ESSENTIALS

HOFFBRAND'S ESSENTIAL HAEMATOLOGY

A. VICTOR HOFFBRAND | PAUL A.H. MOSS

SEVENTH EDITION

WILEY Blackwell

Hoffbrand's Essential Haematology

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Cover image: P242/0207. Blood cells, SEM. NATIONAL CANCER INSTITUTE/SCIENCE PHOTO LIBRARY. Blood cells and platelets. Coloured Scanning Elec-tron micrograph (SEM) of human blood showing red and white cells and platelets. Red blood cells (erythrocytes) have a characteristic biconcave- disc shape and are numerous. These large cells contain haemoglobin, a red pigment by which oxygen is transported around the body. They are more numerous than white blood cells (yellow). White blood cells (leucocytes) are rounded cells with microvilli projections from the cell surface. Leucocytes play an important role in the immune response of the body. Platelets are smaller cells (pink) that play a major role in blood clotting.

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Preface to the Seventh Edition

There have been remarkable advances in the understanding of the pathogenesis of diseases of the blood and lymphatic system and in the treatment of these diseases, since the 6th Edition of *Essential Haematology* was published in 2011. This new knowledge is due largely to the application of next generation sequencing of DNA which has enabled the detection of the genetic mutations, inherited or acquired, that underlie these diseases. As examples, sequencing has revealed the *CALR* mutation underlying a substantial proportion of patients with myeloproliferative diseases and the *MYD88* mutation present in almost all cases of Waldenström's macrogobulinaemia. Multiple 'driver' gene mutations affecting signalling pathways and epigenetic reactions involved in cell proliferation and survival have been discovered which underlie myelodysplasia, acute myeloid and lymphoblastic leukaemias, chronic lymphocytic leukaemia and the lymphomas. The complexity of the molecular changes underlying the malignant diseases and the relevance of this to their sensitivity or resistance to therapy is becoming apparent.

This new knowledge has been accompanied by spectacular improvements in therapy. Inhibition of the B cell receptor signalling pathway has transformed the life expectancy in many patients with resistant chronic lymphocytic leukaemia and some of the B cell lymphomas resistant to other therapy. *JAK2* inhibitors are improving the quality of life and survival in primary myelofibrosis. Survival in myeloma is improving remarkably with new proteasome inhibitory and immunomodulatory drugs. Life expectancy has also improved for patients with diseases such as thalassaemia major receiving multiple transfusions with the worldwide introduction of orally active iron chelating agents. New anticoagulants which directly inhibit at a single point in the coagulation cascade and rarely need monitoring are now used commonly in preference to warfarin for the treatment and prevention of arterial and venous thrombosis.

These advances in knowledge have been incorporated as new text, diagrams and tables for this seventh edition. New multiple choice questions have been added to the website and short summary boxes are included at the end of each chapter.

We thank Dr Trevor Baglin for his helpful suggestions for the coagulation section of the book. We wish to thank our publishers Wiley-Blackwell and the staff who have helped us with the production of this 7th Edition. We also thank Jane Fallows for once more producing clear, expertly drawn scientific diagrams. We hope it will be widely used both by undergraduates and by postgraduates in medicine and related sciences wishing to gain a grounding in one of the most exciting and advanced fields of medicine.

Victor Hoffbrand
Paul Moss

Preface to the First Edition

The major changes that have occurred in all fields of medicine over the last decade have been accompanied by an increased understanding of the biochemical, physiological and immunological processes involved in normal blood cell formation and function and the disturbances that may occur in different diseases. At the same time, the range of treatment available for patients with diseases of the blood and blood-forming organs has widened and improved substantially as understanding of the disease processes has increased and new drugs and means of support care have been introduced.

We hope the present book will enable the medical student of the 1980s to grasp the essential features of modern clinical and laboratory haematology and to achieve an understanding of how many of the manifestations of blood diseases can be explained with this new knowledge of the disease processes.

We would like to thank many colleagues and assistants who have helped with the preparation of the book. In particular, Dr H.G. Prentice cared for the patients whose haematological responses are illustrated in Figs 5.3 and 7.8 and Dr J. McLaughlin supplied Fig. 8.6. Dr S. Knowles reviewed critically the final manuscript and made many helpful suggestions. Any remaining errors are, however, our own. We also thank Mr J.B. Irwin and R.W. McPhee who drew many excellent diagrams, Mr Cedric Gilson for expert photomicrography, Mrs T. Charalambos, Mrs B. Elliot, Mrs M. Evans and Miss J. Allaway for typing the manuscript, and Mr Tony Russell of Blackwell Scientific Publications for his invaluable help and patience.

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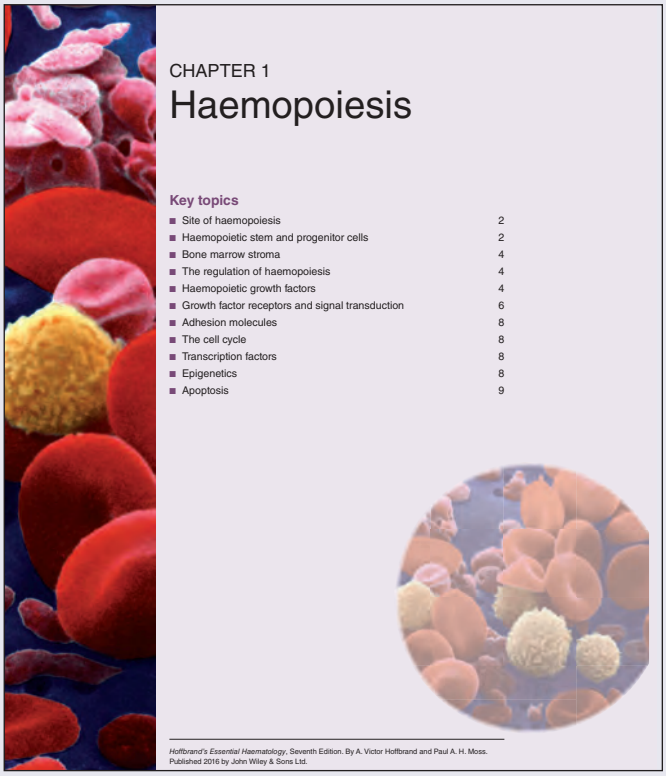

CHAPTER 1

Haemopoiesis

Key topics

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◀ Every chapter begins with a list of **Key topics** of the chapter.

proteins that are involved in mediating apoptosis following DNA damage, such as p53 and ATM, are also frequently mutated and therefore inactivated in haemopoietic malignancies.

Necrosis is death of cells and adjacent cells due to ischaemia, chemical trauma or hyperthermia. The cells swell, the plasma membrane loses integrity. There is usually an inflammatory infiltrate in response to spillage of cell contents. Autophagy is the digestion of cell organelles by lysosomes. It may be involved in cell death but in some situations also in maintaining cell survival by recycling nutrients.

SUMMARY

- Haemopoiesis (blood cell formation) arises from pluripotent stem cells in the bone marrow. Stem cells give rise to progenitor cells which, after cell divisions and differentiation, form red cells, granulocytes (neutrophils, eosinophils and basophils), monocytes, platelets and B and T lymphocytes.
- Haemopoetic tissue occupies about 50% of the marrow space in normal adult marrow. Haemopoiesis in adults is confined to the central skeleton but in infants and young children haemopoietic tissue extends down the long bones of the arms and legs.
- Stem cells reside in the bone marrow in niches formed by stromal cells and circulate in the blood.
- Growth factors attach to specific cell receptors and produce a cascade of phosphorylation events to the cell nucleus. Transcription factors carry the message to those genes that are to be 'switched on', to stimulate cell division, differentiation, functional activity or suppress apoptosis.
- Adhesion molecules are a large family of glycoproteins that mediate attachment of marrow precursors and mature leucocytes and platelets to extracellular matrix, endothelium and to each other.
- Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence. Histone modification and DNA methylation are two important examples relevant to haemopoiesis and haematological malignancies.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families.
- Apoptosis is a physiological process of cell death resulting from activation of caspases. The intracellular ratio of pro-apoptotic proteins (e.g. BAX) to anti-apoptotic proteins (e.g. BCL-2) determines the cell susceptibility to apoptosis.

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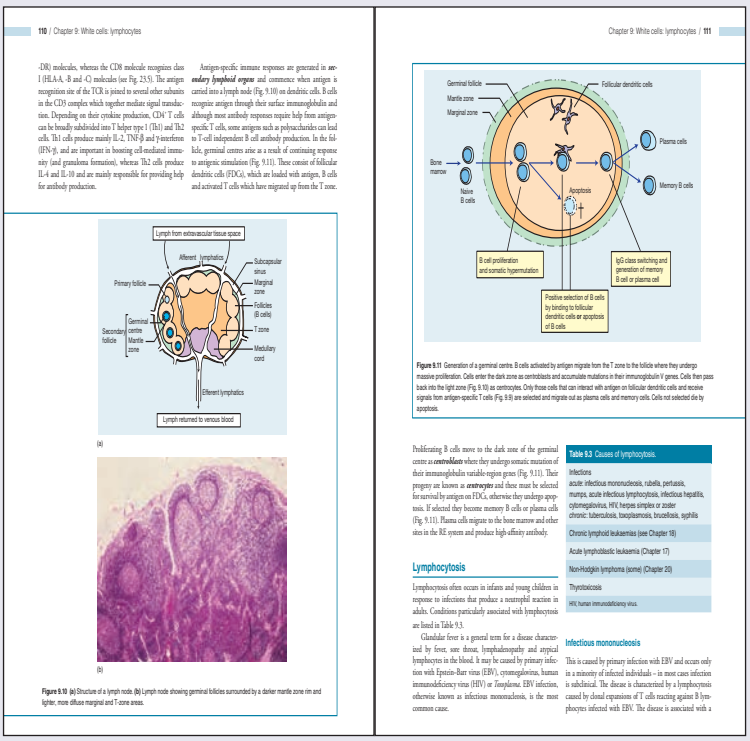

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-DR) molecules, whereas the CD8 molecule recognizes class I (HLA-A, -B and -C) molecules (see Fig. 23.5). The antigen recognition site of the TCR is joined to several other subunits in the CD3 complex which together mediate signal transduction. Depending on their cytokine production, $CD4^+$ T cells can be broadly subdivided into T helper type 1 (Th1) and Th2 cells. Th1 cells produce mainly IL-2, TNF-β and γ-interferon (IFN-γ), and are important in boosting cell-mediated immunity (and granuloma formation), whereas Th2 cells produce IL-4 and IL-10 and are mainly responsible for providing help for antibody production.

Antigen-specific immune responses are generated in ***secondary lymphoid organs*** and commence when antigen is carried into a lymph node (Fig. 9.10) on dendritic cells. B cells recognize antigen through their surface immunoglobulin and although most antibody responses require help from antigen-specific T cells, some antigens such as polysaccharides can lead to T-cell independent B cell antibody production. In the follicle, germinal centres arise as a result of continuing response to antigenic stimulation (Fig. 9.11). These consist of follicular dendritic cells (FDCs), which are loaded with antigen, B cells and activated T cells which have migrated up from the T zone.

