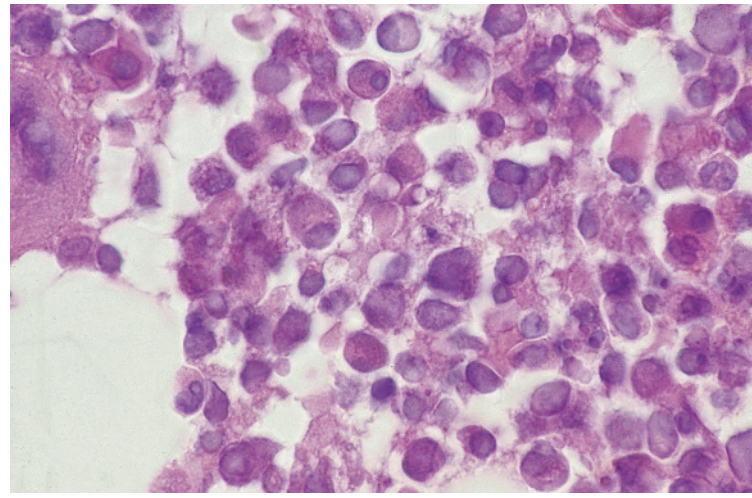


**Fig. 1.65** BM trephine biopsy section showing nuclei that appear glassy and homogeneous as a consequence of fixation artefact. H&E ×100.

distinguished from haemosiderin, which is golden-brown (Fig. 1.66). If biopsy specimens are fixed in mercury-based fixatives, such as B5, inadequate washing may lead to cells being obscured by a precipitate [118]; however, it should be noted that in many countries mercury-based fixatives are prohibited on environmental and safety grounds.

Both excessive and inadequate decalcification can lead to artefactual changes. Excessive decalcification leads to loss of cellular detail (particularly nuclear detail) and poor uptake of haematoxylin. Inadequate decalcification leads to the presence of a central core of undecalcified bone in the centre of bony spicules. This makes it difficult to produce high quality thin sections and the sections tend to tear.

Artefacts induced during processing are most often a problem in paraffin-embedded tissue. Some degree of shrinkage artefact is usual. This is most apparent with erythroblasts for which the halo that surrounds the nucleus can be an aid to identification. Shrinkage artefact also leads to megakaryocytes appearing within large empty spaces (Fig. 1.67). The use of a blunt knife can lead to tearing of sections or to the sections appearing banded (Fig. 1.68). Bony trabeculae may be lost during processing leaving gaps in the section (Fig. 1.69).

Artefactual inclusion of extraneous tissue in the biopsy specimen is not uncommon. Trephine biopsy samples, particularly from children, may include cartilage (Fig. 1.70). Pieces of skin (Fig. 1.71), adipose tissue,

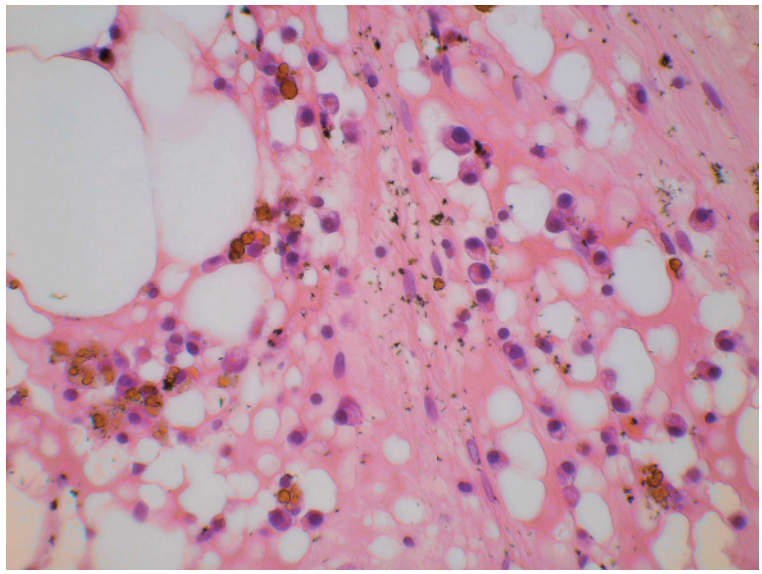


**Fig. 1.66** BM section from a patient with multiple myeloma showing both increased haemosiderin (golden brown) and formalin pigment (black). H&E ×50.

