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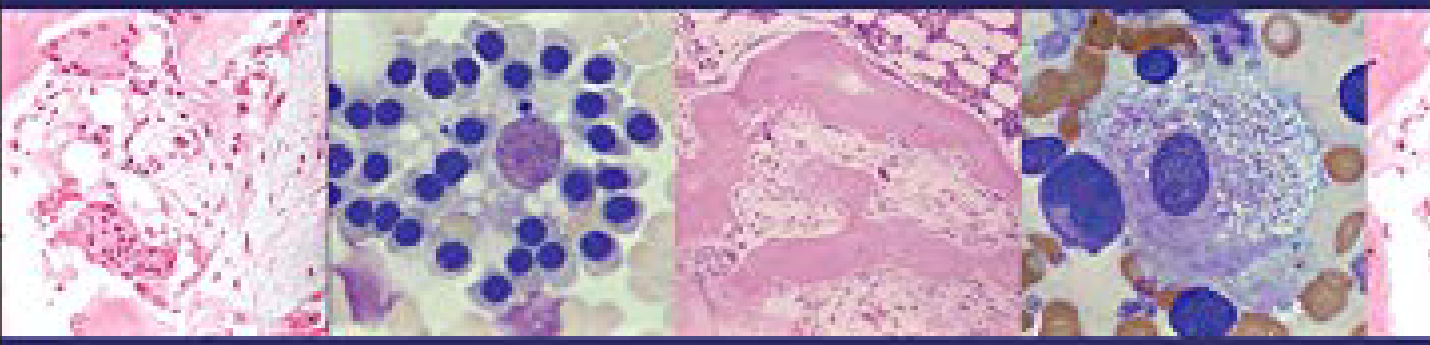

Bone Marrow Pathology

Barbara J. Bain
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WILEY Blackwell

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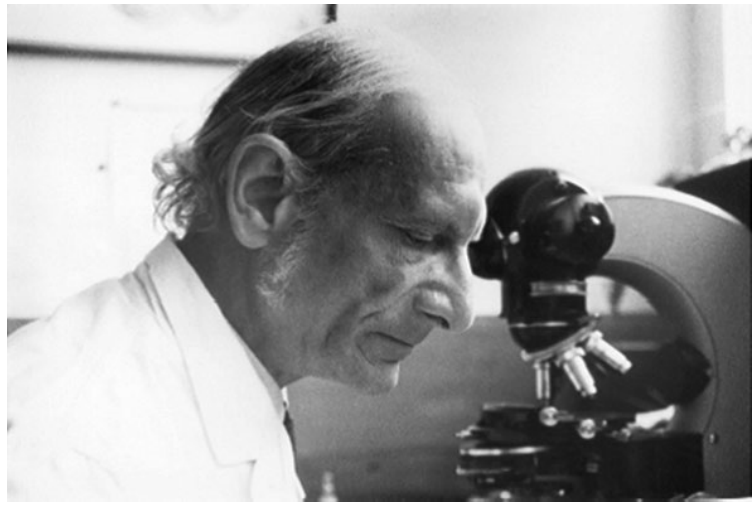

This book is dedicated to the late Professor David A. G. Galton

BONE MARROW PATHOLOGY

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PREFACE TO THE FIFTH 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 2016 World Health Organization classification of tumours of haematopoietic and lymphoid tissues.

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 edition, 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, diagnostic algorithms and summary boxes.

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

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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, Hammersmith Hospital, Nottingham University Hospital, Path Links and St Thomas' 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 thanks go to 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 has 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 much missed.

BJB, DMC, BSW

ABBREVIATIONS

ABC	activated B-cell-like
aCML	atypical chronic myeloid leukaemia
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
ANAE	alpha-naphthyl acetate esterase
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
BDCA-2	blood dendritic cell antigen 2
BFU-E	burst-forming unit, erythroid
BL	Burkitt lymphoma
BM	bone marrow
B-PLL	B-cell prolymphocytic leukaemia
c	cytoplasmic
CAE	chloroacetate esterase
CAR T cell	chimaeric antigen receptor-redirected T cell
CD	cluster of differentiation
CDA	congenital dyserythropoietic anaemia
CEA	carcino-embryonic antigen
CEL	chronic eosinophilic leukaemia
CFU-E	colony-forming unit, erythroid
CFU-GM	colony-forming unit, granulocyte–macrophage
CFU-Meg	colony-forming unit, megakaryocyte
CGH	comparative genomic hybridization
CHAD	cold haemagglutinin disease
cIg	cytoplasmic immunoglobulin
CK	cytokeratin
CLL	chronic lymphocytic leukaemia
CLL/PL	chronic lymphocytic leukaemia, mixed cell type
CML	chronic myeloid leukaemia
CMML	chronic myelomonocytic leukaemia
CMV	cytomegalovirus
CNS	central nervous system
CT	computed tomography
DAB	diaminobenzidine tetrachloride
del	deletion
DIC	disseminated intravascular coagulation
DLBCL	diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
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
ER	oestrogen receptor
ET	essential thrombocythaemia
FAB	French–American–British (co-operative group)
FBC	full blood count
FDC	follicular dendritic cell

FHL	follicular helper lymphocyte
FISH	fluorescence *in situ* hybridization
FPD/AML	familial platelet disorder with propensity to acute myeloid leukaemia
GCB	germinal centre B-cell-like
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte–macrophage colony-stimulating factor
GMS	Grocott's methenamine silver (stain)
GPI	glycosyl phosphatidylinositol
GVHD	graft-versus-host disease
Hb	haemoglobin concentration
H&E	haematoxylin and eosin (stain)
HEMPAS	hereditary erythroid multinuclearity with positive acidified serum lysis test
HER2	human epidermal growth factor receptor 2
HHV	human herpesvirus
HIV	human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	human leucocyte antigen
HPLC	high performance liquid chromatography
HRS	Hodgkin/Reed–Sternberg
HTLV-1	human T-cell lymphotropic virus 1
i	isochromosome
ICOS	inducible T-cell co-stimulator
ICSH	International (Committee) Council for Standardization in Haematology
idic	isodicentric chromosome
Ig	immunoglobulin
IHC	immunohistochemistry
IL	interleukin
inv	inversion
IPI	International Prognostic Index
IPSID	immunoproliferative small intestinal disease
ISH	*in situ* hybridization
ITD	internal tandem duplication
JMML	juvenile myelomonocytic leukaemia
KIR	killer inhibitory receptor
LANA	latency-associated nuclear antigen
LDH	lactate dehydrogenase
LE	lupus erythematosus
LEF1	lymphoid enhancer-binding factor 1
LGL	large granular lymphocyte/s
LMP	latent membrane protein
LP	lymphocyte predominant
MALT	mucosa-associated lymphoid tissue
McAb	monoclonal antibody/ies
MCV	mean cell volume
MDS	myelodysplastic syndrome/s
MDS-EB	myelodysplastic syndrome with excess blasts
MDS-EB-F	myelodysplastic syndrome with excess blasts and fibrosis
MDS-F	myelodysplastic syndrome with fibrosis
MDS-MLD	myelodysplastic syndrome with multilineage dysplasia
MDS/MPN	myelodysplastic / myeloproliferative neoplasm
MDS/MPN-RS-T	myelodysplastic/ myeloproliferative neoplasm with ring sideroblasts and thrombocytosis
MDS-RS-MLD	myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia
MDS-RS-SLD	myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia
MDS-SLD	myelodysplastic syndrome with single lineage dysplasia
MDS-U	myelodysplastic syndrome, unclassifiable
M : E	myeloid : erythroid
MF	myelofibrosis
MGG	May–Grünwald–Giemsa (stain)
MGUS	monoclonal gammopathy of undetermined significance
MPAL	mixed phenotype acute leukaemia
MPN	myeloproliferative neoplasm/s
MPO	myeloperoxidase
MRD	minimal residual disease
mRNA	messenger ribonucleic acid
NEC	non-erythroid cell/s
NGS	next generation sequencing
NHL	non-Hodgkin lymphoma
NK	natural killer

