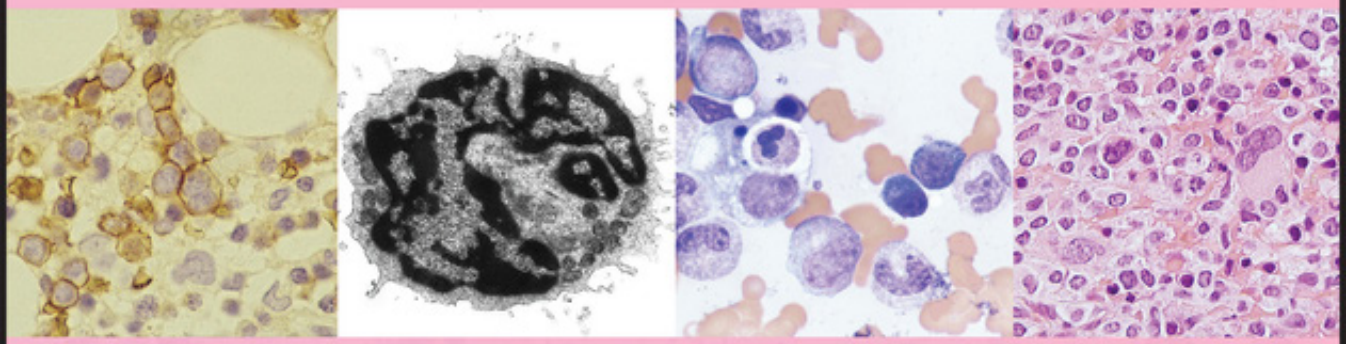
Sixth Edition

Bone Marrow Pathology



Barbara J. Bain | David M. Clark
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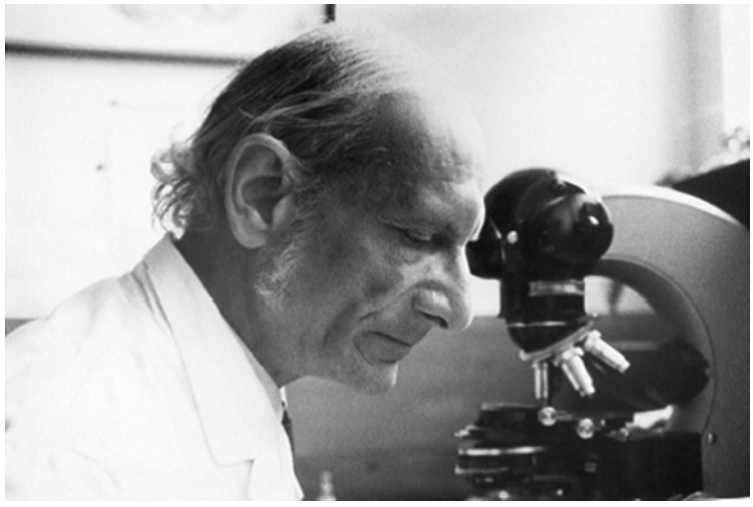

This book is dedicated to the late Professor David A. G. Galton

BONE MARROW PATHOLOGY

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PREFACE TO THE SIXTH EDITION

In this book we have set out to provide a practical guide to bone marrow diagnosis, based on an integrated assessment of peripheral blood and bone marrow aspirate films, trephine biopsy sections and supplementary investigations. We believe that a trephine biopsy specimen should not be examined and interpreted in isolation. We have therefore discussed the clinical context of bone marrow diagnosis and have given equal weight to cytological and histological features. Since bone marrow diagnosis is no longer based on morphological features alone, we have also discussed in detail the role of immunophenotypic, cytogenetic and molecular genetic analysis. We have incorporated the diagnostic criteria and terminology of the 2022 World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues (Beta version) and of the 2022 International Consensus Classification of these disorders.

We have dealt very fully with haematological disorders for which bone marrow examination is commonly performed. However, we have also sought to be comprehensive, including information on uncommon and rare disorders so that the book will serve as a useful reference source. When possible, we have illustrated rare as well as common conditions and have cited the relevant scientific literature extensively. As in the previous editions, magnifications are given as the microscopic objective used rather than the magnification on the printed page. Unless otherwise specified, trephine biopsy sections were embedded in paraffin wax. We have retained and expanded problems and pitfalls.

We hope that haematologists, histopathologists and haematopathologists will continue to find *Bone Marrow Pathology* a useful aid in their day-to-day practice and that trainees in these disciplines will find it indispensable. Cytogeneticists, molecular geneticists and scientists working in flow cytometry laboratories should find that it provides them with a context in which to interpret their findings.

BJB, DMC, BSW, VS

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We should like to thank our many friends and colleagues in North and South America, Europe, Africa, the Middle East, Asia, Australia and New Zealand who have provided illustrations or have permitted us to photograph microscopic slides from their personal collections. They are individually acknowledged in the legends of specific figures. In addition we should like to thank our technical and medical colleagues in St Mary's Hospital and Nottingham University Hospital for the direct and indirect help they have given us in the preparation of this edition. We are also grateful for the comments and feedback given to us by readers of the previous editions and individuals attending the postgraduate courses on which we teach.

Our special acknowledgement goes to the late Dr Irvin Lampert, for his invaluable contributions as co-author of the first three editions of this book, and for the friendship and wisdom he generously shared with us for more than 20 years.

This book is dedicated to the late Professor David Galton (1922–2006) who taught us and countless other haematologists and histopathologists a great deal over many years. Those who had the opportunity to work with him admired him for his exceptional diagnostic skills, his humility and his mindfulness of patients as individuals. He is still much missed.