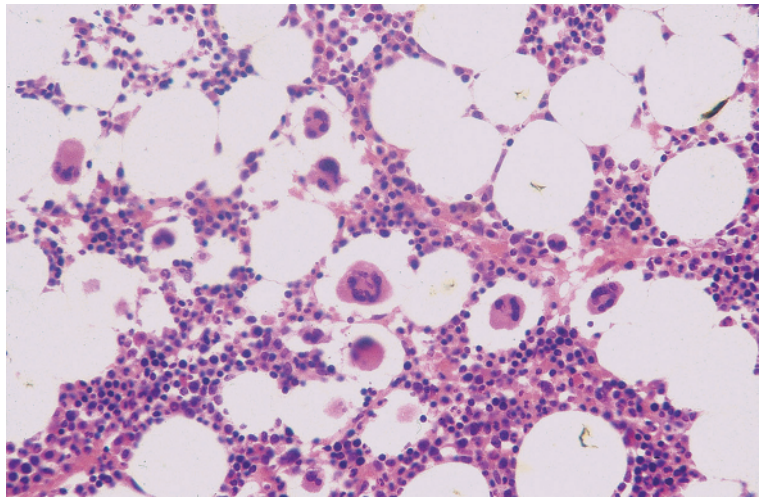


**Fig. 1.67** BM trephine biopsy section showing megakaryocytes surrounded by an empty space as a consequence of shrinkage artefact. H&E ×40.

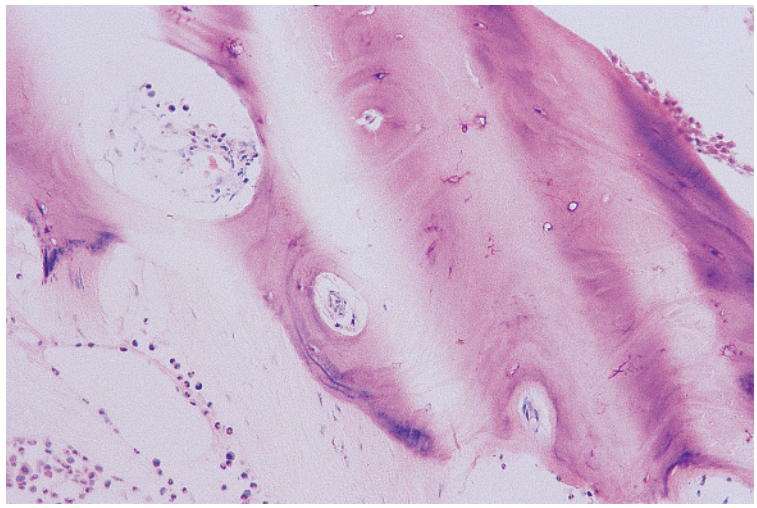


**Fig. 1.68** BM trephine biopsy specimen showing an artefact caused by using a blunt knife. H&E ×40.

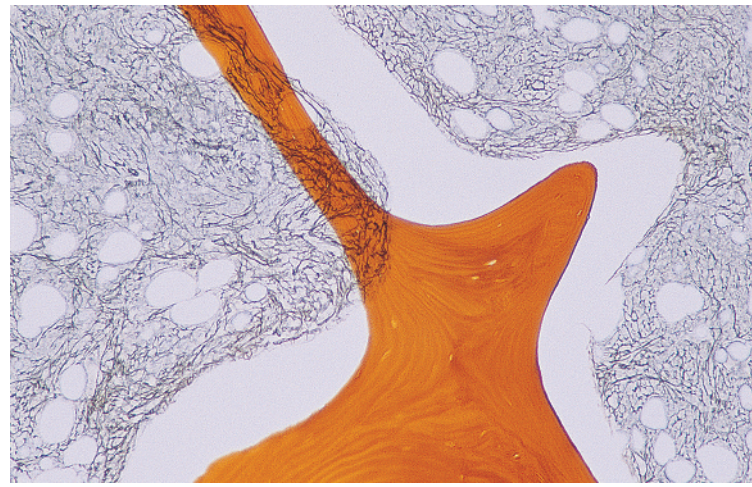


**Fig. 1.69** BM trephine biopsy specimen showing displacement of bony trabeculae; if trabeculae are completely displaced confusion with dilated sinusoids can occur. Reticulin stain ×10.