NLPHL	nodular lymphocyte-predominant Hodgkin lymphoma
NOS	not otherwise specified
NRBC	nucleated red blood cell
NSE	non-specific esterase
PAS	periodic acid–Schiff (stain)
PB	peripheral blood
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEL	primary effusion lymphoma
PET	positron emission tomography
PGP9.5	protein gene product 9.5
Ph	Philadelphia (chromosome)
PLL	prolymphocytic leukaemia
PMF	primary myelofibrosis
PNET	primitive neuroectodermal tumour/s
PNH	paroxysmal nocturnal haemoglobinuria
POEMS	polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes (syndrome)
PRINS	primed *in situ* hybridization
PSA	prostate-specific antigen
PSAP	prostate-specific acid phosphatase
PTLD	post-transplant lymphoproliferative disorder
PV	polycythaemia vera
RA	refractory anaemia
RAEB	refractory anaemia with excess of blasts
RAEB-T	refractory anaemia with excess of blasts in transformation
RARS	refractory anaemia with ring sideroblasts
RARS-T	refractory anaemia with ring sideroblasts and marked thrombocytosis
RCC	refractory cytopenia of childhood
RCMD	refractory cytopenia with multilineage dysplasia
RCUD	refractory cytopenia with unilineage dysplasia
RN	refractory neutropenia
RNA	ribonucleic acid
RQ-PCR	real-time quantitative polymerase chain reaction
RT	refractory thrombocytopenia
RT-PCR	reverse transcriptase polymerase chain reaction
SBB	Sudan black B (stain)
SCID	severe combined immune deficiency
SD	standard deviation
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	side scatter
t	translocation
T-ALL	T acute lymphoblastic leukaemia
t-AML	therapy-related acute myeloid leukaemia
TAR	thrombocytopenia-absent radii (syndrome)
TBS	Tris-buffered saline
TCR	T-cell receptor
TdT	terminal deoxynucleotidyl transferase
TEMPI	telangiectasia, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collection and intrapulmonary shunting (syndrome)
TKI	tyrosine kinase inhibitor
T-LGL	T-cell large granular lymphocytic (leukaemia)
t-MDS	therapy-related myelodysplastic syndrome
TNF	tumour necrosis factor
T-PLL	T-cell prolymphocytic leukaemia
TRAP	tartrate-resistant acid phosphatase
VEGF	vascular endothelial growth factor
WBC	white blood cell count
WHIM	warts, hypogammaglobulinaemia, infections and myelokathexis (syndrome)
WHO	World Health Organization
XLP	X-linked lymphoproliferative disease
ZN	Ziehl–Neelsen (stain)

ONE

THE NORMAL BONE MARROW

"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

The cortex and the medulla differ functionally as well as histologically. 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 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 cortical bone is covered on the external surface by periosteum, which has

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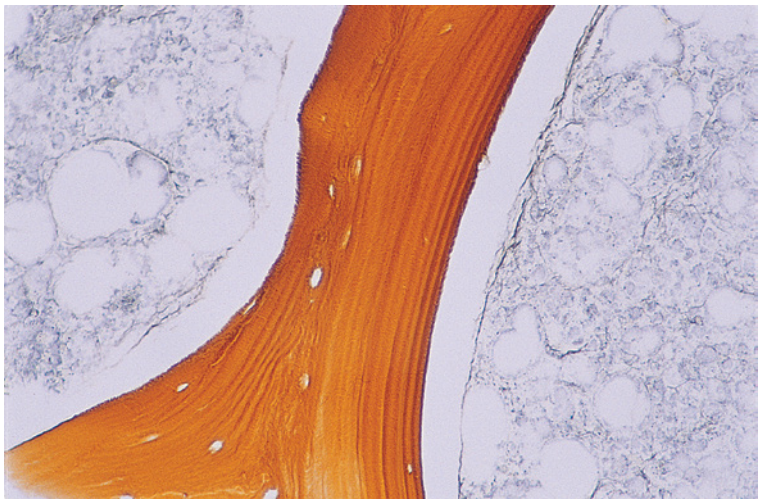

Fig. 1.1 Bone marrow (BM) trephine biopsy section showing normal bone structure; the trabeculae are composed of lamellar bone. Reticulin stain ×20.

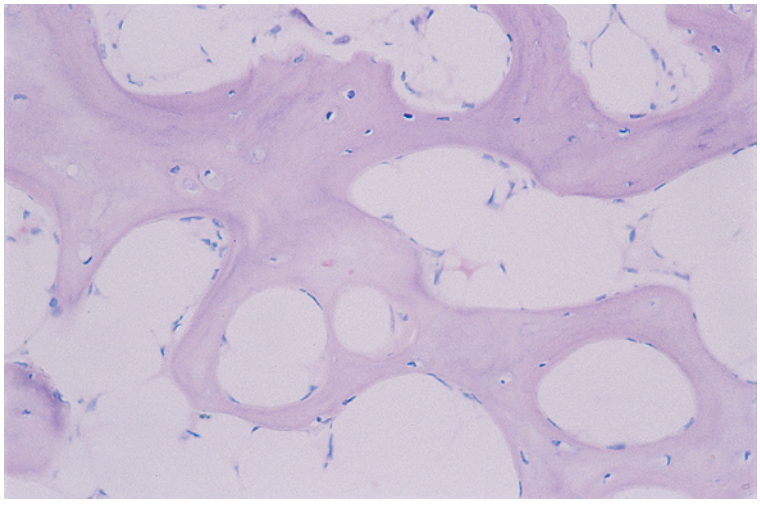

Fig. 1.2 BM trephine biopsy section showing woven bone (pale pink; without lamellae) in a hypocellular but otherwise unremarkable bone marrow. Haematoxylin and eosin (H&E) ×20.

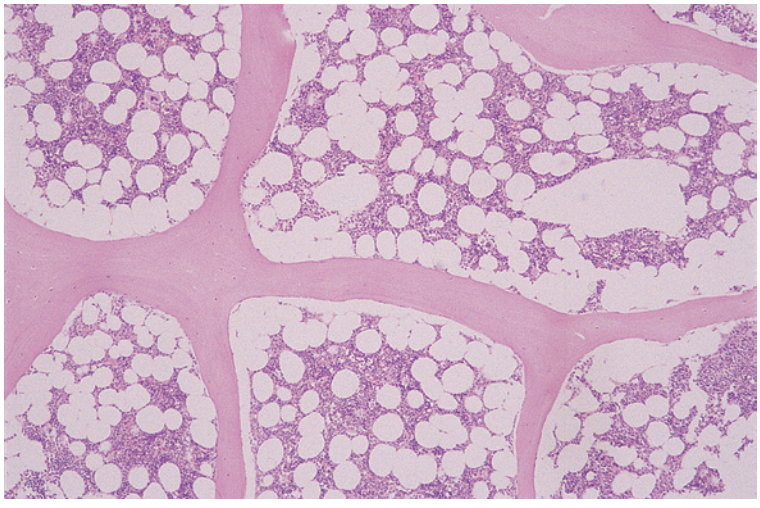

Fig. 1.3 BM trephine biopsy section showing normal bone structure; there are anastomosing bony trabeculae. H&E ×5.

an outer fibrous layer and an inner osteogenic layer. At articular surfaces, and more extensively in younger patients, 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's 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. The difference between the two 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).