Figure 9.10 **(a)** Structure of a lymph node. **(b)** Lymph node showing germinal follicles surrounded by a darker mantle zone rim and lighter, more diffuse marginal and T-zone areas.

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Figure 9.11 Generation of a germinal centre. B cells activated by antigen migrate from the T zone to the follicle where they undergo massive proliferation. Cells enter the dark zone as centroblasts and accumulate mutations in their immunoglobulin V genes. Cells then pass back into the light zone (Fig. 9.10) as centrocytes. Only those cells that can interact with antigen on follicular dendritic cells and receive signals from antigen-specific T cells (Fig. 9.9) are selected and migrate out as plasma cells and memory cells. Cells not selected die by apoptosis.

Proliferating B cells move to the dark zone of the germinal centre as ***centroblasts*** where they undergo somatic mutation of their immunoglobulin variable-region genes (Fig. 9.11). Their progeny are known as ***centrocytes*** and these must be selected for survival by antigen on FDCs, otherwise they undergo apoptosis. If selected they become memory B cells or plasma cells (Fig. 9.11). Plasma cells migrate to the bone marrow and other sites in the RE system and produce high-affinity antibody.

Table 9.3 Causes of lymphocytosis.

Infections acute: infectious mononucleosis, rubella, pertussis, mumps, acute infectious lymphocytosis, infectious hepatitis, cytomegalovirus, HIV, herpes simplex or zoster chronic: tuberculosis, toxoplasmosis, brucellosis, syphilis
Chronic lymphoid leukaemias (see Chapter 18)
Acute lymphoblastic leukaemia (Chapter 17)
Non-Hodgkin lymphoma (some) (Chapter 20)
Thyrotoxicosis

HIV, human immunodeficiency virus.

Lymphocytosis

Lymphocytosis often occurs in infants and young children in response to infections that produce a neutrophil reaction in adults. Conditions particularly associated with lymphocytosis are listed in Table 9.3.

Glandular fever is a general term for a disease characterized by fever, sore throat, lymphadenopathy and atypical lymphocytes in the blood. It may be caused by primary infection with Epstein-Barr virus (EBV), cytomegalovirus, human immunodeficiency virus (HIV) or *Toxoplasma*. EBV infection, otherwise known as infectious mononucleosis, is the most common cause.

Infectious mononucleosis

This is caused by primary infection with EBV and occurs only in a minority of infected individuals – in most cases infection is subclinical. The disease is characterized by a lymphocytosis caused by clonal expansions of T cells reacting against B lymphocytes infected with EBV. The disease is associated with a

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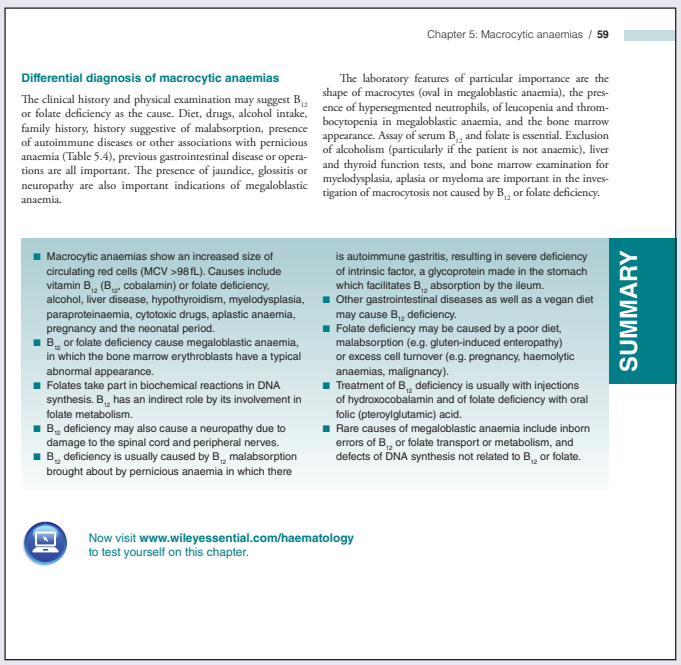

Differential diagnosis of macrocytic anaemias

The clinical history and physical examination may suggest B_{12} or folate deficiency as the cause. Diet, drugs, alcohol intake, family history, history suggestive of malabsorption, presence of autoimmune diseases or other associations with pernicious anaemia (Table 5.4), previous gastrointestinal disease or operations are all important. The presence of jaundice, glossitis or neuropathy are also important indications of megaloblastic anaemia.

The laboratory features of particular importance are the shape of macrocytes (oval in megaloblastic anaemia), the presence of hypersegmented neutrophils, of leucopenia and thrombocytopenia in megaloblastic anaemia, and the bone marrow appearance. Assay of serum B_{12} and folate is essential. Exclusion of alcoholism (particularly if the patient is not anaemic), liver and thyroid function tests, and bone marrow examination for myelodysplasia, aplasia or myeloma are important in the investigation of macrocytosis not caused by B_{12} or folate deficiency.

SUMMARY

- Macrocytic anaemias show an increased size of circulating red cells (MCV >98 fL). Causes include vitamin B_{12} (B_{12}, cobalamin) or folate deficiency, alcohol, liver disease, hypothyroidism, myelodysplasia, paraproteinaemia, cytotoxic drugs, aplastic anaemia, pregnancy and the neonatal period.
- B_{12} or folate deficiency cause megaloblastic anaemia, in which the bone marrow erythroblasts have a typical abnormal appearance.
- Folates take part in biochemical reactions in DNA synthesis. B_{12} has an indirect role by its involvement in folate metabolism.
- B_{12} deficiency may also cause a neuropathy due to damage to the spinal cord and peripheral nerves.
- B_{12} deficiency is usually caused by B_{12} malabsorption brought about by pernicious anaemia in which there is autoimmune gastritis, resulting in severe deficiency of intrinsic factor, a glycoprotein made in the stomach which facilitates B_{12} absorption by the ileum.
- Other gastrointestinal diseases as well as a vegan diet may cause B_{12} deficiency.
- Folate deficiency may be caused by a poor diet, malabsorption (e.g. gluten-induced enteropathy) or excess cell turnover (e.g. pregnancy, haemolytic anaemias, malignancy).
- Treatment of B_{12} deficiency is usually with injections of hydroxocobalamin and of folate deficiency with oral folic (pteroylglutamic) acid.
- Rare causes of megaloblastic anaemia include inborn errors of B_{12} or folate transport or metabolism, and defects of DNA synthesis not related to B_{12} or folate.

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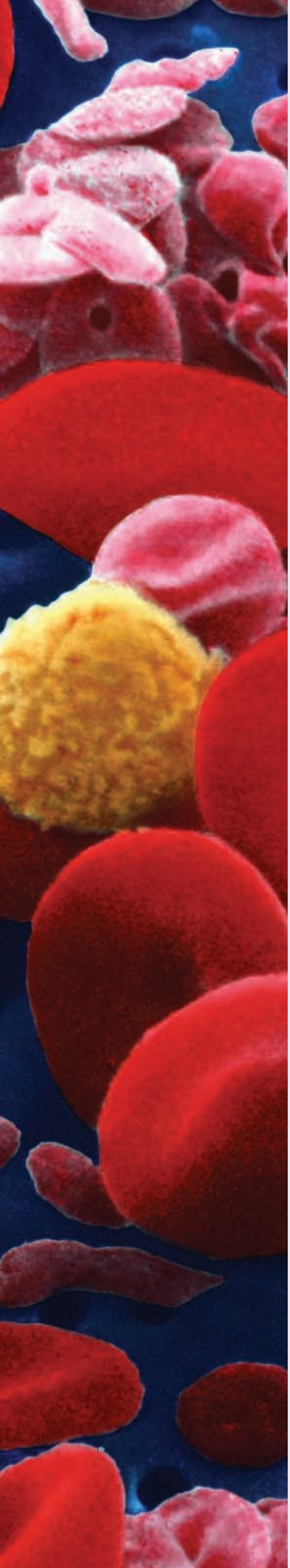
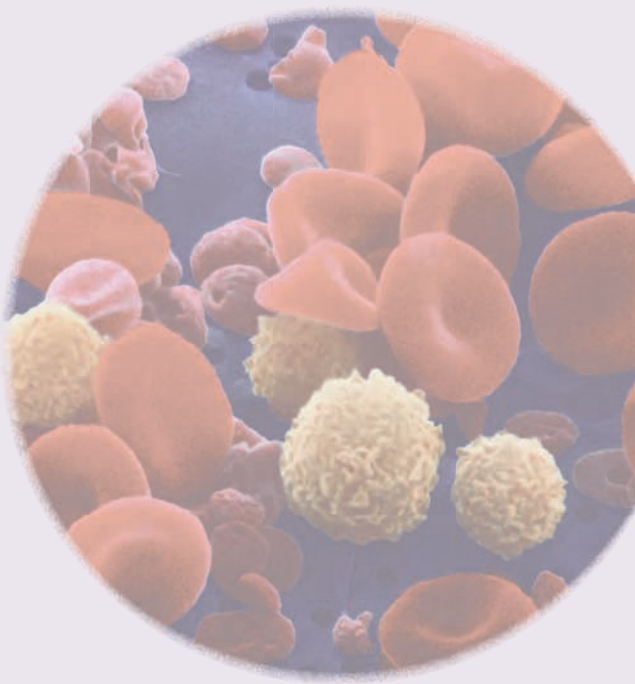


CHAPTER 1

Haemopoiesis

Key topics



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This first chapter is concerned with the general aspects of blood cell formation (haemopoiesis). The processes that regulate haemopoiesis and the early stages of formation of red cells (erythropoiesis), granulocytes and monocytes (myelopoiesis) and platelets (thrombopoiesis) are also discussed.

Site of haemopoiesis

In the first few weeks of gestation the yolk sac is a transient site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow. From 6 weeks until 6–7 months of fetal life, the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth (Table 1.1; see Fig. 7.1b). The placenta also contributes to fetal haemopoiesis. The bone marrow is the most important site from 6–7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation.

In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri (Table 1.1). Even in these haemopoietic areas, approximately 50% of the marrow consists of fat (Fig. 1.1). The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extramedullary haemopoiesis').

Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotential stem cell that can by asymmetric cell division self-renew but also give rise to the separate cell lineages. These cells are able to repopulate a bone marrow from which all stem cells have been eliminated by lethal irradiation or chemotherapy. This ***haemopoietic stem cell*** (HSC) is rare, perhaps 1 in every 20 million nucleated cells in bone marrow. Many of the cells are dormant and in mice it has been estimated that they enter cell cycle approximately every 20 weeks. Although its exact phenotype is unknown, on immunological testing the HSC is $CD34^+$ $CD38^-$ and negative for lineage markers (Lin^-) and has the appearance of a small or medium-sized lymphocyte (see Fig. 23.3). The cells reside in specialized osteoblastic or vascular 'niches'.