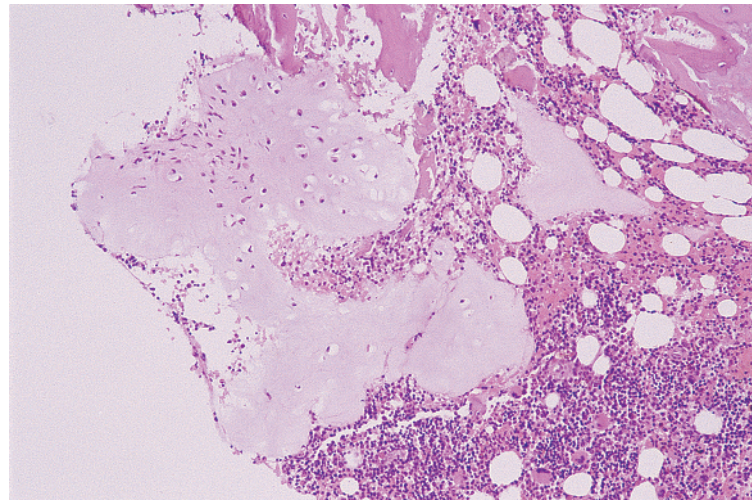


**Fig. 1.70** An inclusion of paediatric cartilage in a trephine biopsy specimen. H&E ×20.

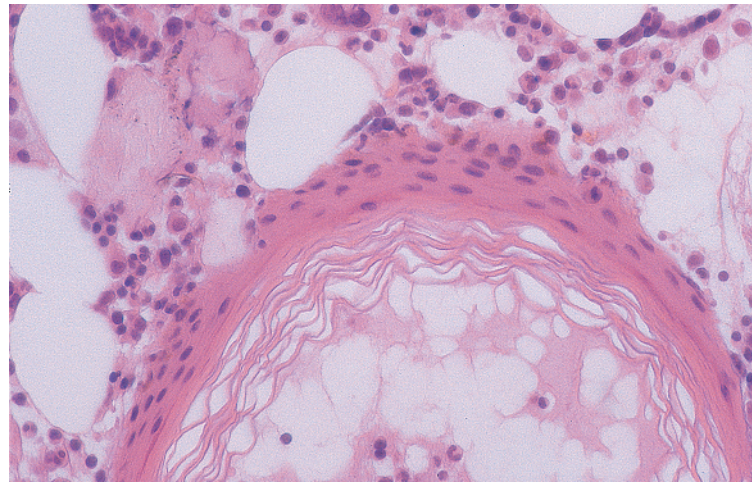


**Fig. 1.71** BM trephine biopsy showing a piece of epidermis which has been driven into the biopsy specimen. H&E ×50.

striated muscle (Fig. 1.72), hair follicles (Fig. 1.73) and sweat glands (Figs 1.73 and 1.74) can be introduced into the biopsy specimen during the biopsy process. Occasionally synovium (Fig. 1.75), or even a gouty tophus (Fig. 1.76), is included in the specimen; however it should be noted that deposition of uric acid crystals within the bone marrow has also been described (see page 662). Other extraneous material can be transferred from the blade used in cutting sections and can be embedded with the bone marrow biopsy specimen (Fig. 1.77). Tissue from other biopsy specimens can contaminate the water bath in which sections are floated prior to mounting on glass slides. Such tissue may adhere to the trephine biopsy specimen or to the glass slide adjacent to the section and may thus appear to represent part of the trephine biopsy specimen. Sometimes the abnormal tissue that is inadvertently included is dysplastic or neoplastic. Examination of reticulin stains can be helpful if there is doubt as to whether or not abnormal tissue is an intrinsic part of the biopsy specimen. If foreign tissue has been transferred with a knife, it will not be present if repeat sections are cut. However, sometimes extraneous tissue that was floating in a solvent solution is actually included in the block and will therefore also be present in repeat sections. Histopathology laboratories need good practices for dealing with small friable biopsy specimens to avoid this problem and both haematologists and histopathologists must be aware of this potential problem. In a last resort the unexplained tissue can be dug out of the