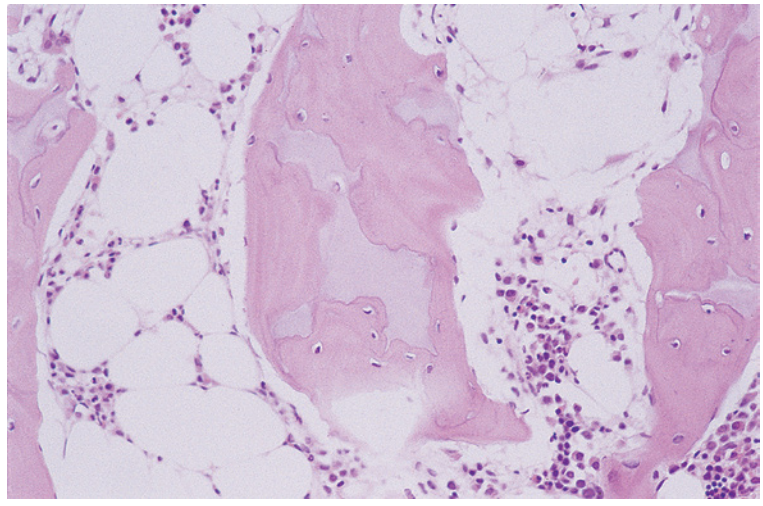

Fig. 1.4 BM trephine biopsy section from a child showing endochondrial ossification in an island of cartilage. H&E ×20.

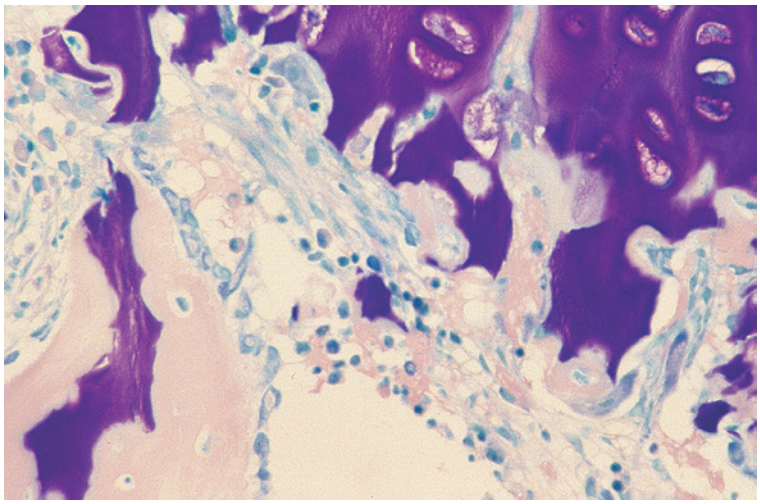

Fig. 1.5 BM trephine biopsy section from a child showing endochondrial ossification; a bony spicule with a core of cartilage is lined by osteoblasts. Giemsa stain ×40.

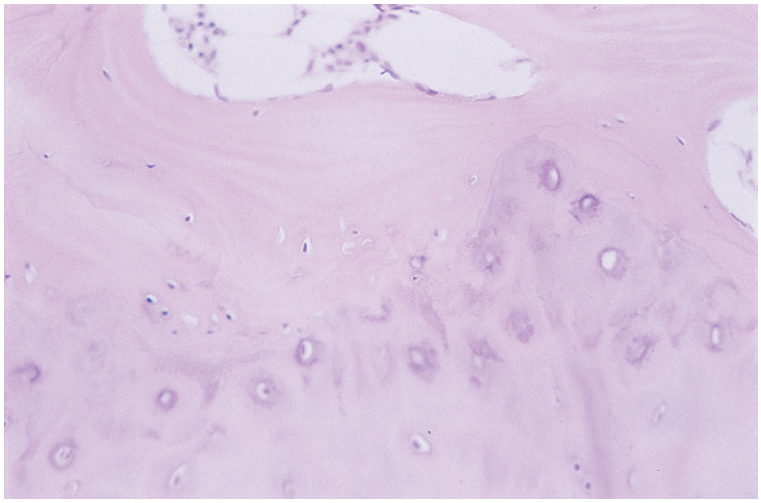

Fig. 1.6 BM trephine biopsy section from an adult showing cartilage adjacent to the cortex. By contrast with childhood appearances, a well-defined layer of cortical bone separates this cartilage from the bone marrow. Cartilage cells are dispersed singly or in small groups and are not aligned into columns,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

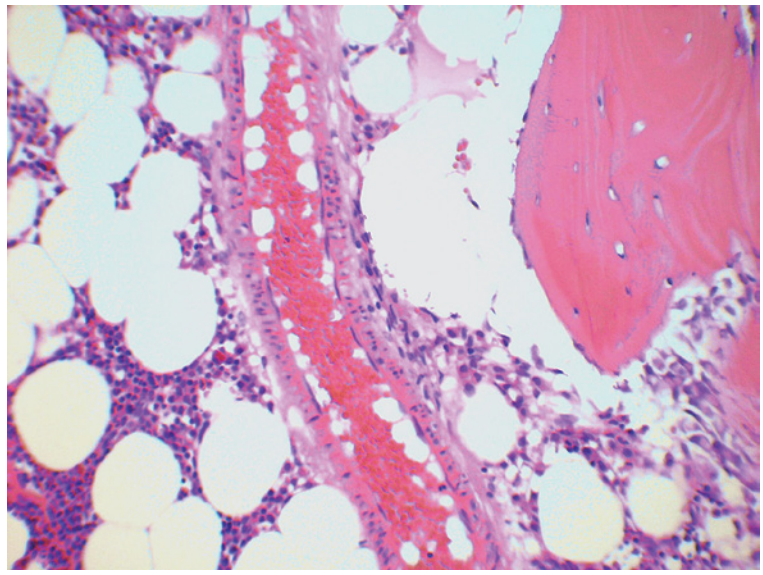

Fig. 1.7 BM trephine biopsy section showing a longitudinal section of an arteriole. H&E ×20.

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 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.

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. In man, 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.

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 from Germany by P. Wolff, following puncture of the femur and tibia respectively [5]. 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. 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 [6,7]. In another study the quality of specimens was equivalent but pain was less [8].

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 [9]. Local anaesthesia supplemented by inhaled nitrous oxide anaesthesia is also an option [10]. In children, aspiration and trephine biopsies are often 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. 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 [11]. Sternal aspiration is more dangerous at any age (see later), 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 [12].

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 [13]. 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 [14], 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.

Trephine biopsy of bone marrow

Trephine or needle biopsy is most easily carried out on the iliac crest, either posteriorly or anteriorly, as described above. The posterior approach (Fig. 1.8) appears now to be more generally preferred. It both gives longer specimens with a larger area for examination and is less painful for the patient [15]. Disposable needles are now generally 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. Ranfac's Snarecoil needle also has a capturing device [16]. There are also powered devices, one of which (OnControl, Vidacare Corporation) was found in a meta-analysis of five randomized controlled studies to produce a longer biopsy specimen with the procedure being less painful [17]. However in a subsequent study, although the specimen was

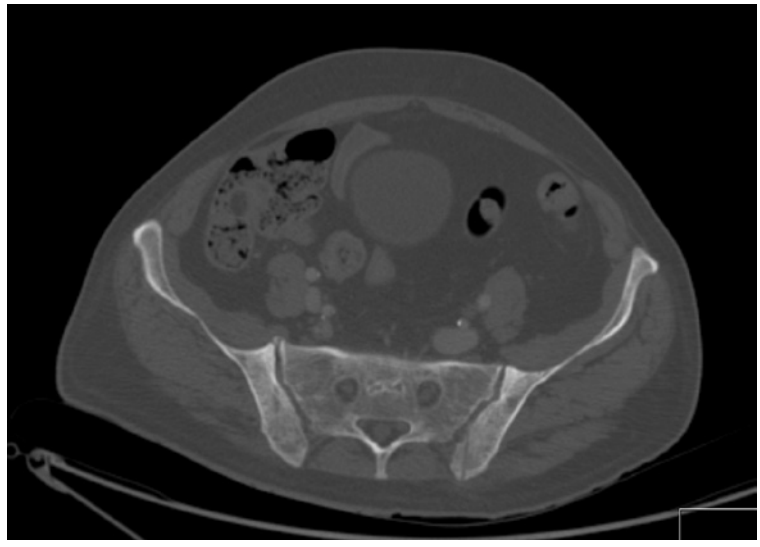

Fig. 1.8 Computed tomography (CT) scan of the pelvis showing a trephine biopsy needle track through the posterior iliac crest. (With thanks to Dr Marc Heller, London.)

longer, the length of evaluable marrow was greater with a manual technique [18]. 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 needle 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 [19]. 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 [20]. In obese patients, ultrasound can be used to localize the posterior iliac crest [20]. 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 [21] 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 [22]. 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. Frozen sections of trephine biopsy specimens are possible but they are not usually very satisfactory because of technical problems, including difficulty in cutting sections, poor adhesion of sections to glass slides during staining procedures and poor preservation of morphological detail. They are rarely used now that immunohistochemistry can be readily applied to fixed tissues. Histological sections may be prepared from fixed biopsy specimens which have either been decalcified and paraffin-embedded or have been embedded in resin without prior decalcification.