BJB, DMC, BSW, VS

ABBREVIATIONS

ABC	activated B-cell-like
aCML	atypical chronic myeloid leukaemia
aCML/MDS/MPN-N	atypical chronic myeloid leukaemia/ myelodysplastic/ myeloproliferative neoplasm with neutrophilia
AESOP	adenopathy and extensive skin patch overlying a plasmacytoma
AI	artificial intelligence
AIDS	acquired immune deficiency syndrome
ALCL	anaplastic large cell lymphoma
ALIP	abnormal localization of immature precursors
ALL	acute lymphoblastic leukaemia
ALPS	autoimmune lymphoproliferative syndrome
AML	acute myeloid leukaemia
AML-MR	acute myeloid leukaemia, myelodysplasia related
ANAE	alpha naphthyl acetate esterase
ANCA	anti-neutrophilic cytoplasmic antibody
APAAP	alkaline phosphatase–anti-alkaline phosphatase
ATLL	adult T-cell leukaemia/ lymphoma
ATRA	all-*trans*-retinoic acid
B-ALL	B-acute lymphoblastic leukaemia
B-ALL/LBL	B-lymphoblastic leukaemia/lymphoma
BCG	bacillus Calmette–Guérin
BCR	breakpoint cluster region gene
BDCA-2	blood dendritic cell antigen 2
BFU-E	erythroid burst-forming unit
BM	bone marrow
BMU	basic multicellular unit
bp	base pair
BPDCN	blastic plasmacytoid dendritic cell neoplasm
B-PLL	B-cell prolymphocytic leukaemia
BSH	British Society for Haematology
bZIP	basic leucine zipper
CAE	chloroacetate esterase
CAR-T cells	chimaeric antigen receptor T cells
CCMUS	clonal cytopenia with monocytosis of undetermined significance
CCUS	clonal cytopenia of undetermined significance
CD	cluster of differentiation
CDA	congenital dyserythropoietic anaemia
CEL	chronic eosinophilic leukaemia
CFU-E	erythroid colony-forming unit
CFU-GM	granulocyte–macrophage colony-forming unit

CFU-Meg	megakaryocyte colony-forming unit
CFU-mix	mixed colony-forming unit
CGH	comparative genomic hybridization
CHAD	cold haemagglutinin disease
CLL	chronic lymphocytic leukaemia
cMDS-IB	childhood MDS with increased blasts
cMDS-LB	childhood MDS with low blasts
CML	chronic myeloid leukaemia
CMML	chronic myelomonocytic leukaemia
CMUS	clonal monocytosis of undetermined significance
CMV	cytomegalovirus
CNS	central nervous system
COVID-19	corona virus disease 2019
CT	computed tomography
DAB	diaminobenzidine tetrachloride
DEXA	dual-energy X-ray absorptiometry
DIC	disseminated intravascular coagulation
DLBCL	diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
dUTP	deoxyuridine triphosphate
EBER	Epstein–Barr virus early RNA
EBNA	Epstein–Barr virus nuclear antigen
EBV	Epstein–Barr virus
EDTA	ethylene diamine tetra-acetic acid
EGIL	European Group for the Immunological Characterization of Leukemias
EMA	epithelial membrane antigen
ERK	extracellular signal-regulated kinase
ET	essential thrombocythaemia
FAB	French–American–British (co-operative group)
FBC	full blood count
FDC	follicular dendritic cell
FICTION	fluorescence immunophenotyping and interphase cytogenetics
FISH	fluorescence *in situ* hybridization
FLAER	fluorescein-labelled aerolysin
FPD	familial platelet disorder
GCB	germinal centre B-cell-like
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte–macrophage colony-stimulating factor
GMS	Grocott methenamine silver (stain)
GPI	glycosyl phosphatidylinositol
GVHD	graft-versus-host disease
H&E	haematoxylin and eosin (stain)
Hb	haemoglobin concentration
HEMPAS	hereditary erythroid multinuclearity with positive acidified serum lysis test
HER2	human epidermal growth factor receptor 2
HHV	human herpesvirus
HHV8-LNA-1	human herpesvirus 8 latent nuclear antigen-1
HIV	human immunodeficiency virus
HLA	human leucocyte antigen

HPLC	high performance liquid chromatography
HTLV-1	human T-cell lymphotropic virus 1
ICC	International Consensus Classification
ICUS	idiopathic cytopenia of unknown significance
Ig	immunoglobulin
IL	interleukin
IMS	image management software
INCTR	International Network for Cancer Treatment and Research
IPI	International Prognostic Index
IPSID	immunoproliferative small intestinal disease
ICSH	International Council for Standardization in Haematology
ISH	*in situ* hybridization
ITD	internal tandem duplication
ITP	autoimmune thrombocytopenic purpura
JMML	juvenile myelomonocytic leukaemia
KSHV	Kaposi sarcoma herpesvirus
LANA	latency-associated nuclear antigen
LE	lupus erythematosus
LEF1	lymphoid enhancer-binding factor 1
LGL	large granular lymphocytes
LIMS	laboratory information management system
LMP	latent membrane protein
L-NGFR	low affinity nerve growth factor receptor
LP	lymphocyte predominant
LTC-IC	long-term culture-initiating cell
M : E	myeloid : erythroid (ratio)
MALT	mucosa-associated lymphoid tissue
McAb	monoclonal antibody
MCV	mean cell volume
MDS	myelodysplastic neoplasm/syndrome
MDS/MPN	myelodysplastic/myeloproliferative neoplasm
MDS/MPN-N	MDS/MPN with neutrophilia
MDS/MPN-RS-T	MDS/MPN with ring sideroblasts and thrombocytosis
MDS-EB	MDS with excess blasts
MDS-f	MDS with increased blasts and fibrosis
M-FISH	multicolour fluorescence *in situ* hybridization
MGG	May–Grünwald–Giemsa (stain)
MGUS	monoclonal gammopathy of undetermined significance
MPAL	mixed phenotype acute leukaemia
MPN	myeloproliferative neoplasm
MPO	myeloperoxidase
MRD	measurable (minimal) residual disease
mRNA	messenger ribonucleic acid
MSB	Martius scarlet blue (stain)
M-TEL	multicolour fluorescence *in situ* hybridization
NGS	next generation sequencing
NHL	non-Hodgkin lymphoma
NICE	National Institute for Health and Care Excellence
NK	natural killer
NLPHL	nodular lymphocyte-predominant Hodgkin lymphoma