Table 1.1 Sites of haemopoiesis.

Fetus	0–2 months (yolk sac)
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	Bone marrow (practically all bones)
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

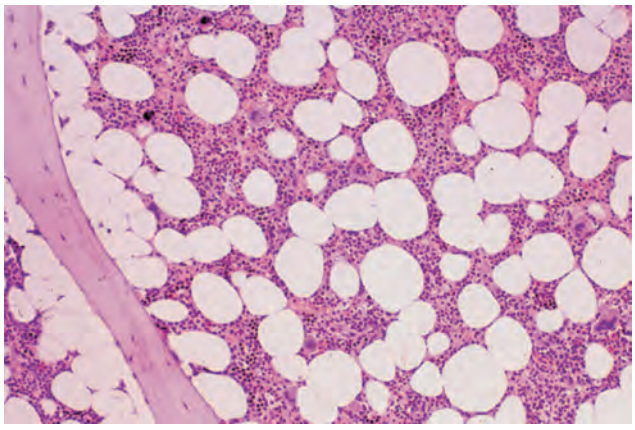

Figure 1.1 Normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% is fat.

Cell differentiation occurs from the stem cell via committed ***haemopoietic progenitors*** which are restricted in their developmental potential (Fig. 1.2). The existence of the separate progenitor cells can be demonstrated by *in vitro* culture techniques. Very early progenitors are assayed by culture on bone marrow stroma as long-term culture initiating cells, whereas late progenitors are generally assayed in semi-solid media. An example is the earliest detectable mixed myeloid precursor which gives rise to granulocytes, erythrocytes, monocytes and megakaryocytes and is termed CFU (colony-forming unit)-GEMM (Fig. 1.2). The bone marrow is also the primary site of origin of lymphocytes, which differentiate from a common lymphoid precursor. The spleen, lymph nodes and thymus are secondary sites of lymphocyte production (see Chapter 9).

The stem cell has the capability for ***self-renewal*** (Fig. 1.3) so that marrow cellularity remains constant in a normal healthy steady state. There is considerable amplification in the system: one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions (Fig. 1.3). In humans HSCs are capable of about 50 cell divisions, telomere shortening affecting viability. Under normal conditions most are dormant. With aging, the number of stem cells falls and the relative proportion giving rise to lymphoid rather than myeloid progenitors also falls. Stem cells also accumulate genetic mutations with age, an average of 8 at age 60, and these, either passenger or driver, may be present in tumours arising from these stem cells (see Chapter 11). The precursor cells are capable of responding to haemopoietic growth factors with increased production of one

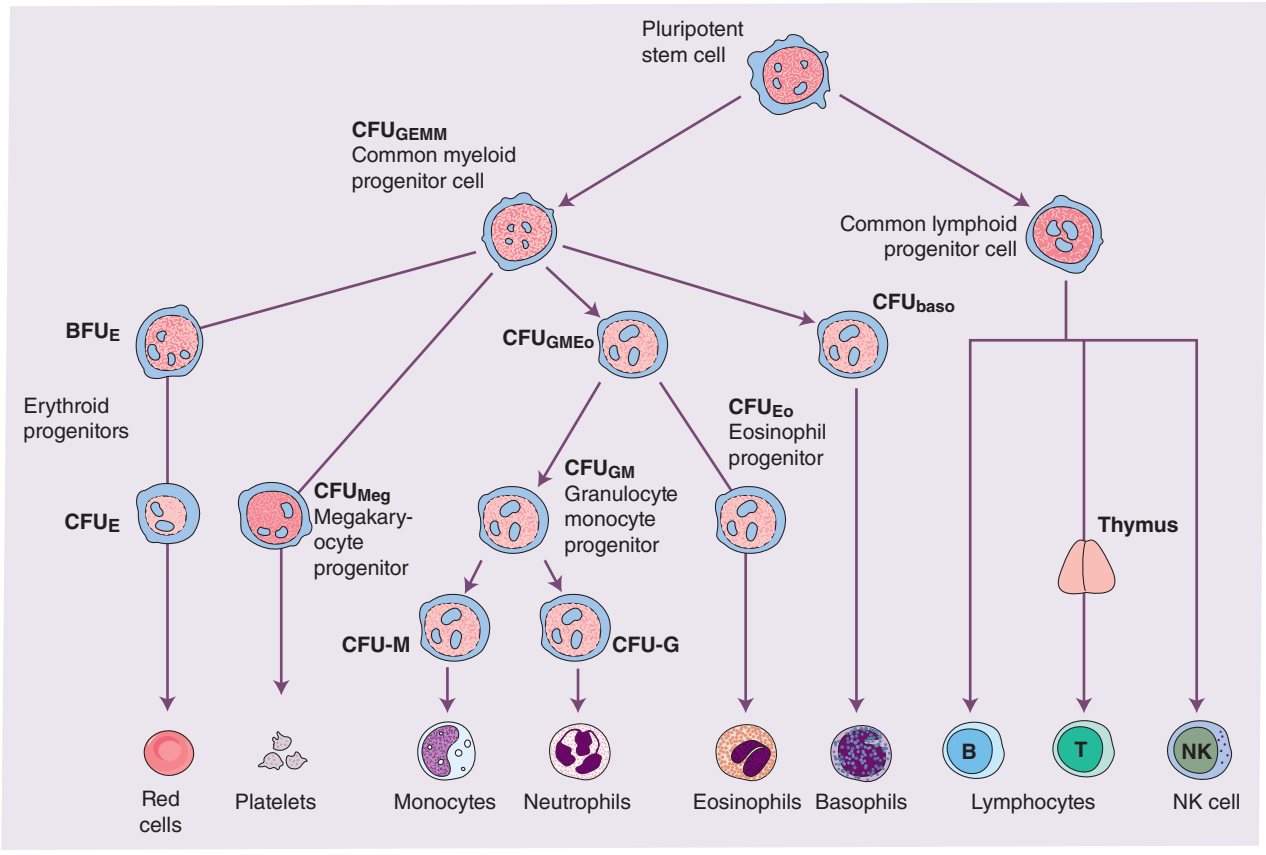

Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cell and the cell lines that arise from it. Various progenitor cells can be identified by culture in semi-solid medium by the type of colony they form. It is possible that an erythroid/megakaryocytic progenitor may be formed before the common lymphoid progenitor diverges from the mixed granulocytic/monocyte/eosinophil myeloid progenitor. Baso, basophil; BFU, burst-forming unit; CFU, colony-forming unit; E, erythroid; Eo, eosinophil; GEMM, granulocyte, erythroid, monocyte and megakaryocyte; GM, granulocyte, monocyte; Meg, megakaryocyte; NK, natural killer.

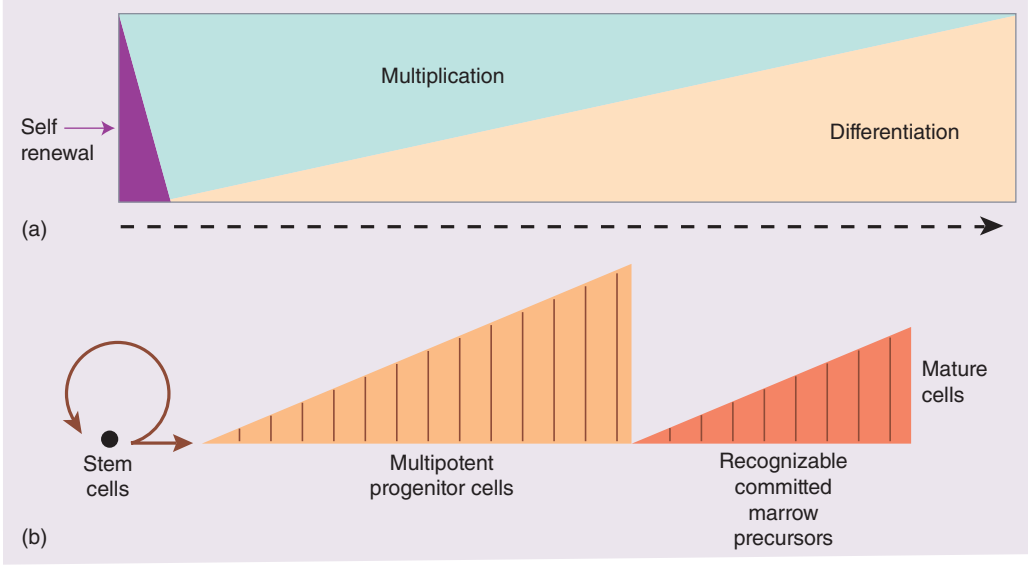

Figure 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

or other cell line when the need arises. The development of the ***mature cells*** (red cells, granulocytes, monocytes, megakaryocytes and lymphocytes) is considered further in other sections of this book.

Bone marrow stroma

The bone marrow forms a suitable environment for stem cell survival, self-renewal and formation of differentiated progenitor cells. It is composed of stromal cells and a microvascular network (Fig. 1.4). The stromal cells include mesenchymal stem cells, adipocytes, fibroblasts, osteoblasts, endothelial cells and macrophages and they secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronic acid and chondroitin derivatives) to form an extracellular matrix. In addition, stromal cells secrete several growth factors necessary for stem cell survival.

Mesenchymal stem cells are critical in stromal cell formation. Together with osteoblasts or endothelial cells they form niches and provide the growth factors, adhesion molecules and cytokines which support stem cells, e.g. the protein jagged, on stromal cells, binds to a receptor NOTCH1 on stem cells which then becomes a transcription factor involved in the cell cycle.

Stem cells are able to traffic around the body and are found in peripheral blood in low numbers. In order to exit the bone marrow, cells must cross the blood vessel endothelium and this process of ***mobilization*** is enhanced by administration of growth factors such as granulocyte colony-stimulating factor (G-CSF) (see p. 91). The reverse process of stem cell ***homing*** appears to depend on a chemokine gradient in which the stromal-derived factor 1 (SDF-1) which binds to its receptor CXCR4 on HSC is critical. Several critical interactions maintain stem cell viability and production in the stroma including stem cell factor (SCF) and jagged proteins expressed on stroma and their respective receptors KIT and NOTCH expressed on stem cells.

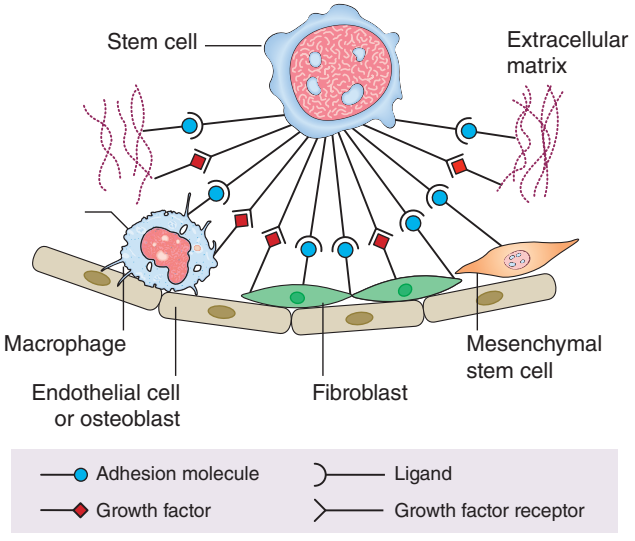

Figure 1.4 Haemopoiesis occurs in a suitable microenvironment ('niche') provided by a stromal matrix on which stem cells grow and divide. The niche may be vascular (lined by endothelium) or endosteal (lined by osteoblasts). There are specific recognition and adhesion sites (see p. 8); extracellular glycoproteins and other compounds are involved in the binding.