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, e.g. formic acid and acetic acid, or by chelation, e.g. 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 no 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. Although excellent results are 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 authors' laboratories. Resins with differing qualities are available for embedding. Methyl methacrylate requires lengthy processing and is therefore not very suitable for routine diagnostic laboratories. Glycol methacrylate is more satisfactory; however, when cellularity is low, sections tend to tear and, in this circumstance,

a small amount of decalcification may be useful. Methods which 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 [23]. 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 [24]. 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 [25,26]. Haemorrhage may be either intra-abdominal [27], retroperitoneal [25] (rarely with secondary haemothorax) [28] or into the buttock and thigh [25], in the latter two circumstances with the risk of nerve compression [25,29,30]. Pseudoaneurysm formation [31,32] and creation of an arteriovenous fistula with associated haemorrhage [33] have been reported and can require intervention; selective embolization may be useful to control bleeding in such cases. Risk factors are heparin or warfarin therapy, coagulation factor deficiencies, von Willebrand's 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 antiplatelet 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's 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 is a rare complication. Other rare complications include avulsion fracture at the biopsy site [34], pneumoretroperitoneum [35], implantation of malignant cells in the track of the

biopsy needle in plasmacytoma and non-Hodgkin lymphoma [36–38], prolonged leak of serous fluid in a patient with nephrotic syndrome [39], bone marrow embolism [40], cerebrospinal fluid leak [41] and later development of exostosis [42].

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 often 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 [43]. Specimens that are suitable for histological assessment of cellularity are: aspirated fragments; needle 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 [44].

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 [44,45]. Cellularity can also be assessed subjectively. Such estimates are less reproducible and may lead to some under-estimation 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 [44]. 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

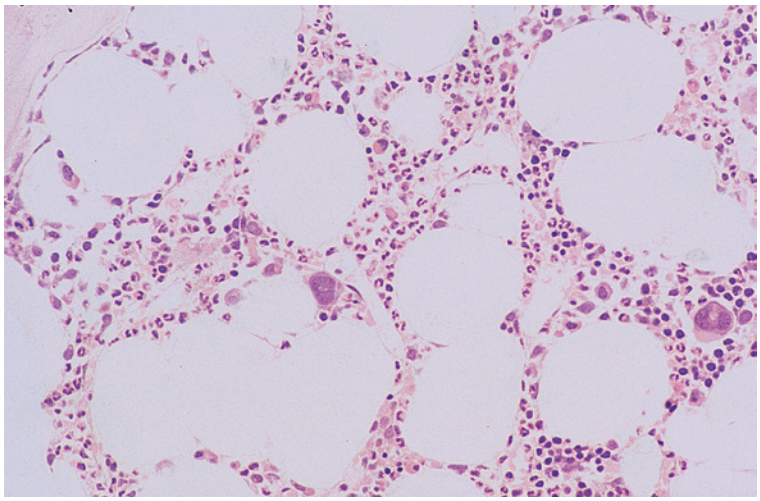

Fig. 1.9 Section of normal BM: normal distribution of all three haemopoietic lineages; note the megakaryocyte adjacent to a sinusoid. Resin-embedded, H&E ×20.

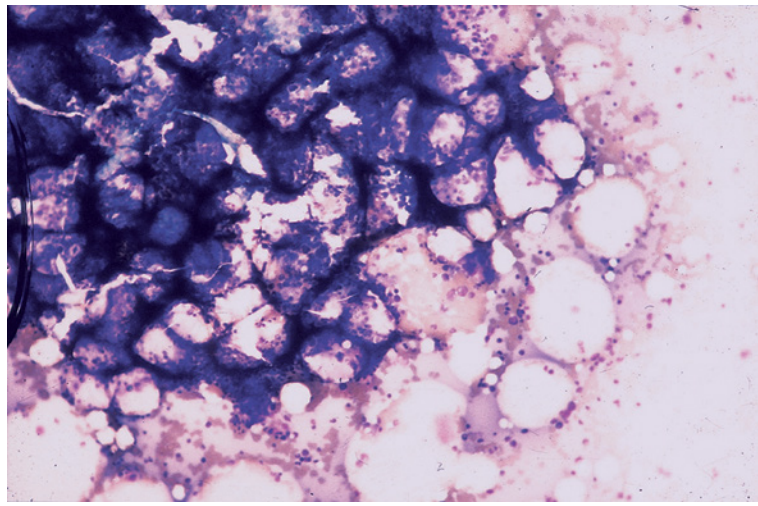

Fig. 1.10 Aspirate of normal BM: fragment showing normal cellularity. May–Grünwald–Giemsa (MGG) ×40.

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) [46] or as a percentage of the marrow cavity [44,47]. There are advantages in the latter approach, in which the area occupied by bone is excluded from the calculation, since the percentages obtained are then directly comparable with measurements made on histological sections of aspirated fragments or estimates made from fragments in bone marrow films.

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 [46–50] (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 [48]. Average cellularity in the bone marrow of children, assessed on core biopsy or clot sections, is 80% at 2 years, 69% at 2–4 years, 59% at 5–9 years and around

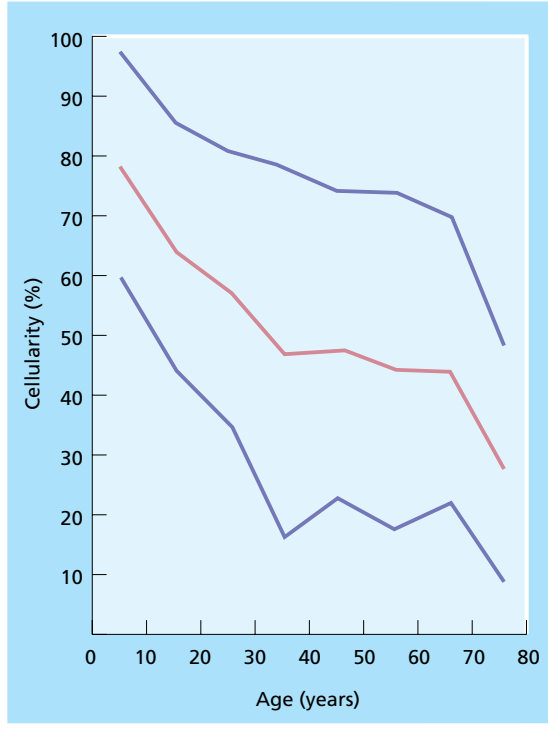

Fig. 1.11 Mean and 95% range of cellularity at various ages of anterior iliac crest bone marrow which has been decalcified and paraffin-embedded. Cellularity is expressed as a percentage of the bone marrow cavity. (Calculated from Hartsock *et al.* [47].) (Reproduced with permission of Oxford University Press.)