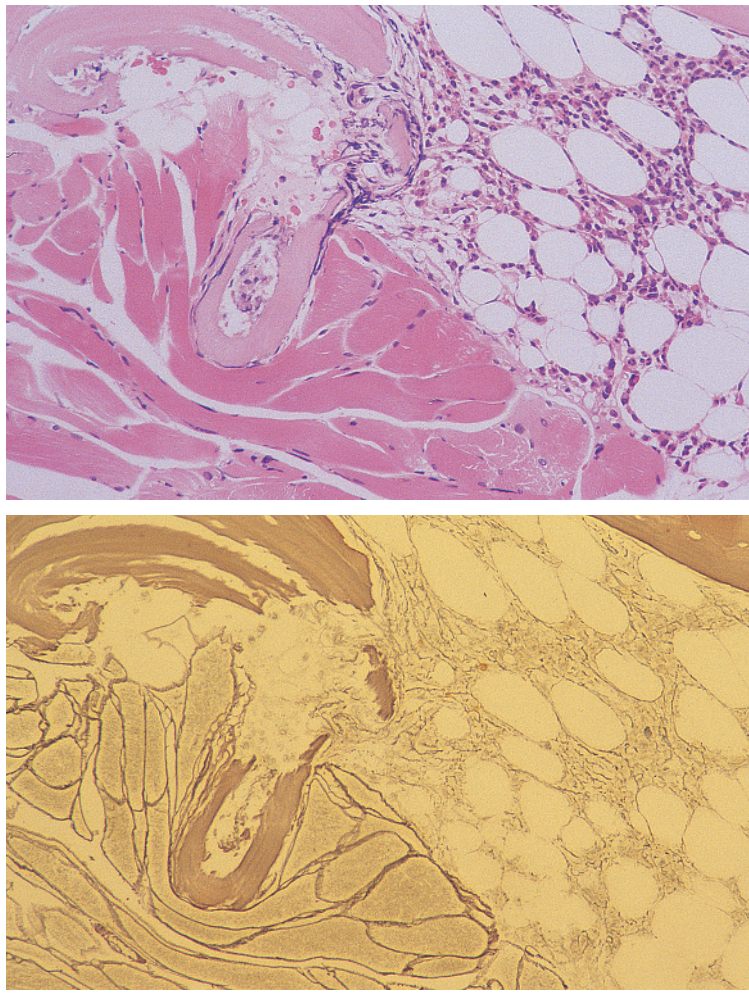



**Fig. 1.72** Trephine biopsy showing striated muscle which has been driven into the biopsy specimen. (a) H&E ×40. (b) Reticulin stain ×40.

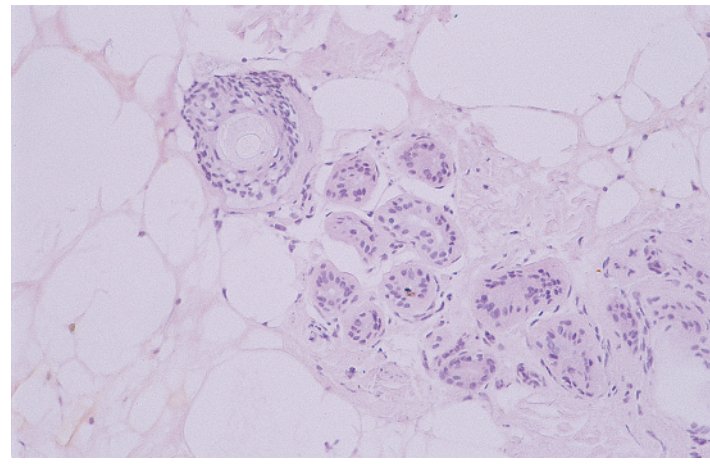
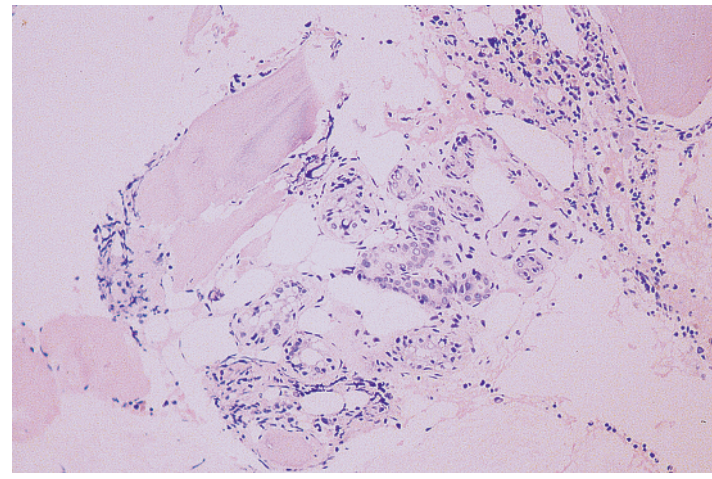
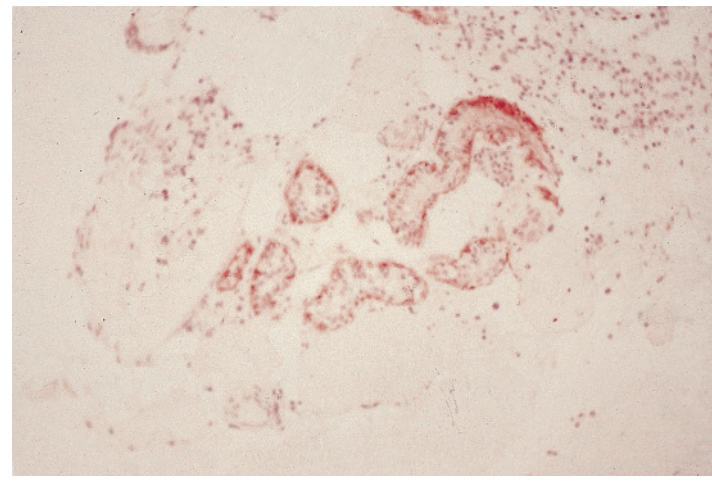