NOS	not otherwise specified
NRBC	nucleated red blood cells
NSE	non-specific esterase
PAS	periodic acid–Schiff (stain)
PB	peripheral blood
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PET	positron emission tomography
PGP9.5	protein gene product 9.5
Ph	Philadelphia (chromosome)
PMF	primary myelofibrosis
PNET	primitive neuroectodermal tumour
PNH	paroxysmal nocturnal haemoglobinuria
POEMS	polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes (syndrome)
PRINS	primed *in situ* hybridization
PTCL	peripheral T-cell lymphoma
PV	polycythaemia vera
qRT-PCR	real-time quantitative reverse transcriptase polymerase chain reaction
RAEB-T	refractory anaemia with excess of blasts in transformation
RARS	refractory anaemia with ring sideroblasts
R-CHOP	rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone/prednisolone
RNA	ribonucleic acid
RQ-PCR	real-time quantitative polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome corona virus 2
SBB	Sudan black B (stain)
SCID	severe combined immune deficiency
SD	standard deviation
SKY	spectral karyotyping
SLL	small lymphocytic lymphoma
SLVL	splenic lymphoma with villous lymphocytes
Sm	surface membrane
SmIg	surface membrane immunoglobulin
SMZL	splenic marginal zone lymphoma
SNP	single nucleotide polymorphism
SSC	sideways scatter
TAFRO	thrombocytopenia, anasarca, fever, reticulin fibrosis, organomegaly (syndrome)
t-AML	therapy-related acute myeloid leukaemia
TAR	thrombocytopenia-absent radii
TBS	Tris-buffered saline
TCR	T-cell receptor
TdT	terminal deoxynucleotidyl transferase
TEMPI	telangiectasia, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collection, intrapulmonary shunting (syndrome)
TKI	tyrosine kinase inhibitor
T-LGLL	T-cell large granular lymphocytic leukaemia
t-MDS	therapy-related myelodysplastic neoplasm/syndrome
TNF	tumour necrosis factor
T-PLL	T-cell prolymphocytic leukaemia
TPO	thrombopoietin

TRAP	tartrate-resistant acid phosphatase
TROCI	tryptase-positive compact round cell infiltrates
UHD	ultra-high definition
VEGF	vascular endothelial growth factor
VEXAS	vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (syndrome)
WBC	white blood cell count
WHIM	warts, hypogammaglobulinaemia, infections, myelokathexis (syndrome)
WHO	World Health Organization
WSI	whole slide digital images
ZN	Ziehl–Neelsen (stain)

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

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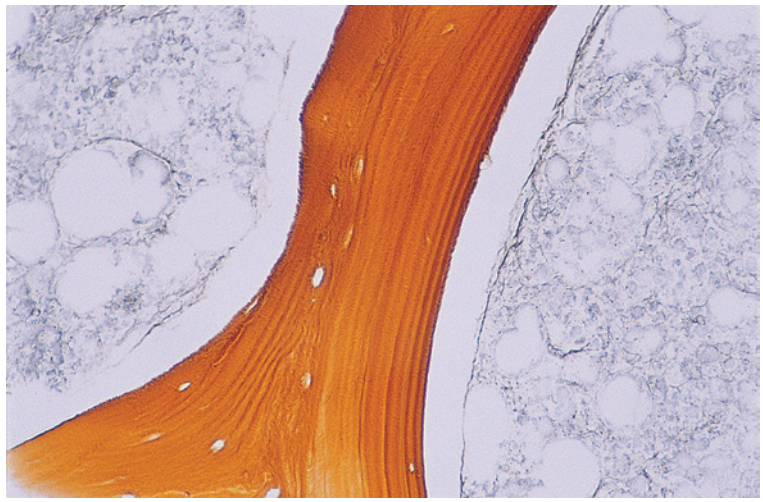

Fig. 1.1 Bone marrow (BM) trephine biopsy section showing normal bone structure; trabeculae are composed of lamellar bone. Reticulin stain ×20 objective.