The regulation of haemopoiesis

Haemopoiesis starts with stem cell division in which one cell replaces the stem cell (*self-renewal*) and the other is committed to differentiation. These early committed progenitors express low levels of transcription factors that may commit them to discrete cell lineages. Which cell lineage is selected for differentiation may depend both on chance and on the external signals received by progenitor cells. Several transcription factors (see p. 8) regulate survival of stem cells (e.g. SCL, GATA-2, NOTCH-1), whereas others are involved in differentiation along the major cell lineages. For instance, PU.1 and the CEBP family commit cells to the myeloid lineage, whereas GATA-2 and then GATA-1 and FOG-1 have essential roles in erythropoietic and megakaryocytic differentiation. These transcription factors interact so that reinforcement of one transcription programme may suppress that of another lineage. The transcription factors induce synthesis of proteins specific to a cell lineage. For example, the erythroid-specific genes for globin and haem synthesis have binding motifs for GATA-1.

Haemopoietic growth factors

The haemopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells. They may act locally at the site where they are produced by cell–cell contact or circulate in plasma. They also bind to the extracellular matrix to form niches to which stem and progenitor cells adhere. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells (Fig. 1.5).

They share a number of common properties (Table 1.2) and act at different stages of haemopoiesis (Table 1.3; Fig. 1.6). Stromal cells are the major source of growth factors except for erythropoietin, 90% of which is synthesized in the kidney, and thrombopoietin, made largely in the liver. An important feature of growth factor action is that two or more factors may synergize in stimulating a particular cell to proliferate or differentiate. Moreover, the action of one growth factor on a cell may stimulate production of another growth factor or growth factor receptor. SCF and FLT3 ligand (FLT3-L) act locally on the pluripotential stem cells and on early myeloid and lymphoid progenitors (Fig. 1.6). Interleukin-3 (IL-3) and

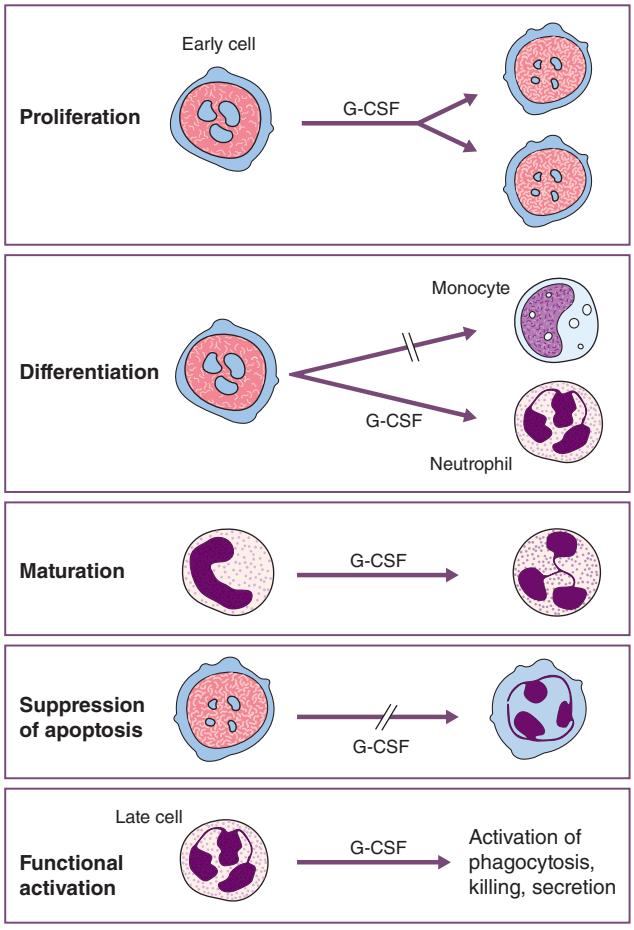

Figure 1.5 Growth factors may stimulate proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF) for an early myeloid progenitor and a neutrophil.

Table 1.2 General characteristics of myeloid and lymphoid growth factors.

Glycoproteins that act at very low concentrations
Act hierarchically
Usually produced by many cell types
Usually affect more than one lineage
Usually active on stem/progenitor cells and on differentiated cells
Usually show synergistic or additive interactions with other growth factors
Often act on the neoplastic equivalent of a normal cell
Multiple actions: proliferation, differentiation, maturation, functional activation, prevention of apoptosis of progenitor cells

granuloctye–macrophage colony-stimulating factor (GM-CSF) are multipotential growth factors with overlapping activities. G-CSF and thrombopoietin enhance the effects of SCF, FLT-L, IL-3 and GM-CSF on survival and differentiation of the early haemopoietic cells.

These factors maintain a pool of haemopoietic stem and progenitor cells on which later-acting factors, erythropoietin, G-CSF, macrophage colony-stimulating factor (M-CSF), IL-5 and thrombopoietin, act to increase production of one or other cell lineage in response to the body's need. Granulocyte and monocyte formation, for example, can be stimulated by infection or inflammation through release of IL-1 and tumour necrosis factor (TNF) which then stimulate stromal cells to produce growth factors in an interacting network (see Fig. 8.4). In contrast, cytokines, such as transforming growth factor-β (TGF-β) and γ-interferon (IFN-γ), can exert a negative effect on haemopoiesis and may have a role in the development of aplastic anaemia (see p. 244).

Table 1.3 Haemopoietic growth factors.

Act on stromal cells	
	IL-1
	TNF
Act on pluripotential stem cells	
	SCF
	FLT3-L
	VEGF
Act on multipotential progenitor cells	
	IL-3
	GM-CSF
	IL-6
	G-CSF
	Thrombopoietin
Act on committed progenitor cells	
	G-CSF*
	M-CSF
	IL-5 (eosinophil-CSF)
	Erythropoietin
	Thrombopoietin*

CSF, colony-stimulating factor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.
* These also act synergistically with early acting factors on pluripotential progenitors.

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target cells. Many receptors (e.g. erythropoietin (epo) receptor (R), GMCSF-R) are from the ***haematopoietin receptor superfamily*** which dimerize after binding their ligand.

Dimerization of the receptor leads to activation of a complex series of intracellular signal transduction pathways, of which the three major ones are the JAK/STAT, the mitogen-activated protein (MAP) kinase and the phosphatidylinositol 3 (PI3) kinase pathways (Fig. 1.7; see Fig. 15.2). The Janus-associated kinase (JAK) proteins are a family of four tyrosine-specific protein kinases that associate with the intracellular domains of the growth factor receptors (Fig. 1.7). A growth factor molecule binds simultaneously to the extracellular domains of two or three receptor molecules, resulting in their aggregation. Receptor aggregation induces activation of the JAKs which now phosphorylate members of the signal transducer and activator of transcription (STAT) family of transcription factors. This results in their dimerization and translocation from the cell cytoplasm across the nuclear membrane to the cell nucleus. Within the nucleus STAT dimers activate transcription of specific genes. A model for control of gene expression by a transcription factor is shown in Fig. 1.8. The clinical importance of

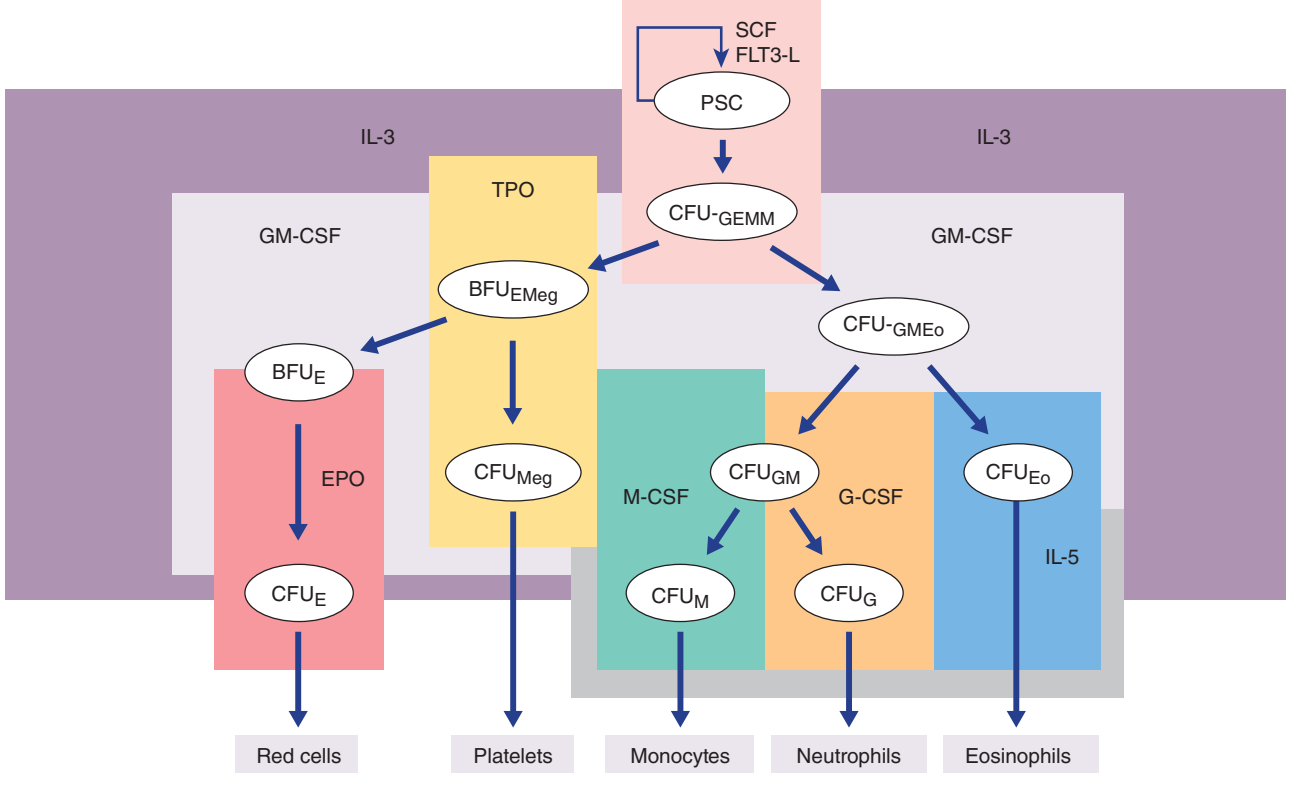

Figure 1.6 A diagram of the role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; PSC, pluripotential stem cell; SCF, stem cell factor; TPO, thrombopoietin; FLT3-L, FLT3 ligand. For other abbreviations see Fig. 1.2.