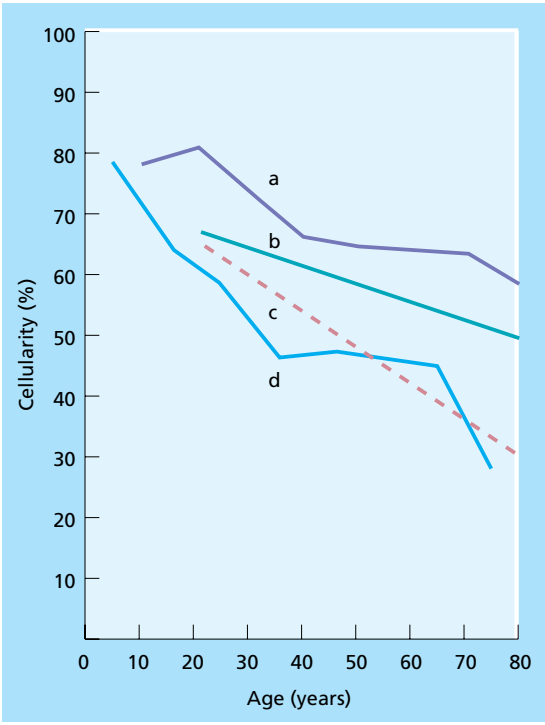

Fig. 1.12 Mean value of bone marrow cellularity at various ages expressed as a percentage of bone marrow cavity: (a) iliac crest, autopsy, not decalcified (recalculated from Frisch *et al.* [46]); (b) iliac crest, autopsy, not decalcified [49]; (c) sternum, biopsy, not decalcified [50]; and (d) ilium, autopsy, decalcified [47].

60% thereafter [51]. 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 [47]; from 85% at age 20 years to 40% at age 60, also in the iliac crest [48]; and from 66% at age 20 to 30% at age 80 in the sternum [50].

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 [14]. Vertebrae are also more 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.* [11], 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 [46–50]. It should be noted that the lowest estimates of iliac crest cellularity are from a study using decalcified, paraffin-embedded bone marrow specimens [47] while the highest estimates are from a study using non-decalcified, resin-embedded specimens [46]. Some studies have been conducted on biopsy specimens [50] and others on specimens obtained at autopsy [46,47,49]. 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 [52]. The British

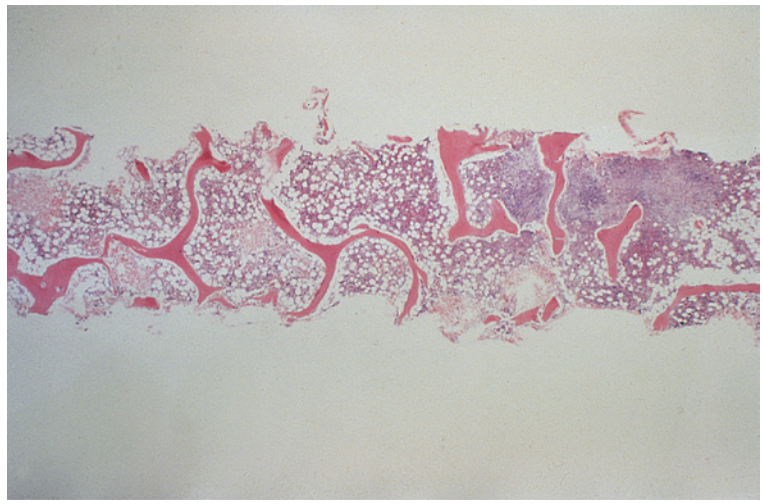

Fig. 1.13 A section of a trephine biopsy specimen of adequate size from a patient with Hodgkin lymphoma showing only a small area of infiltration at one end of the specimen, illustrating how a small biopsy may miss focal lesions. H&E ×2.5. (With thanks to Dr Ken Maclennan.)

Committee for Standards in Haematology and Royal College of Pathologists guidelines recommend at least 16 mm [53].

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 '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 above.

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 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 [54]. 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.

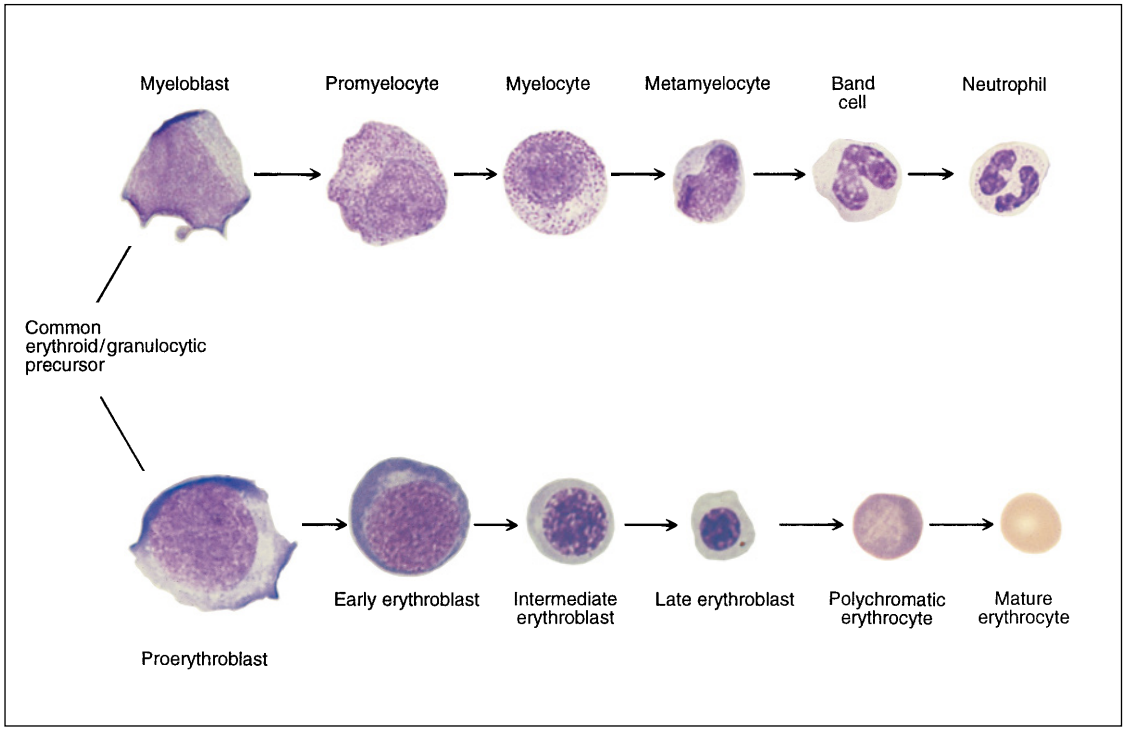

Fig. 1.14 A semi-diagrammatic representation of granulopoiesis and erythropoiesis. Cell division occurs up to the myelocyte and intermediate erythroblast stages.

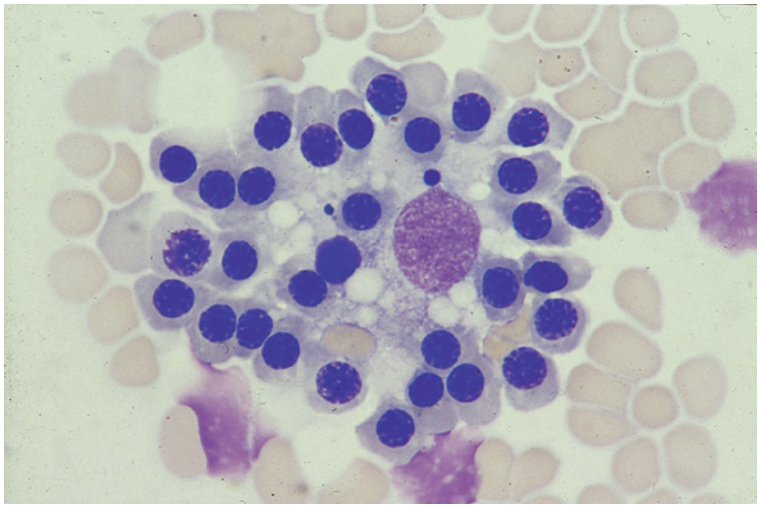

Fig. 1.15 BM aspirate: an erythroid island. MGG ×100.