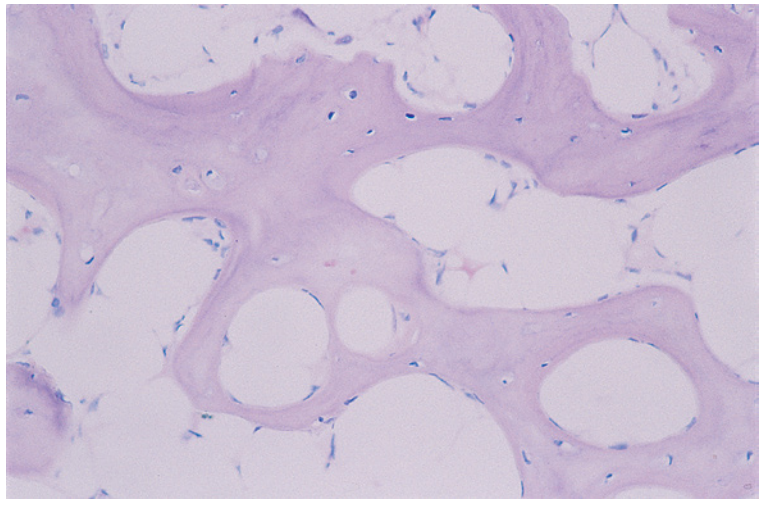

Fig. 1.2 BM trephine biopsy section showing woven bone (pale pink; without lamellae) in a hypocellular but otherwise unremarkable bone marrow. Haematoxylin and eosin (H&E) ×20.

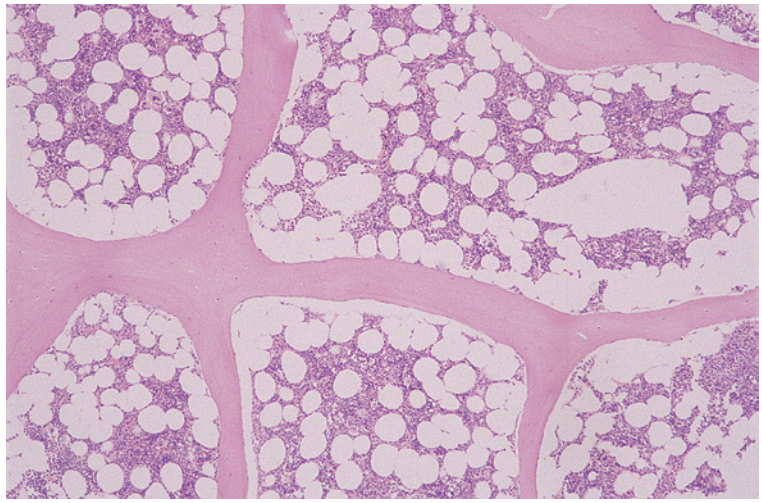

Fig. 1.3 BM trephine biopsy section showing normal bone structure; there are anastomosing bony trabeculae. H&E ×5.

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).

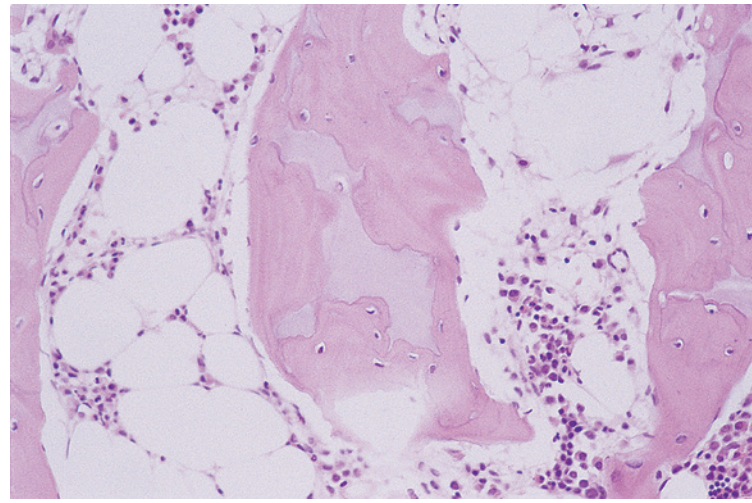

Fig. 1.4 BM trephine biopsy section from a child showing endochondrial ossification in an island of cartilage. H&E ×20.

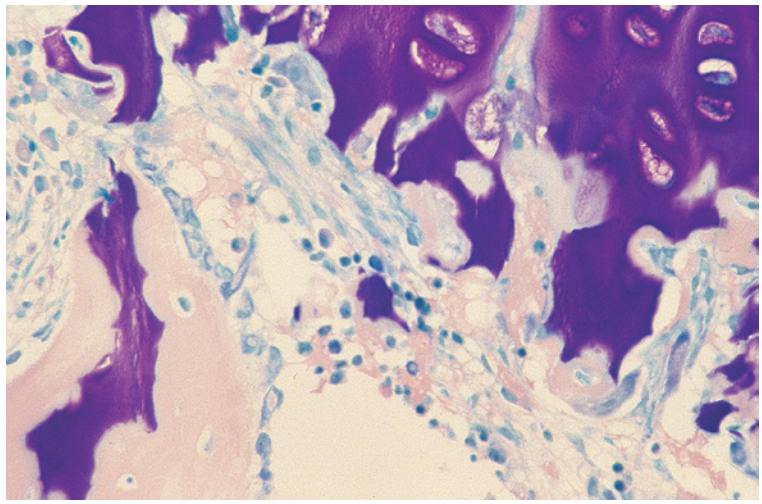

Fig. 1.5 BM trephine biopsy section from a child showing endochondrial ossification; a bony spicule with a core of cartilage is lined by osteoblasts. Giemsa stain ×40.