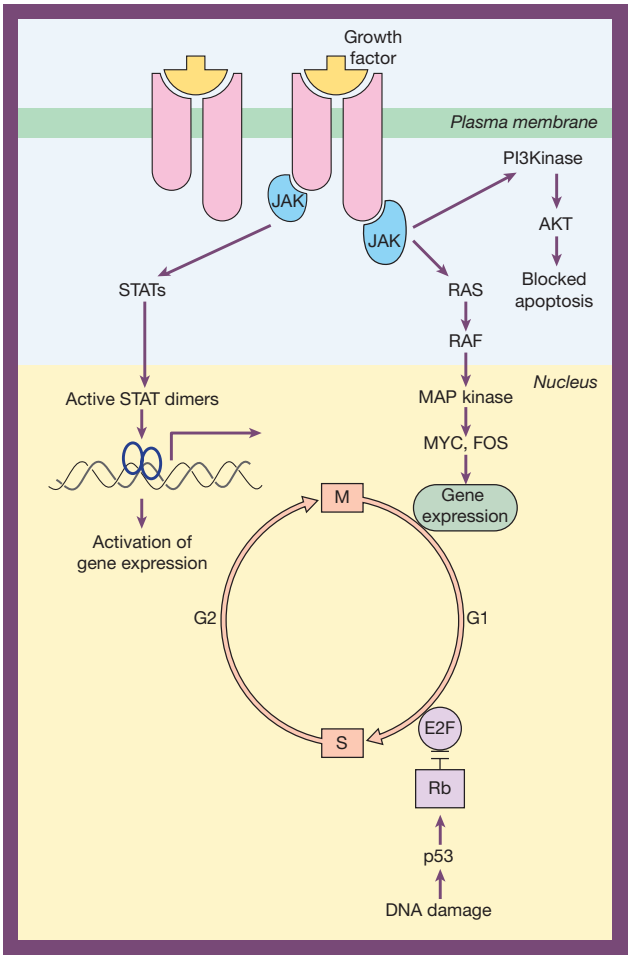

Figure 1.7 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and phosphatidyl-inositol 3-kinase (PI3K) pathways (see Fig. 15.2) which leads to transcriptional activation of specific genes. E2F is a transcription factor needed for cell transition from G1 to S phase. E2F is inhibited by the tumour suppressor gene Rb (retinoblastoma) which can be indirectly activated by p53. The synthesis and degradation of different cyclins stimulates the cell to pass through the different phases of the cell cycle. The growth factors may also suppress apoptosis by activating AKT (protein kinase B).

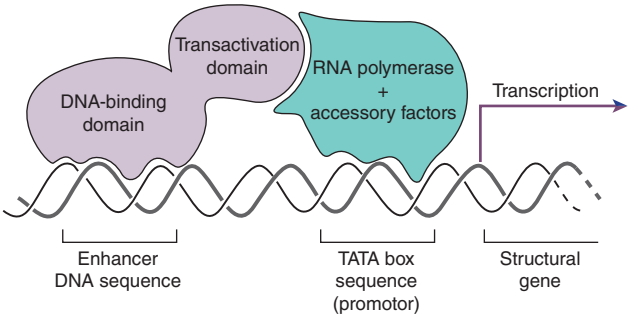

Figure 1.8 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box. The RNA polymerase now initiates transcription of the structural gene to form mRNA. Translation of the mRNA by the ribosomes generates the protein encoded by the gene.

this pathway is revealed by the finding of an activating mutation of the *JAK2* gene as the cause of polycythaemia rubra vera (see p. 166).

JAK can also activate the MAPK pathway, which is regulated by RAS and controls proliferation. PI3 kinases phophorylate inositol lipids which have a wide range of downstream effects including activation of AKT leading to block of apoptosis and other actions (Fig. 1.7; see Fig. 15.2). Different domains of the intracellular receptor protein may signal for the different processes (e.g. proliferation or suppression of apoptosis) mediated by growth factors.

A second smaller group of growth factors, including SCF, FLT-3L and M-CSF (Table 1.3), bind to receptors that have an extracellular immunoglobulin-like domain linked via a transmembrane bridge to a cytoplasmic tyrosine kinase domain. Growth factor binding results in dimerization of these receptors and consequent activation of the tyrosine kinase domain. Phosphorylation of tyrosine residues in the receptor itself generates binding sites for signalling proteins which initiate complex cascades of biochemical events resulting in changes in gene expression, cell proliferation and prevention of apoptosis.

Adhesion molecules

A large family of glycoprotein molecules termed adhesion molecules mediate the attachment of marrow precursors, leucocytes and platelets to various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The adhesion molecules on the surface of leucocytes are termed receptors and these interact with proteins termed ligands on the surface of target cells, e.g. endothelium. The adhesion molecules are important in the development and maintenance of inflammatory and immune responses, and in platelet–vessel wall and leucocyte–vessel wall interactions.

The pattern of expression of adhesion molecules on tumour cells may determine their mode of spread and tissue localization (e.g. the pattern of metastasis of carcinoma cells or non-Hodgkin lymphoma cells into a follicular or diffuse pattern). The adhesion molecules may also determine whether or not cells circulate in the bloodstream or remain fixed in tissues. They may also partly determine whether or not tumour cells are susceptible to the body's immune defences.

The cell cycle

The cell division cycle, generally known simply as the ***cell cycle***, is a complex process that lies at the heart of haemopoiesis. Dysregulation of cell proliferation is also the key to the development of malignant disease. The duration of the cell cycle is variable between different tissues but the basic principles remain constant. The cycle is divided into the mitotic phase (***M phase***), during which the cell physically divides, and ***interphase***, during which the chromosomes are duplicated and cell growth occurs prior to division (Fig. 1.7). The M phase is further partitioned into classical ***mitosis***, in which nuclear division is accomplished, and ***cytokinesis***, in which cell fission occurs.

Interphase is divided into three main stages: a ***G_1 phase***, in which the cell begins to commit to replication, an ***S phase***, during which DNA content doubles and the chromosomes replicate, and the ***G_2 phase***, in which the cell organelles are copied and cytoplasmic volume is increased. If cells rest prior to division they enter a G_0 state where they can remain for long periods of time. The number of cells at each stage of the cell cycle can be assessed by exposing cells to a chemical or radiolabel that gets incorporated into newly generated DNA or by flow cytometry.

The cell cycle is controlled by two ***checkpoints*** which act as brakes to coordinate the division process at the end of the G_1 and G_2 phases. Two major classes of molecules control these checkpoints, ***cyclin-dependent protein kinases*** (Cdk), which phosophorylate downstream protein targets, and ***cyclins***, which bind to Cdks and regulate their activity. An example of the importance of these systems is demonstrated by mantle cell lymphoma which results from the constitutive activation of cyclin D1 as a result of a chromosomal translocation (see p. 223).

Transcription factors

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families (Fig. 1.8). Typically, they contain at least two domains: a ***DNA-binding domain***, such as a leucine zipper or helix–loop–helix motif which binds to a specific DNA sequence, and an ***activation domain***, which contributes to assembly of the transcription complex at a gene promoter. Mutation, deletion or translocation of transcription factors underlie many cases of haematological neoplasms (see Chapter 11).

Epigenetics

This refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence.

Cellular DNA is packaged by wrapping it around histones, a group of specialized nuclear proteins. The complex is tightly compacted as chromatin. In order for the DNA code to be read, transcription factors and other proteins need to physically attach to DNA. Histones act as custodians for this access and so for gene expression. Histones may be modified by methylation, acetylation and phosphorylation which can result in increased or decreased gene expression and so changes in cell phenotype. Epigenetics also includes changes to DNA itself, such as methylation which regulates gene expression in normal and tumour tissues. The methylation of cytosine residues to methyl cytosine results in inhibition of gene transcription. The genes *DNMT 3A* and *B* are involved in the methylation, and *TET 1,2,3* and *IDH1* and *2* in the hydroxylation and therefore breakdown of methylcytosine and restoration of gene

expression (see Fig. 16.1). These genes are frequently mutated in the myeloid malignancies (see Chapters 13, 15 and 16).

Apoptosis

Apoptosis (programmed cell death) is a regulated process of physiological cell death in which individual cells are triggered to activate intracellular proteins that lead to the death of the cell. Morphologically it is characterized by cell shrinkage, condensation of the nuclear chromatin, fragmentation of the nucleus and cleavage of DNA at internucleosomal sites. It is an important process for maintaining tissue homeostasis in haemopoiesis and lymphocyte development.

Apoptosis results from the action of intracellular cysteine proteases called ***caspases*** which are activated following cleavage and lead to endonuclease digestion of DNA and disintegration of the cell skeleton (Fig. 1.9). There are two major pathways by which caspases can be activated. The first is by signalling through membrane proteins such as Fas or TNF receptor via their intracellular death domain. An example of this mechanism is shown by activated cytotoxic T cells expressing Fas ligand which induces apoptosis in target cells. The second pathway is via the release of cytochrome c from mitochondria. Cytochrome c binds to APAF-1 which then activates caspases. DNA damage induced by irradiation or chemotherapy may act through this pathway. The protein p53 has an important role in sensing DNA damage. It activates apoptosis by raising the cell level of BAX which then increases cytochrome c release (Fig. 1.9). P53 also shuts down the cell cycle to stop the damaged cell from dividing (Fig. 1.7). The cellular level of p53 is rigidly controlled by a second protein, MDM2. Following death, apoptotic cells display molecules that lead to their ingestion by macrophages.

As well as molecules that mediate apoptosis there are several intracellular proteins that protect cells from apoptosis. The best characterized example is BCL-2. BCL-2 is the prototype of a family of related proteins, some of which are anti-apoptotic and some, like BAX, pro-apoptotic. The intracellular ratio of BAX and BCL-2 determines the relative susceptibility of cells to apoptosis (e.g. determines the lifespan of platelets) and may act through regulation of cytochrome c release from mitochondria.

Many of the genetic changes associated with malignant disease lead to a reduced rate of apoptosis and hence prolonged cell survival. The clearest example is the translocation of the *BCL-2* gene to the immunoglobulin heavy chain locus in the t(14;18) translocation in follicular lymphoma (see p. 222). Overexpression of the BCL-2 protein makes the malignant B cells less susceptible to apoptosis. Apoptosis is the normal fate for most B cells undergoing selection in the lymphoid germinal centres.