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. 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

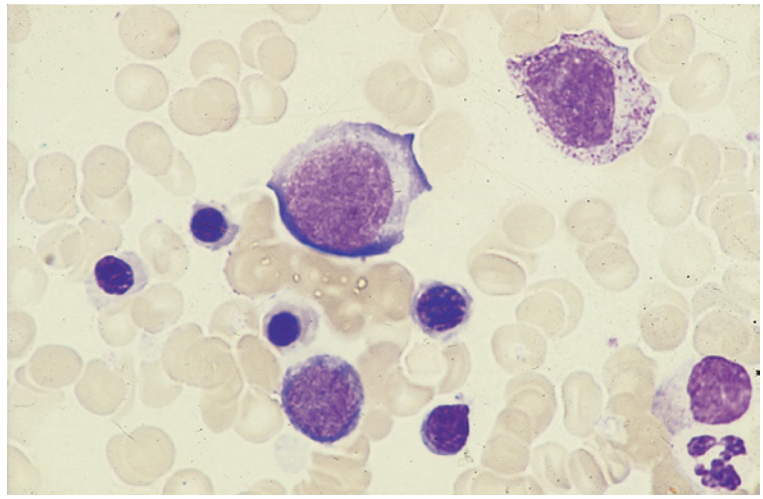

Fig. 1.16 Aspirate of normal BM: a proerythroblast, an intermediate erythroblast, four late erythroblasts, a myelocyte, large and small lymphocytes and a neutrophil. MGG ×100.

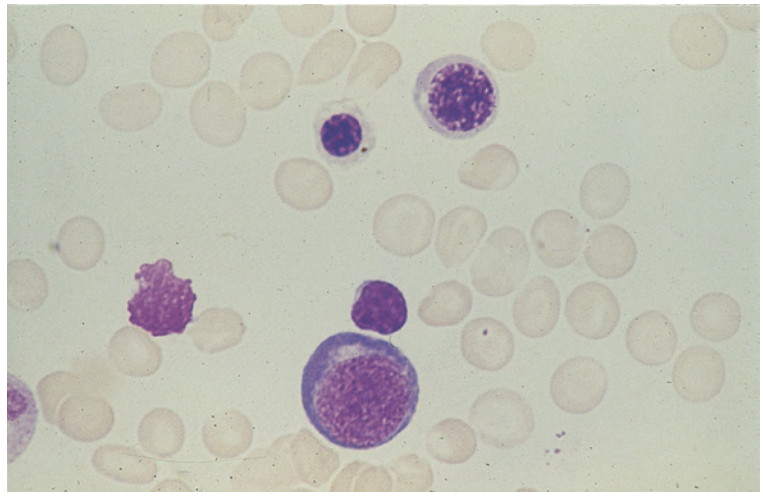

Fig. 1.17 Aspirate of normal BM: early, intermediate and late erythroblasts and a lymphocyte. MGG ×100.

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 [55].

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

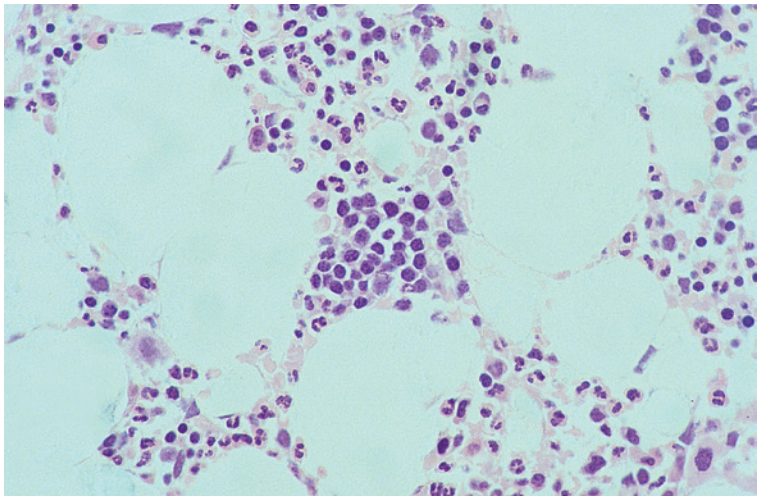

Fig. 1.18 Section of normal BM: an erythroid island (centre). Resin-embedded, H&E ×40.

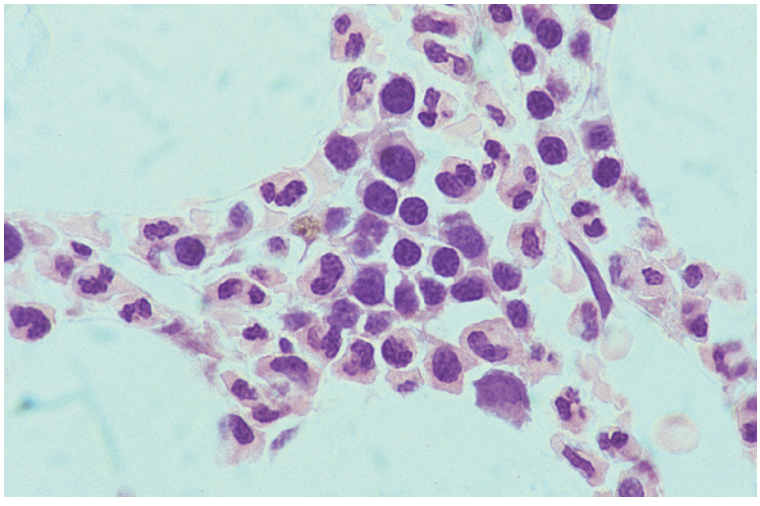

Fig. 1.19 Section of normal BM: an erythroid island containing intermediate and late erythroblasts and a haemosiderin-laden macrophage; a Golgi zone is seen in some of the intermediate erythroblasts. Resin-embedded, H&E ×100.

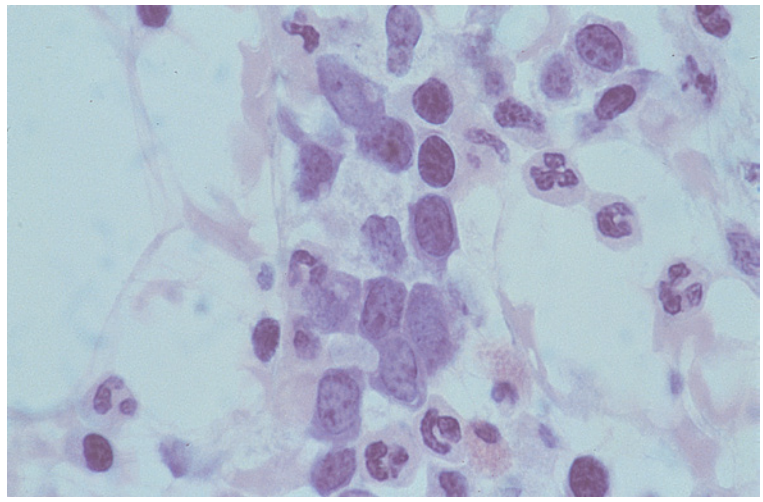

Fig. 1.20 Section of normal BM: an erythroid island containing early and intermediate erythroblasts. Resin-embedded, Giemsa ×100.

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 65) may demonstrate the presence of haemosiderin. Erythropoiesis occurs relatively close to marrow sinusoids although it is probable that, as in the rat [56], 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.

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 present or if they contain only immature cells. When there is any difficulty in recognizing erythroid precursors their identity can be confirmed by immunohistochemical staining (see page 79).

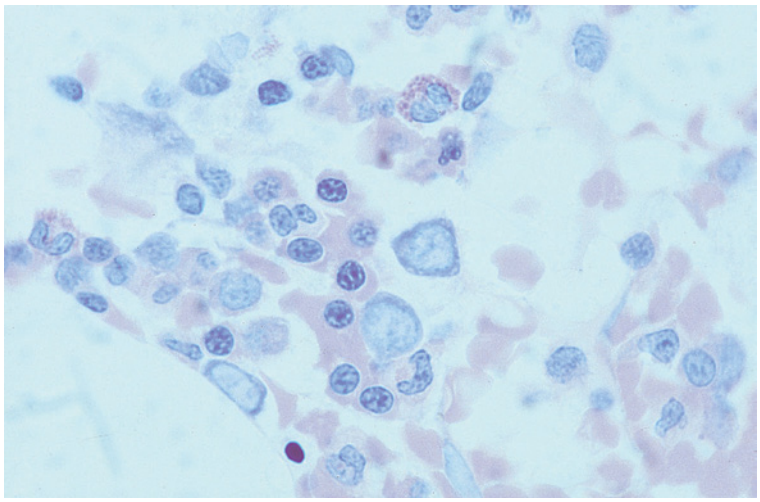

Fig. 1.21 Section of normal BM: erythroid island containing three early, one intermediate and numerous late erythroblasts; note the cytoplasmic basophilia of early erythroblasts. Resin-embedded, Giemsa ×100.