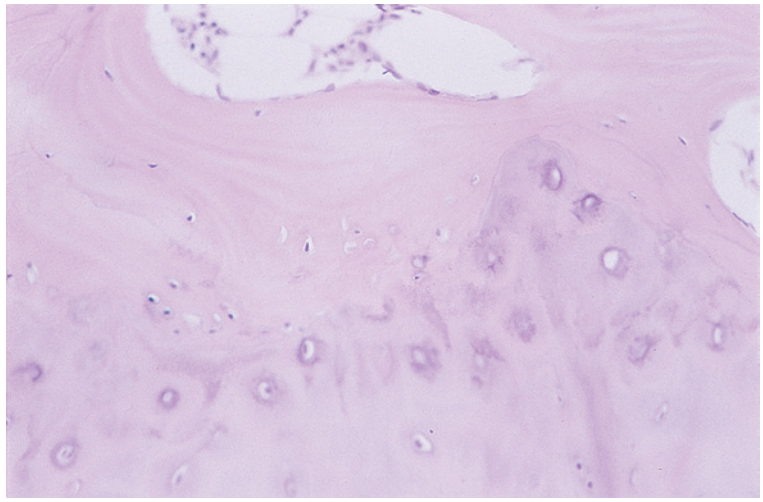

Fig. 1.6 BM trephine biopsy section from an adult showing cartilage adjacent to the cortex. By contrast with childhood appearances, a well-defined layer of cortical bone separates this cartilage from the bone marrow. Cartilage cells are dispersed singly or in small groups and are not aligned into columns,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

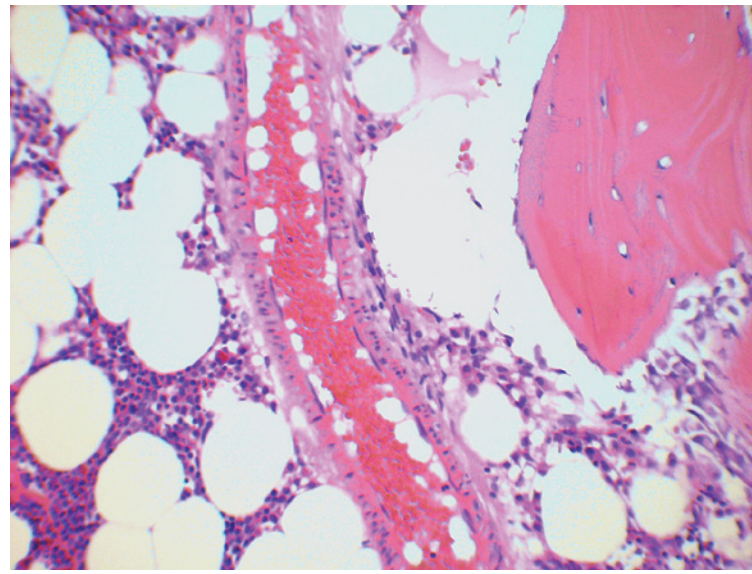

Fig. 1.7 BM trephine biopsy section showing a longitudinal section of an arteriole. H&E ×20.

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

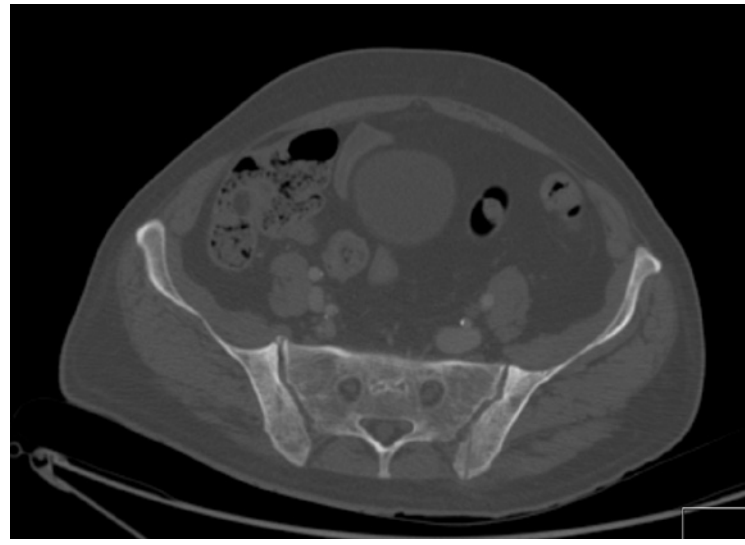

Fig. 1.8 Computed tomography (CT) scan of the pelvis showing a trephine biopsy needle track through the posterior iliac crest. (With thanks to Dr Marc Heller, London.)

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

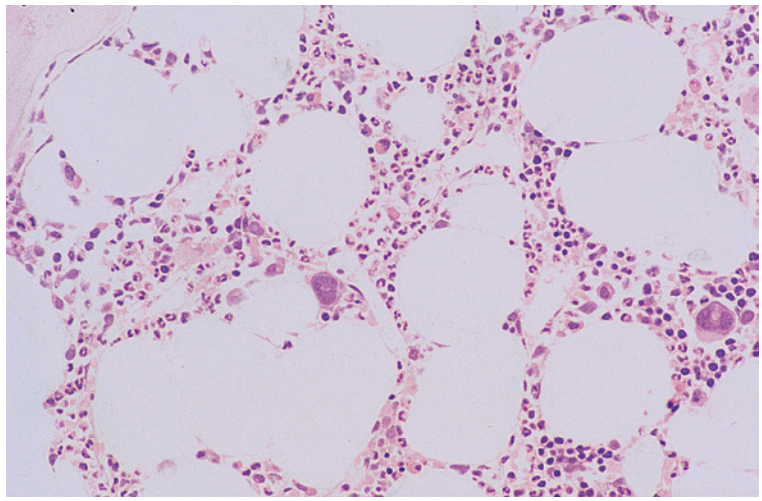

Fig. 1.9 Section of normal BM: normal distribution of all three haemopoietic lineages; note the megakaryocyte adjacent to a sinusoid. Resin-embedded, H&E ×20.