Several translocations leading to the generation of fusion proteins, such as t(9;22), t(1;14) and t(15;17), also result in inhibition of apoptosis (see Chapter 11). In addition, genes encoding

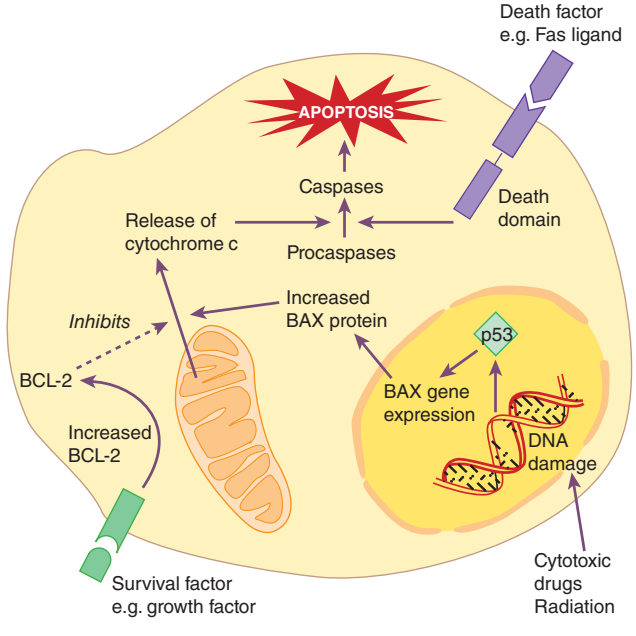

Figure 1.9 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death domain leading to activation of caspases which digest DNA. Cytochrome c binds to the cytoplasmic protein Apaf-1 leading to activation of caspases. The intracellular ratio of pro-apoptotic (e.g. BAX) or anti-apoptotic (e.g. BCL-2) members of the BCL-2 family may influence mitochondrial cytochrome c release. Growth factors raise the level of BCL-2 inhibiting cytochrome c release, whereas DNA damage, by activating p53, raises the level of BAX which enhances cytochrome c release.

proteins that are involved in mediating apoptosis following DNA damage, such as p53 and ATM, are also frequently mutated and therefore inactivated in haemopoietic malignancies.

Necrosis is death of cells and adjacent cells due to ischaemia, chemical trauma or hyperthermia. The cells swell, the plasma membrane loses integrity. There is usually an inflammatory infiltrate in response to spillage of cell contents. Autophagy is the digestion of cell organelles by lysosomes. It may be involved in cell death but in some situations also in maintaining cell survival by recycling nutrients.

SUMMARY

- Haemopoiesis (blood cell formation) arises from pluripotent stem cells in the bone marrow. Stem cells give rise to progenitor cells which, after cell divisions and differentiation, form red cells, granulocytes (neutrophils, eosinophils and basophils), monocytes, platelets and B and T lymphocytes.
- Haemopoetic tissue occupies about 50% of the marrow space in normal adult marrow. Haemopoiesis in adults is confined to the central skeleton but in infants and young children haemopoietic tissue extends down the long bones of the arms and legs.
- Stem cells reside in the bone marrow in niches formed by stromal cells and circulate in the blood.
- Growth factors attach to specific cell receptors and produce a cascade of phosphorylation events to the cell nucleus. Transcription factors carry the message to those genes that are to be 'switched on', to stimulate cell division, differentiation, functional activity or suppress apoptosis.
- Adhesion molecules are a large family of glycoproteins that mediate attachment of marrow precursors and mature leucocytes and platelets to extracellular matrix, endothelium and to each other.
- Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence. Histone modification and DNA methylation are two important examples relevant to haemopoiesis and haematological malignancies.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families.
- Apoptosis is a physiological process of cell death resulting from activation of caspases. The intracellular ratio of pro-apoptotic proteins (e.g. BAX) to anti-apoptotic proteins (e.g. BCL-2) determines the cell susceptibility to apoptosis.

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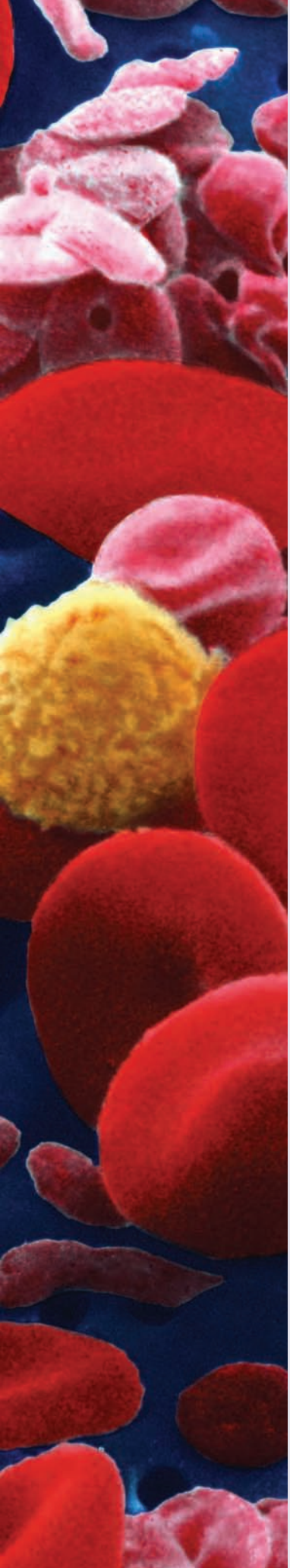
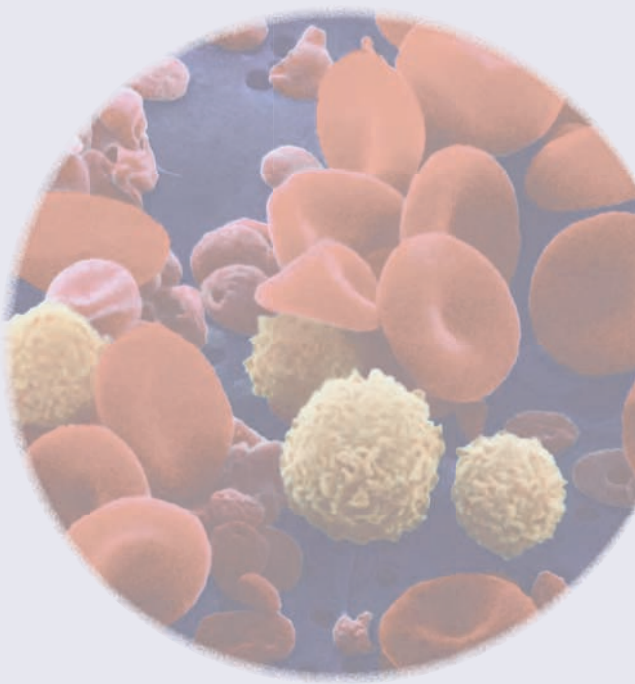


CHAPTER 2

Erythropoiesis and general aspects of anaemia

Key topics



Hoffbrand's Essential Haematology, Seventh Edition. By A. Victor Hoffbrand and Paul A. H. Moss.
Published 2016 by John Wiley & Sons Ltd.

Blood cells

All the circulating blood cells derive from pluripotential stem cells in the marrow. They divide into three main types. The most numerous are red cells which are specialized for carriage of oxygen from the lungs to the tissues and of carbon dioxide in the reverse direction (Table 2.1). They have a 4-month lifespan, whereas the smallest cells, platelets involved in haemostasis, circulate for only 10 days. The white cells are made up of four types of phagocyte, neutrophils, eosinophils, basophils and monocytes, which protect against bacterial and fungal infections, and of lymphocytes, which include B cells, involved in antibody production, and T cells (CD4 helper and CD8 suppressor), concerned with the immune response and in protection against viruses and other foreign cells. White cells have a wide range of lifespan (Table 2.1).

The red cells and platelets are counted and their diameter and other parameters measured by an automated cell counter (Fig. 2.1). This also enumerates the different types of white cell by flow cytometry and detects abnormal cells.

We each make approximately 10^{12} new erythrocytes (red cells) each day by the complex and finely regulated process of erythropoiesis. Erythropoiesis passes from the stem cell through the progenitor cells, colony-forming

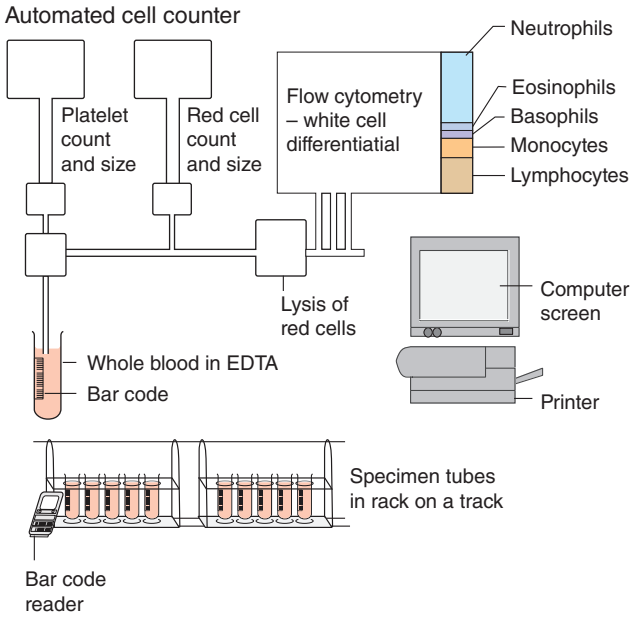


Figure 2.1 Automated blood cell counter. *Source:* Mehta AB & Hoffbrand AV (2014) Haematology at a Glance, 4th edn. Reproduced with permission of John Wiley & Sons.

Table 2.1 The blood cells.

Cell	Diameter (μm)	Lifespan in blood	Number	Function
Red cells	6–8	120 days	Male: $4.5–6.5 \times 10^{12}$/L Female: $3.9–5.6 \times 10^{12}$/L	Oxygen and carbon dioxide transport
Platelets	0.5–3.0	10 days	$140–400 \times 10^{9}$/L	Haemostasis
Phagocytes				
Neutrophils	12–15	6–10 h	$1.8–7.5 \times 10^{9}$/L	Protection from bacteria, fungi
Monocytes	12–20	20-40 h	$0.2–0.8 \times 10^{9}$/L	Protection from bacteria, fungi
Eosinophils	12–15	Days	$0.04–0.44 \times 10^{9}$/L	Protection against parasites
Basophils	12–15	Days	$0.01–0.1 \times 10^{9}$/L	
Lymphocytes B T	7–9 (resting) 12–20 (active)	Weeks or years	$1.5–3.5 \times 10^{9}$/L	B-cells: immunoglobulin synthesis T-cells: protection against viruses; immune functions