**Fig. 1.73** A hair follicle and ducts of a sweat gland which have been driven into a trephine biopsy specimen. H&E ×10.



(a)



(b)

**Fig. 1.74** BM trephine biopsy showing sweat glands which have been driven into the biopsy. (a) Giemsa ×10. (b) Immunohistochemistry with an antibody to smooth muscle actin demonstrating the myoepithelial cells of the sweat gland. Immunoperoxidase ×10.

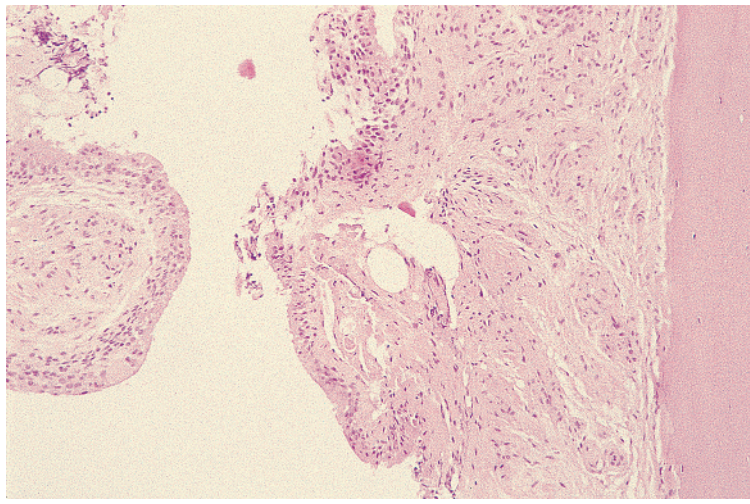


**Fig. 1.75** Section of BM trephine biopsy specimen showing synovium on the periosteal surface of cortical bone. H&E ×10.

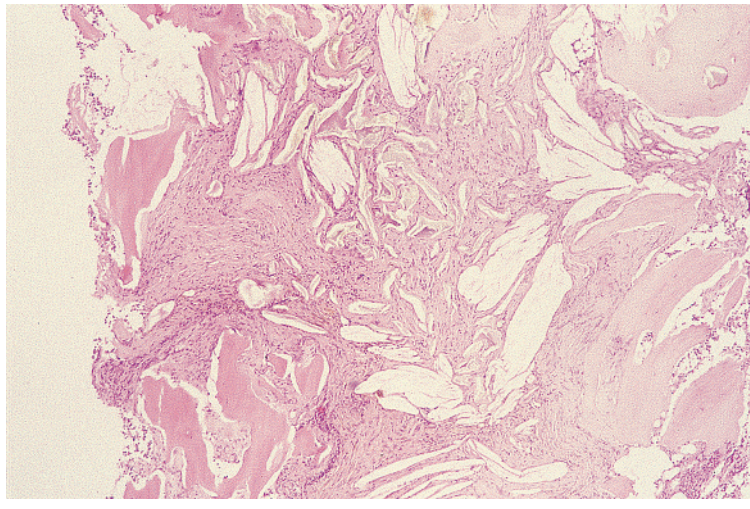


**Fig. 1.76** Section of trephine biopsy specimen showing a gouty tophus which has been driven into the bone marrow; the empty spaces represent areas where uric acid has been removed during processing. H&E ×10.