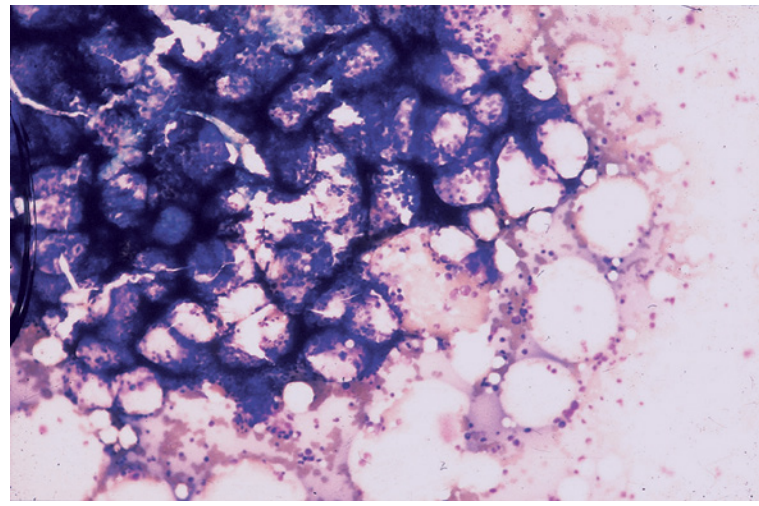

Fig. 1.10 Aspirate of normal BM: fragment showing normal cellularity. May–Grünwald–Giemsa (MGG) ×40.

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].

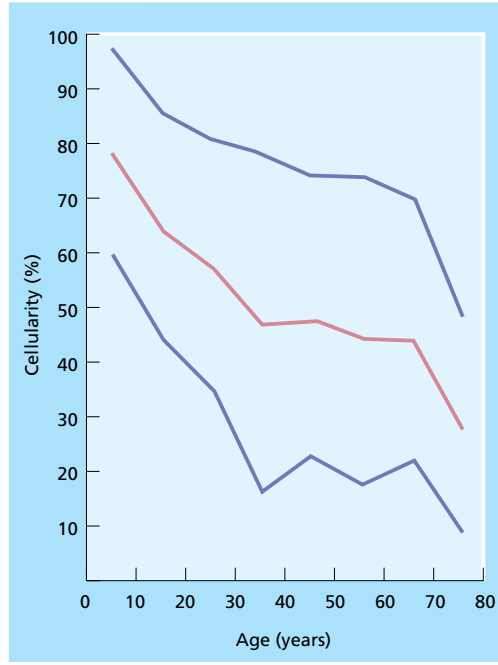

Fig. 1.11 Mean and 95% range of cellularity at various ages of anterior iliac crest bone marrow which has been decalcified and paraffin-embedded. Cellularity is expressed as a percentage of the bone marrow cavity. (Calculated from Hartsock *et al.* [54]/ National Library of Medicine/Public domain.)

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

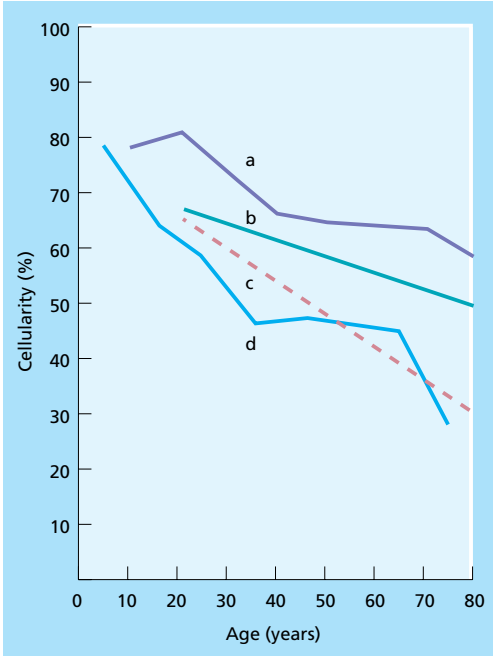

Fig. 1.12 Mean value of bone marrow cellularity at various ages expressed as a percentage of bone marrow cavity: (a) iliac crest, autopsy, not decalcified (recalculated from Frisch *et al.* [53]); (b) iliac crest, autopsy, not decalcified [56]; (c) sternum, biopsy, not decalcified [57]; and (d) ilium,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

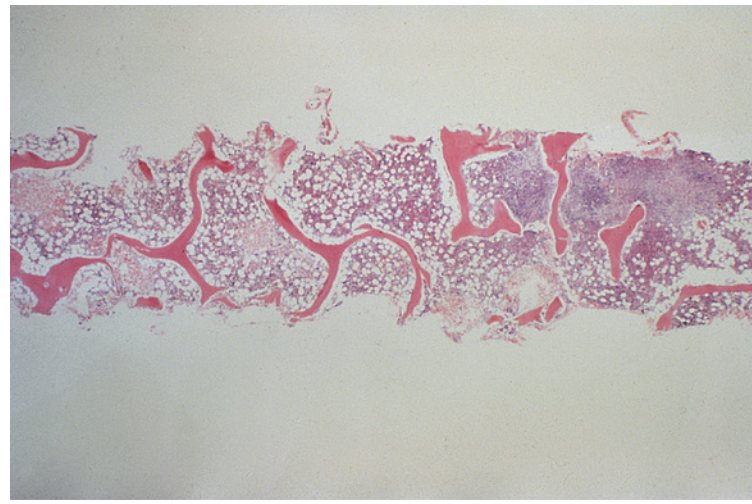

Fig. 1.13 A section of a trephine biopsy specimen of adequate size from a patient with Hodgkin lymphoma showing only a small area of infiltration at one end of the specimen, illustrating how a small biopsy may miss focal lesions. H&E ×2.5. (With thanks to Dr Ken Maclennan.)