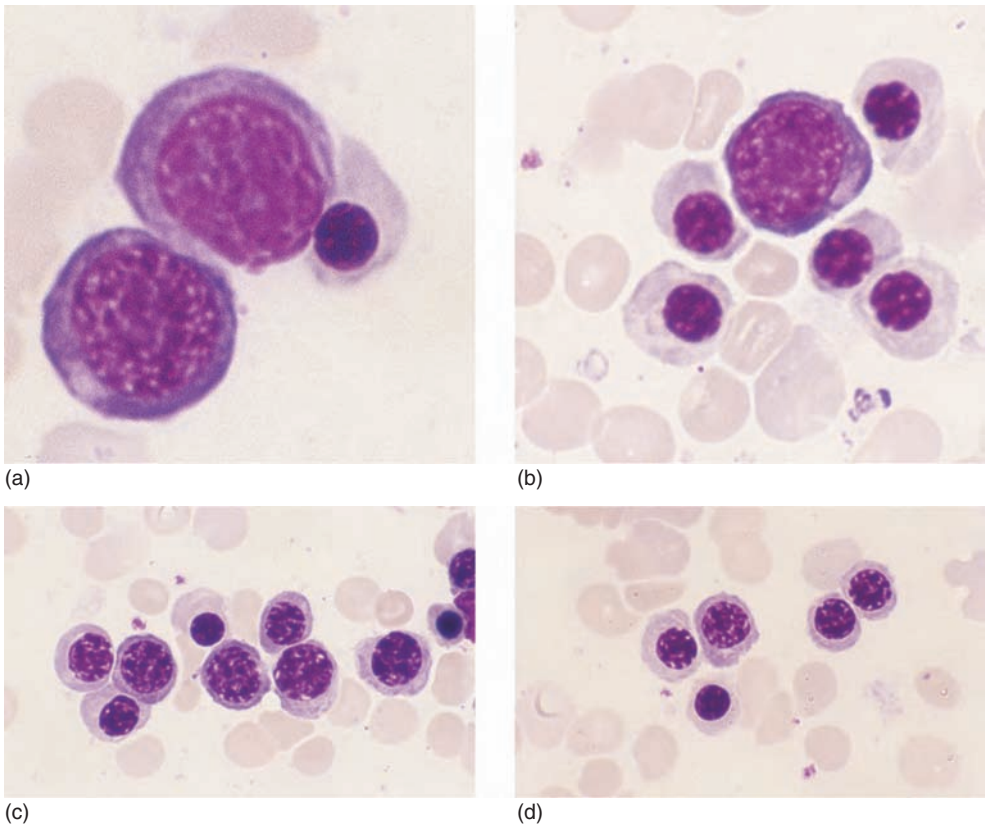


Figure 2.2 Erythroblasts (normoblasts) at varying stages of development. The earlier cells are larger, with more basophilic cytoplasm and a more open nuclear chromatin pattern. The cytoplasm of the later cells is more eosinophilic as a result of haemoglobin formation.

unit granulocyte, erythroid, monocyte and megakaryocyte (CFU_{GEMM}), burst-forming unit erythroid (BFU_E) and erythroid CFU (CFU_E) (Fig. 2.2), to the first recognizable erythrocyte precursor in the bone marrow, the pronormoblast. This process occurs in an erythroid niche in which about 30 erythroid cells at various stages of development surround a central macrophage.

The pronormoblast is a large cell with dark blue cytoplasm, a central nucleus with nucleoli and slightly clumped chromatin (Fig. 2.2). It gives rise to a series of progressively smaller normoblasts by a number of cell divisions. They also contain progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm stains paler blue as it loses its RNA and protein synthetic apparatus while nuclear chromatin becomes more condensed (Figs 2.2 and 2.3). The nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte results, which still contains some ribosomal RNA and is still able to synthesize haemoglobin (Fig. 2.4). This cell is slightly larger than a mature red cell, and circulates in the peripheral blood for 1–2 days before maturing, when RNA is completely lost. A completely pink-staining mature erythrocyte results which is a non-nucleated biconcave disc. One pronormoblast usually gives rise to 16 mature red cells (Fig. 2.3). Nucleated red cells (normoblasts) are not present in normal human peripheral blood (Fig. 2.4). They appear in the blood if erythropoiesis is occurring outside the marrow (extramedullary erythropoiesis) and also with some marrow diseases.

Erythropoietin

Erythropoiesis is regulated by the hormone erythropoietin. Erythropoietin is a heavily glycosylated polypeptide. Normally, 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere. There are no preformed stores and the stimulus to erythropoietin production is the oxygen (O_2) tension in the tissues of the kidney (Fig. 2.5). Hypoxia induces synthesis of hypoxia-inducible factors (HIF-1α and β), which stimulate erythropoietin production and also new vessel formation and transferrin

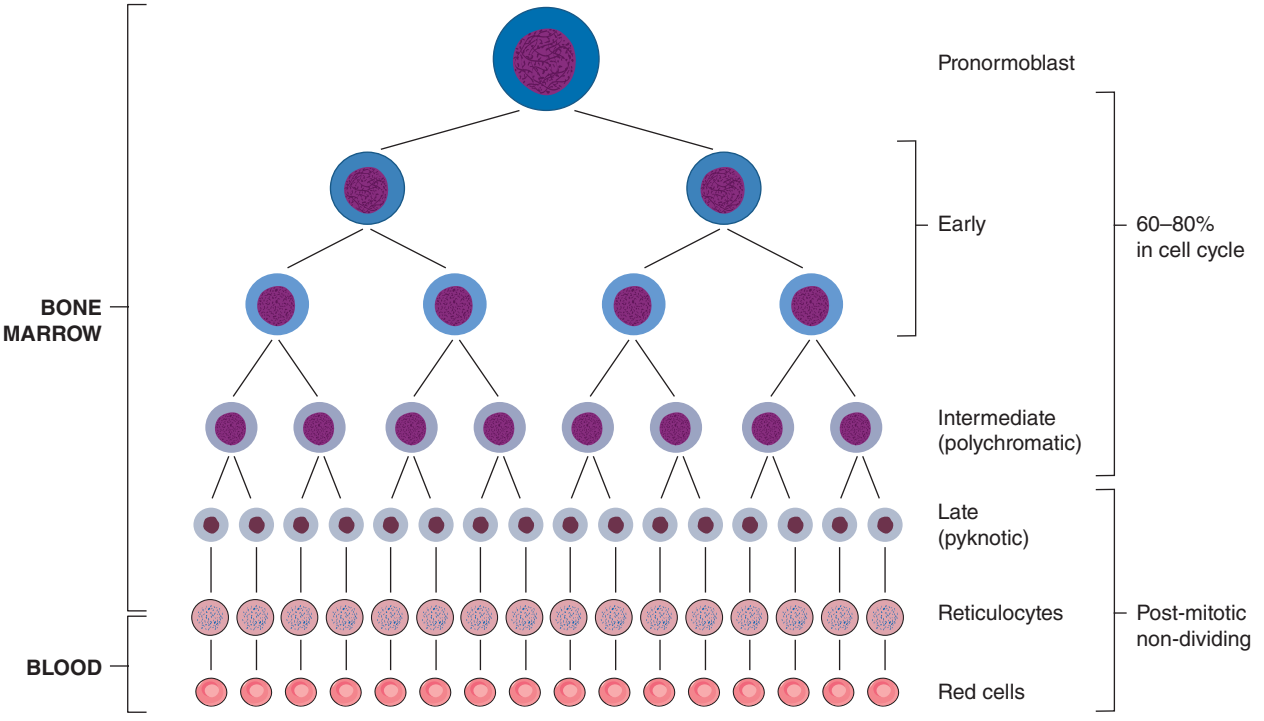
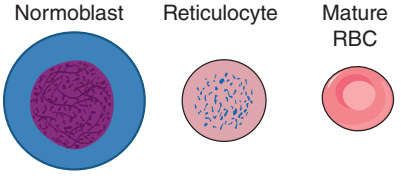


Figure 2.3 The amplification and maturation sequence in the development of mature red cells fromthe pronormoblast.



Nuclear DNA	Yes	No	No
RNA in cytoplasm	Yes	Yes	No
In marrow	Yes	Yes	Yes
In blood	No	Yes	Yes

Figure 2.4 Comparison of the DNA and RNA content, and marrow and peripheral blood distribution, of the erythroblast (normoblast), reticulocyte and mature red blood cell (RBC).

receptor synthesis, and reduces hepcidin synthesis, increasing iron absorption. Von Hippel-Lindau (VHL) protein breaks down HIFs and PHD2 hydroxylates HIF-1α allowing VHL binding (Fig. 2.5). Abnormalities in these proteins may cause polycythaemia (see Chapter 15).

Erythropoietin production therefore increases in anaemia, and also when haemoglobin for some metabolic or structural reason is unable to give up O_2 normally, when atmospheric O_2 is low or when defective cardiac or pulmonary function or damage to the renal circulation affects O_2 delivery to the kidney.

Erythropoietin stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoiesis. The transcription factor GATA-2 is involved in initiating erythroid differentiation from pluripotential stem cells. Subsequently the transcription factors GATA-1 and FOG-1 are activated by erythropoietin receptor stimulation and are important in enhancing expression of erythroid-specific genes (e.g. globin, haem biosynthetic and red cell membrane proteins) and also enhancing expression of anti-apoptotic genes and of the transferrin receptor (CD71). Late BFU_E and CFU_E, which have erythropoietin receptors, are stimulated to proliferate, differentiate and produce haemoglobin. The proportion of erythroid cells in the marrow increases and, in the chronic state, there is anatomical expansion of erythropoiesis into fatty marrow and sometimes into extramedullary sites. In infants, the marrow cavity may expand into cortical bone resulting in bone deformities with frontal bossing and protrusion of the maxilla (see p. 78).

Conversely, increased O_2 supply to the tissues (because of an increased red cell mass or because haemoglobin is able to release its O_2 more readily than normal) reduces the erythropoietin drive. Plasma erythropoietin levels can be valuable in clinical diagnosis. They are high in anaemia unless this is due to renal failure and if a tumour secreting erythropoietin is present, but low in severe renal disease or polycythaemia vera (Fig. 2.6).

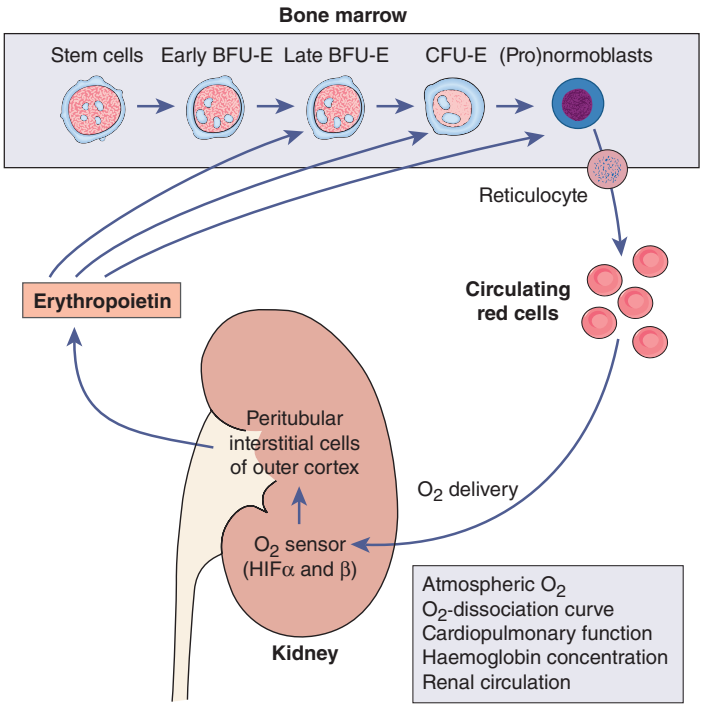


Figure 2.5 The production of erythropoietin by the kidney in response to its oxygen (O_2) supply. Erythropoietin stimulates erythropoiesis and so increases O_2 delivery. BFU_E, erythroid burst-forming unit; CFU_E, erythroid colony-forming unit. Hypoxia induces hypoxia inducible factors (HIFs) α and β, which stimulate erythropoietin production. Von-Hippel–Lindau (VHL) protein breaks down HIFs. PHD2 (prolyl hydroxylase) hydroxylates HIF-1α allowing VHL binding to HIFs. Mutations in VHL, PHD2 or HIF-1α underlie congenital polycythaemia (see p. 171).