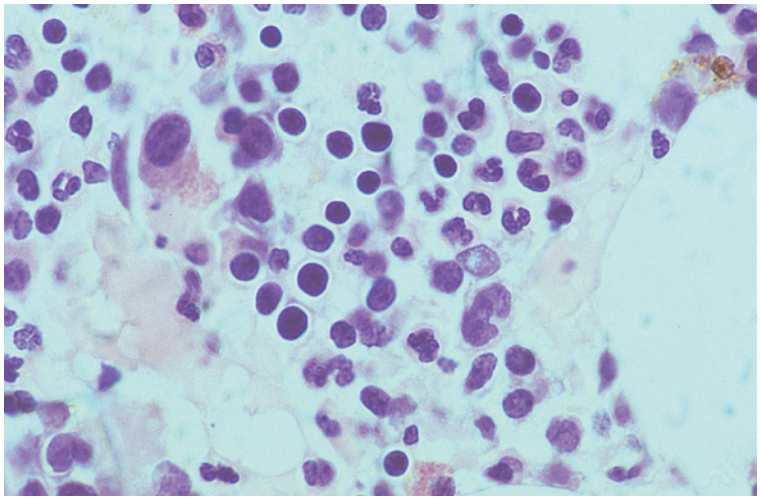

Fig. 1.22 Section of normal BM: erythroid island showing intermediate and late erythroblasts with haloes surrounding the nuclei. H&E ×100.

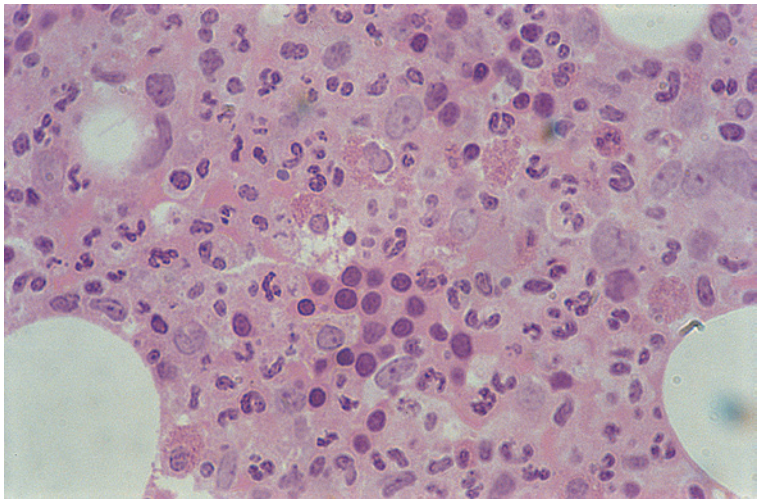

Fig. 1.23 Syncytial appearance of erythroblasts in an erythroid island in sections from a trephine biopsy specimen. Resin-embedded, H&E ×60.

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. 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.

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

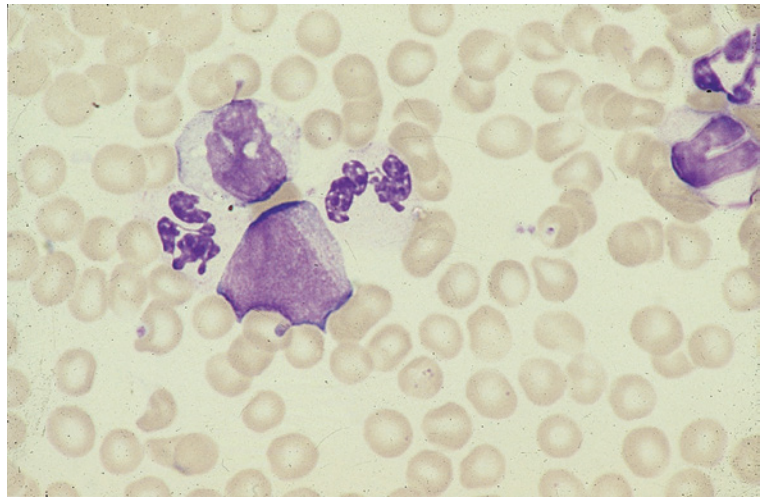

Fig. 1.24 Aspirate of normal BM: a myeloblast, three neutrophils and two monocytes; the myeloblast has a high nucleocytoplasmic ratio, a diffuse chromatin pattern and a nucleolus. MGG ×100.

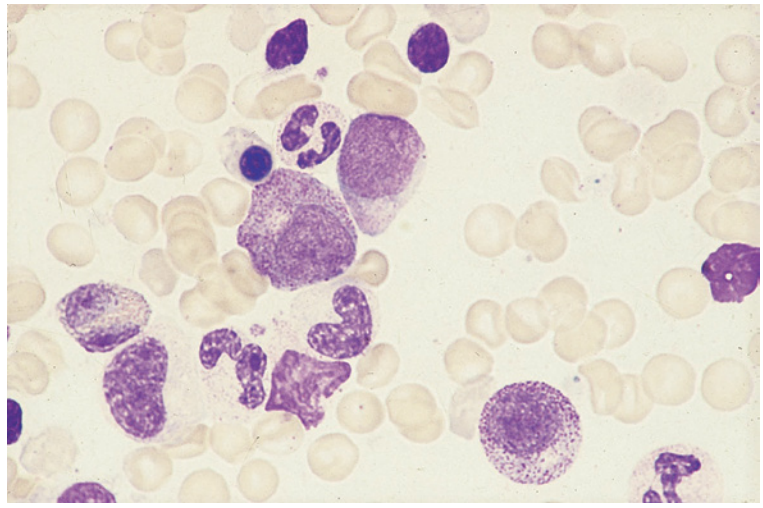

Fig. 1.25 Aspirate of normal BM: a myeloblast and a promyelocyte (centre), a myelocyte (lower right), a metamyelocyte, band forms, a neutrophil and a late erythroblast; the promyelocyte is larger than the myeloblast and is showing some chromatin condensation but with persisting nucleoli, well-developed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

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 the 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 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.

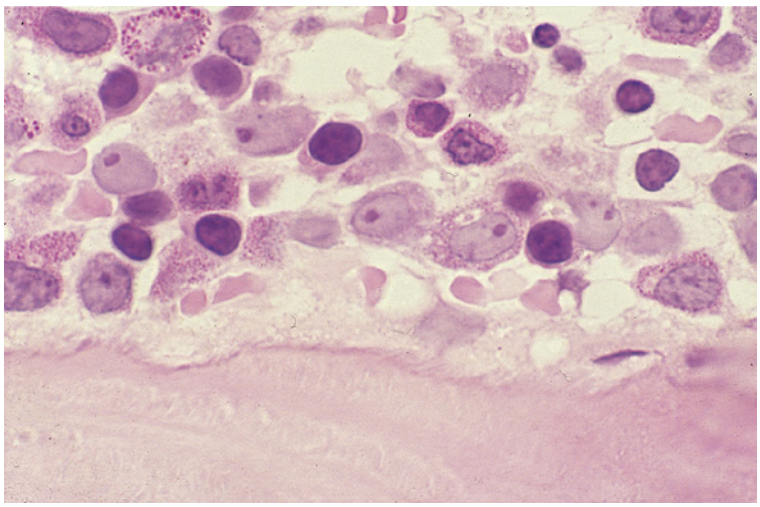

Fig. 1.26 Section of normal BM: myeloblasts and promyelocytes adjacent to a bony trabecula. Resin-embedded, H&E ×100.