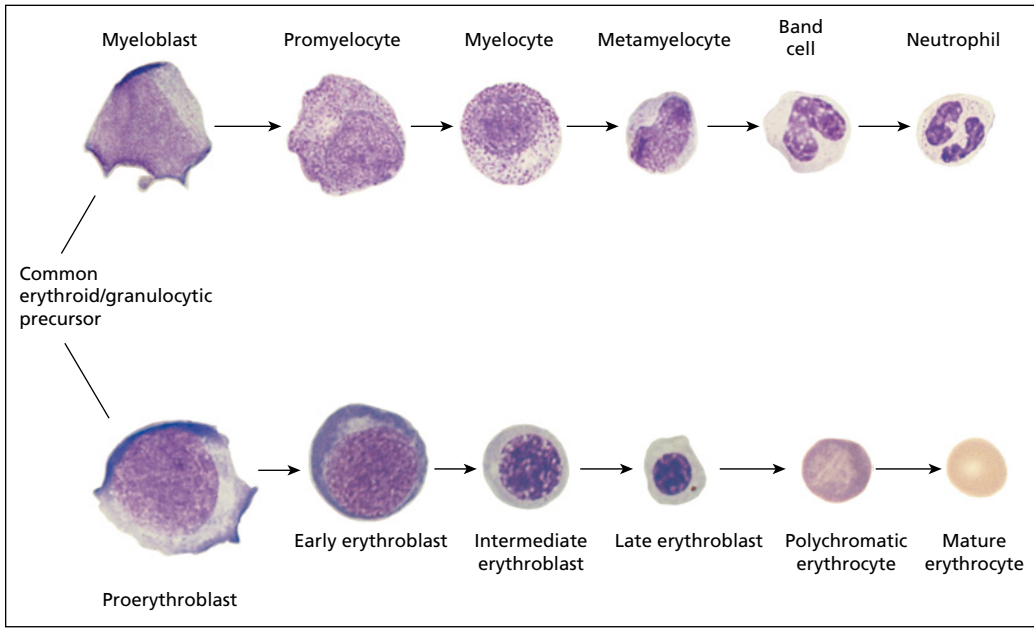

Fig. 1.14 A semi-diagrammatic representation of granulopoiesis and erythropoiesis. Cell division occurs up to the myelocyte and intermediate erythroblast stages.

'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.

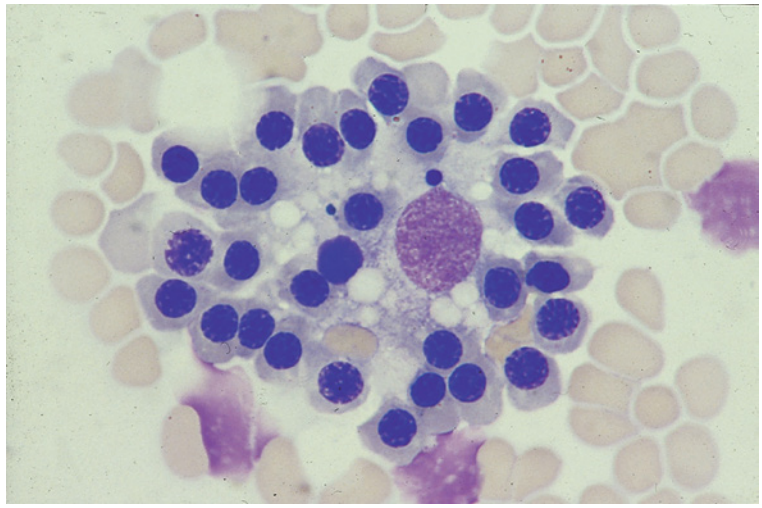

Fig. 1.15 BM aspirate: an erythroid island. MGG ×100.

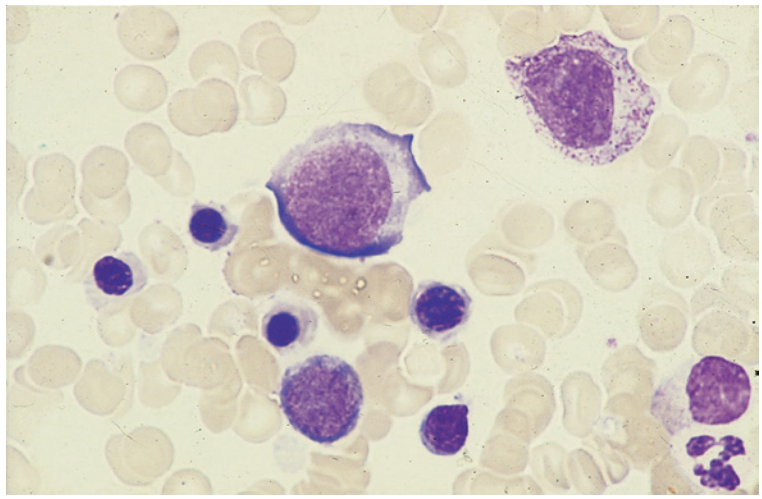

Fig. 1.16 Aspirate of normal BM: a proerythroblast, an intermediate erythroblast, four late erythroblasts, a myelocyte, large and small lymphocytes and a neutrophil. MGG ×100.