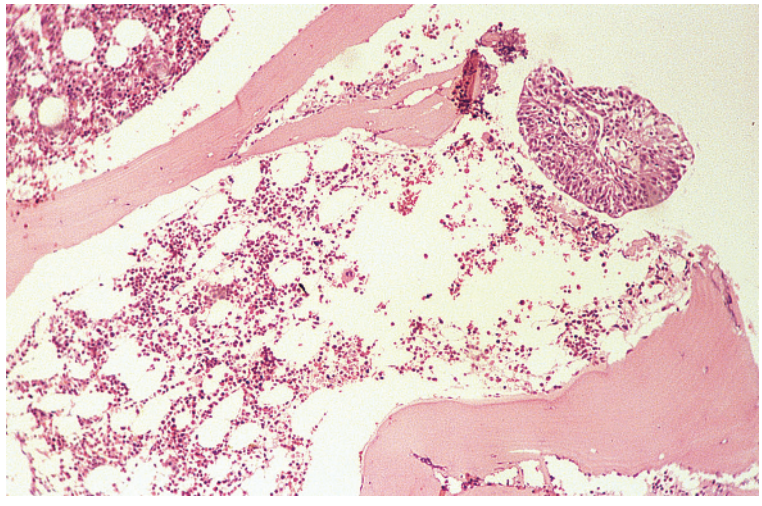


**Fig. 1.77** BM trephine biopsy specimen showing dysplastic bladder epithelium which has been embedded with the biopsy as a result of contamination during processing. H&E ×10.

block and HLA typed against the trephine biopsy specimen or any other candidate specimen to establish its true origin.

Artefacts can be induced by a previous aspiration or trephine biopsy at the same site. A bone marrow aspiration performed immediately before a trephine biopsy usually causes haemorrhage, disruption of the tissue and loss of haemopoietic cells. Occasionally the actual track of the aspiration needle is apparent (Fig. 1.78). This artefact can be avoided if the aspiration and trephine biopsy needles are introduced several millimetres apart and angled differently. This can be done even if they are inserted through the same skin incision. A biopsy performed some time previously may lead to the pathological specimen showing fat necrosis, with focal collections of foamy macrophages, or granulation tissue. A biopsy performed inadvertently at the site of a healing fracture produces a similar histological picture. There is initially granulation tissue, increased reticulin deposition and new bone formation; this can be confused with myelofibrosis [119]. Subsequently, granulation tissue is usually replaced by adipose tissue in which islands of haemopoietic cells develop. A trephine biopsy (Fig. 1.79) or other localized bone marrow damage (Fig. 1.80) can result in a biopsy specimen showing a scar which, in the case of a previous trephine biopsy, may be linear. Scars should not be confused with fibrosis resulting from other pathological processes.

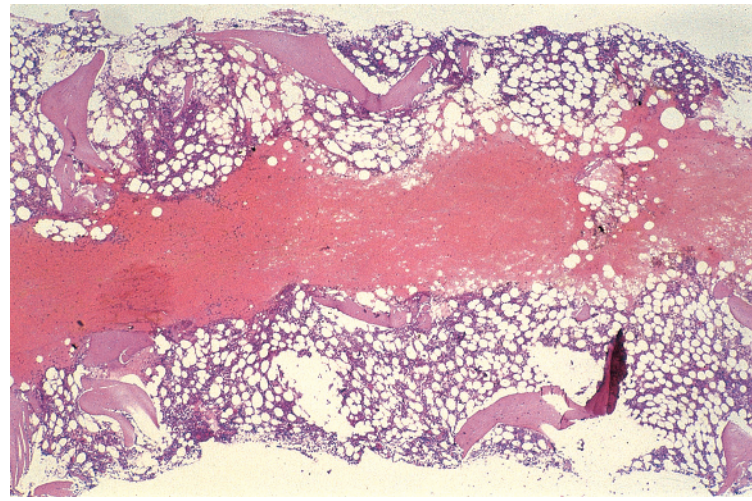


**Fig. 1.78** BM trephine biopsy specimen showing a needle track from a bone marrow aspiration performed immediately before the trephine biopsy. H&E ×4.