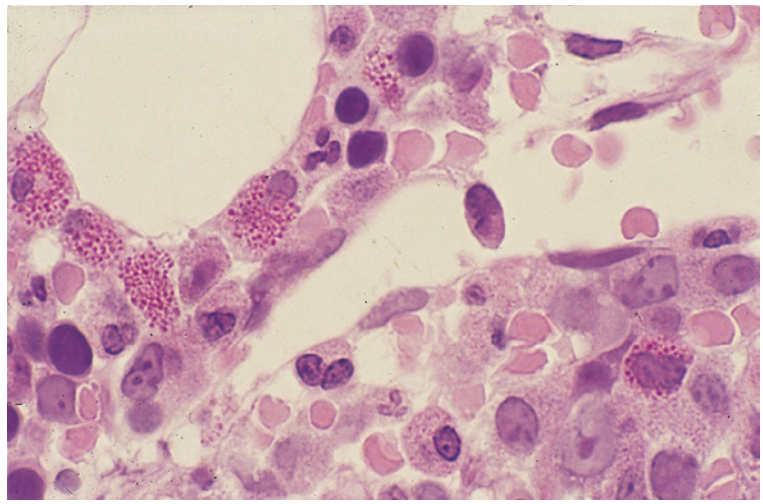

Fig. 1.27 Section of normal BM: promyelocytes, myelocytes and maturing neutrophils and eosinophils adjacent to a sinusoid. Resin-embedded, H&E ×100.

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. The earliest morphologically recognizable precursor is a monoblast, a cell which 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.

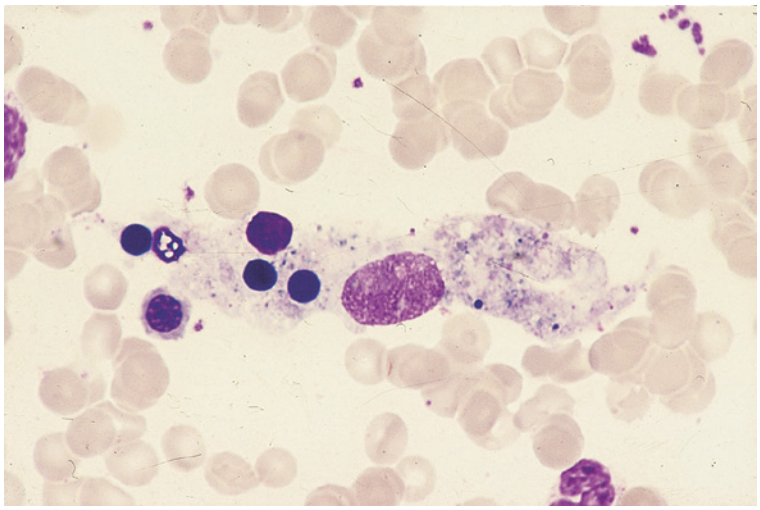

Fig. 1.28 Aspirate of normal BM: a macrophage containing granular and refractile debris and several normoblast nuclei. MGG ×100.

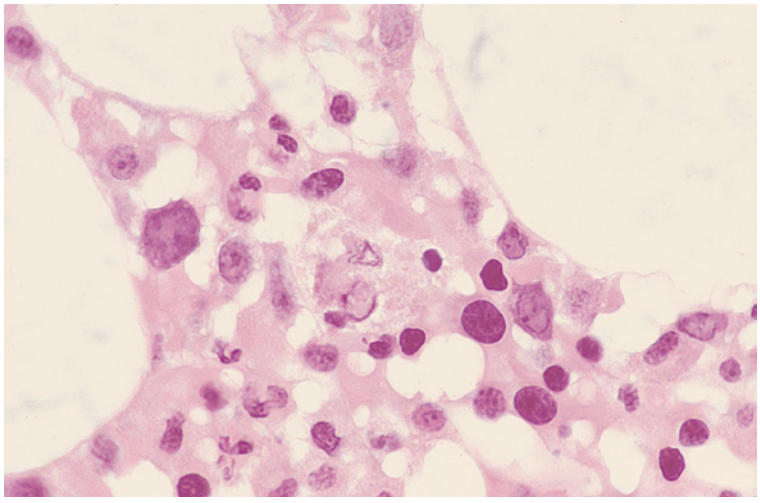

Fig. 1.29 Section of normal BM: a macrophage containing cellular debris. Resin-embedded, H&E ×100.

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 'haemangioblasts', known to be present during embryonic development, that are capable of

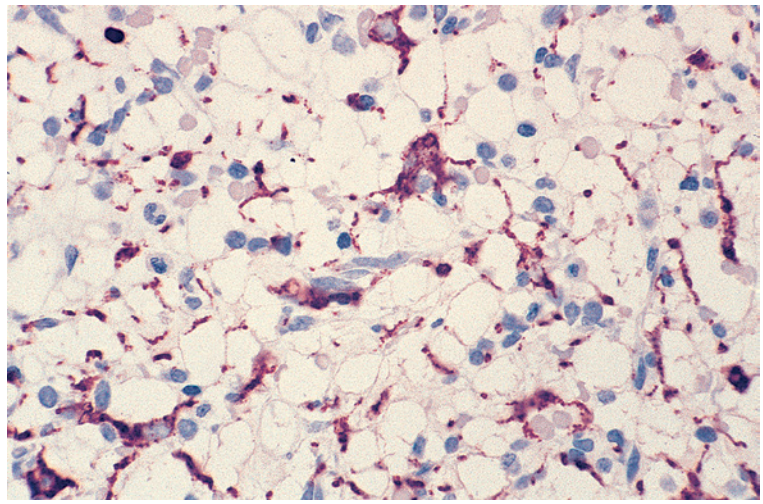

Fig. 1.30 Section of trephine biopsy specimen showing a network of dendritic macrophages. Immunoperoxidase with CD68 monoclonal antibody (McAb) ×50.

differentiating into both myeloid and stromal lineages. Mesenchymal stem cells [57,58] are capable of adipocyte, 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 they 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 mobilization 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 visualize 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

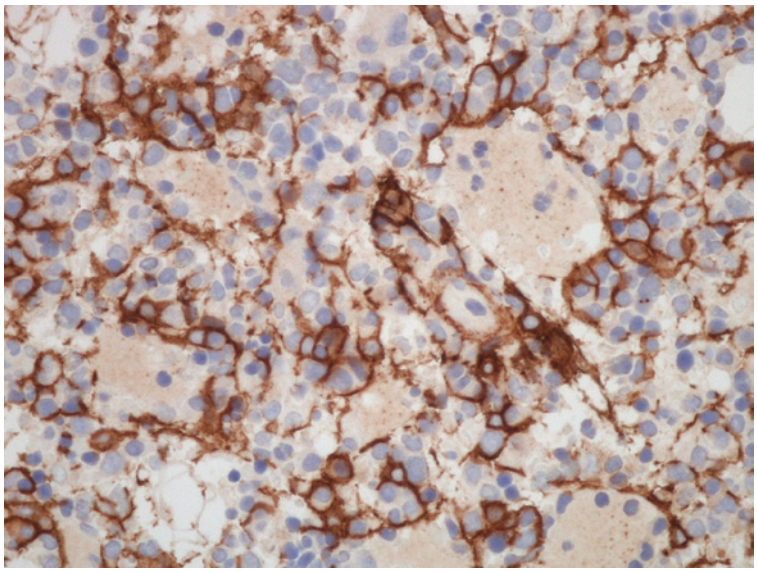

Fig. 1.31 Section of trephine biopsy specimen showing a network of stromal dendritic cells. Immunoperoxidase with CD271 McAb ×40.

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

Megakaryocytes arise from haemopoietic stem cells via a common megakaryocyte–erythroid progenitor cell that gives rise to erythroid precursors and megakaryoblasts. 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 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 [59]. 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 [60]. 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 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. Platelet production involves aggregation of components within the cell