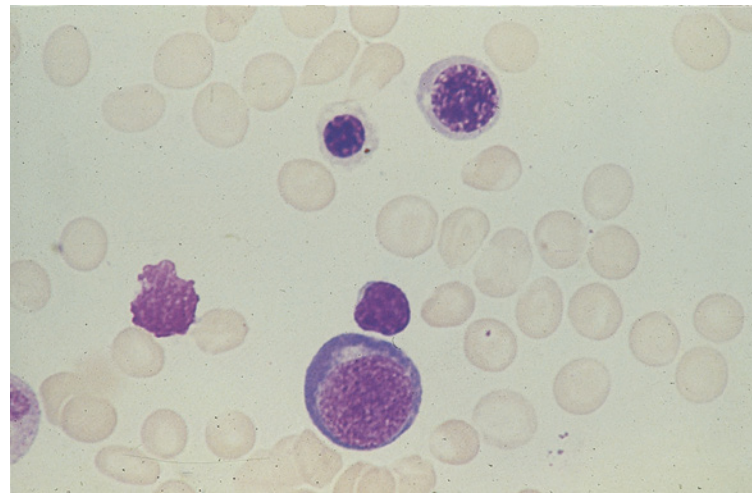

Fig. 1.17 Aspirate of normal BM: early, intermediate and late erythroblasts and a lymphocyte. MGG ×100.

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.

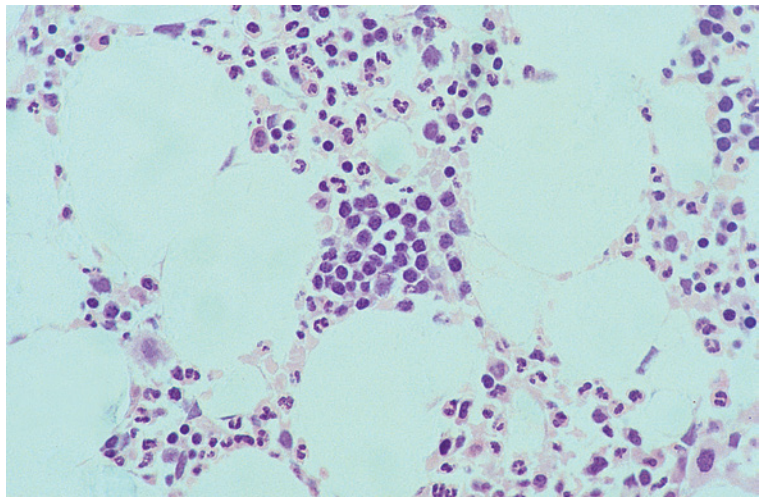

Fig. 1.18 Section of normal BM: an erythroid island (centre). Resin-embedded, H&E ×40.

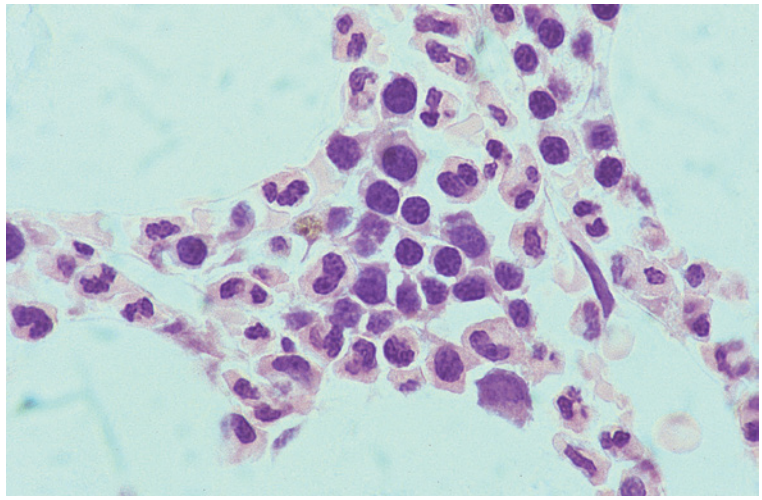

Fig. 1.19 Section of normal BM: an erythroid island containing intermediate and late erythroblasts and a haemosiderin-laden macrophage; a Golgi zone can be seen in some of the intermediate erythroblasts. Resin-embedded, H&E ×100.

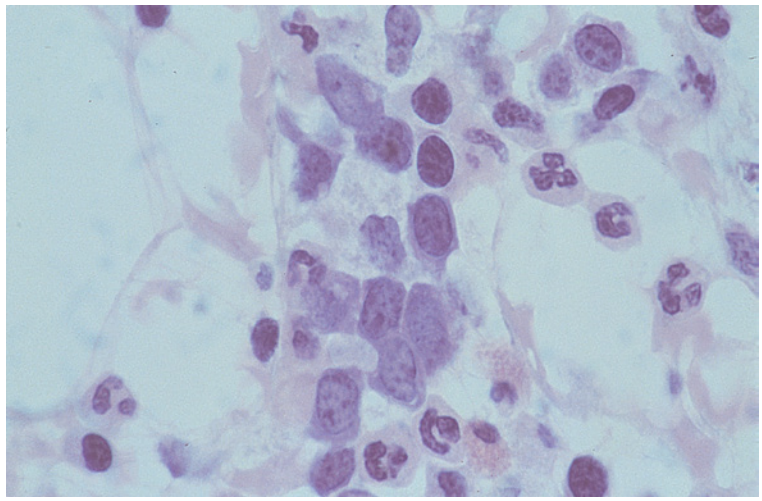

Fig. 1.20 Section of normal BM: an erythroid island containing early and intermediate erythroblasts. Resin-embedded, Giemsa ×100.