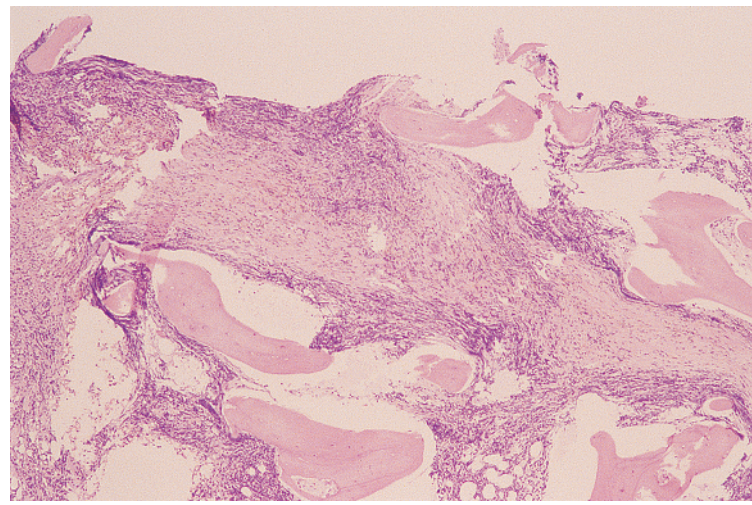
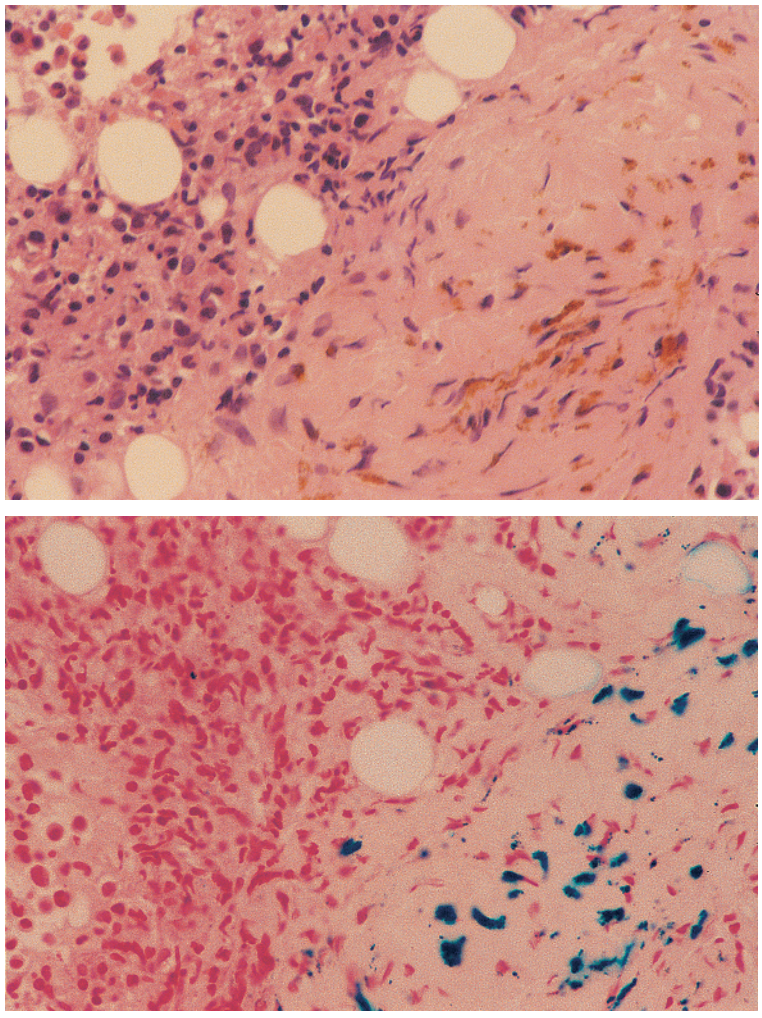


**Fig. 1.79** BM trephine biopsy specimen showing a linear scar resulting from damage by a previous biopsy at the same site. H&E ×5.



(a)

(b)

**Fig. 1.80** Section of BM trephine biopsy specimen from an intravenous drug abuser with Hodgkin lymphoma showing a bone marrow scar; there is deposition of haemosiderin in the scar. (a) H&E ×40. (b) Perls stain ×40.

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