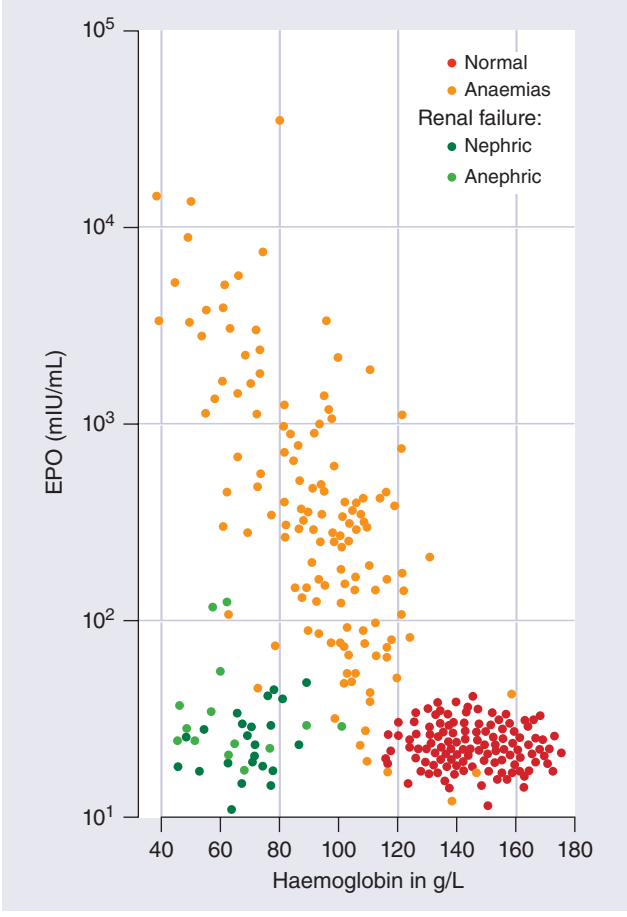


Figure 2.6 The relation between erythropoietin (EPO) in plasma and haemoglobin concentration. Anaemias exclude conditions shown to be associated with impaired production of EPO. Source: Modified from M. Pippard et al. (1992) B J Haematol 82: 445. Reproduced with permission of John Wiley & Sons.

Indications for erythropoietin therapy

Recombinant erythropoietin is needed for treating anaemia resulting from renal disease or from various other causes. It is given subcutaneously either three times weekly or once every 1–2 weeks or every 4 weeks, depending on the indication and on the preparation used (erythropoietin alpha or beta, darbepoetin alpha (a heavily glycosylated longer-acting form), or Micera the longest-acting preparation). The main indication is end-stage renal disease (with or without dialysis). The patients often also need oral or intravenous iron. Other uses are listed in Table 2.2. The haemoglobin level and quality of life may be improved. A low serum erythropoietin level prior to treatment is valuable in predicting an effective response. Side-effects include a rise in blood pressure, thrombosis and local injection site reactions. It has been associated with progression of some tumours which express Epo receptors.

The marrow requires many other precursors for effective erythropoiesis. These include metals such as iron and cobalt, vitamins (especially vitamin B_{12}, folate, vitamin C, vitamin E, vitamin B_6, thiamine and riboflavin) and hormones such as androgens and thyroxine. Deficiency in any of these may be associated with anaemia.

Haemoglobin

Haemoglobin synthesis

The main function of red cells is to carry O_2 to the tissues and to return carbon dioxide (CO_2) from the tissues to the lungs. In order to achieve this gaseous exchange they contain the specialized protein haemoglobin. Each molecule of normal adult haemoglobin A (Hb A) (the dominant haemoglobin in blood after the age of 3–6 months) consists of four polypeptide chains, $\alpha_2\beta_2$, each with its own haem group. Normal adult blood also contains small quantities of two other haemoglobins: Hb F and Hb A_2. These also contain α chains, but with γ and δ chains, respectively, instead of β (Table 2.3). The synthesis of the various globin chains in the fetus and adult is discussed in more detail in Chapter 7.

Table 2.2 Clinical uses of erythropoietin.

Anaemia of chronic renal disease
Myelodysplastic syndrome
Anaemia associated with malignancy and chemotherapy
Anaemia of chronic diseases, e.g. rheumatoid arthritis
Anaemia of prematurity
Perioperative uses

Table 2.3 Normal haemoglobins in adult blood.

	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

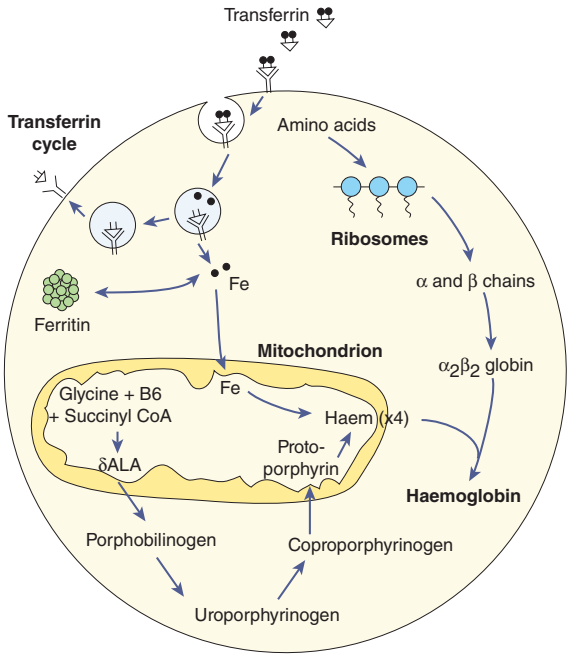


Figure 2.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main sites of protoporphyrin synthesis, iron (Fe) is supplied from circulating transferrin; globin chains are synthesized on ribosomes. δ-ALA, δ-aminolaevulinic acid; CoA, coenzyme A.

Haem synthesis occurs largely in the mitochondria by a series of biochemical reactions commencing with the condensation of glycine and succinyl coenzyme A under the action of the key rate-limiting enzyme δ-aminolaevulinic acid (ALA) synthase (Fig. 2.7). Pyridoxal phosphate (vitamin B_6) is a coenzyme for this reaction. Ultimately, protoporphyrin combines with iron in the ferrous (Fe^{2+}) state to form haem (Fig. 2.8).

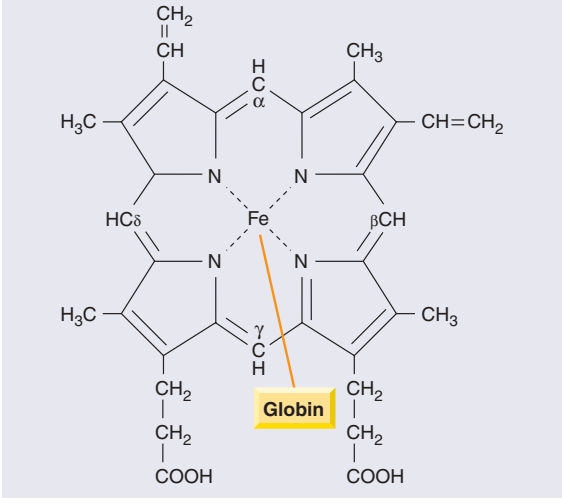


Figure 2.8 The structure of haem.

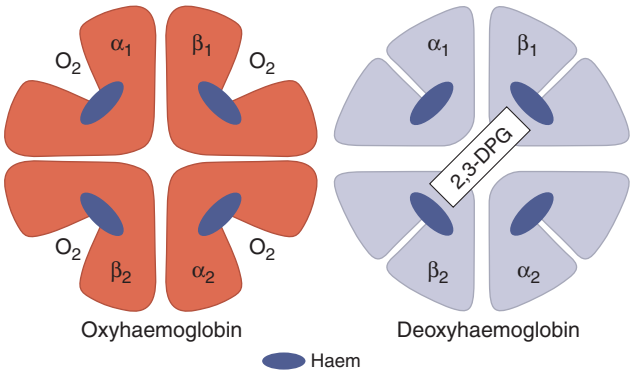


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A). 2,3-DPG, 2,3-diphosphoglycerate.

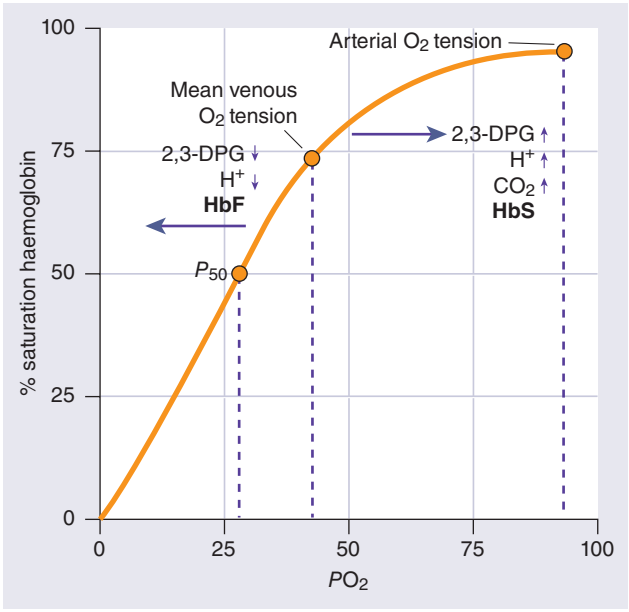


Figure 2.10 The haemoglobin oxygen (O_2) dissociation curve. 2,3-DPG, 2,3-diphosphoglycerate.

A tetramer of four globin chains each with its own haem group in a 'pocket' is then formed to make up a haemoglobin molecule (Fig. 2.9).

Haemoglobin function

The red cells in systemic arterial blood carry O_2 from the lungs to the tissues and return in venous blood with CO_2 to the lungs. As the haemoglobin molecule loads and unloads O_2 the individual globin chains move on each other (Fig. 2.9). The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ contacts stabilize the molecule. When O_2 is unloaded the β chains are pulled apart, permitting entry of the metabolite 2,3-diphosphoglycerate (2,3-DPG) resulting in a lower affinity of the molecule for O_2. This movement is responsible for the sigmoid form of the haemoglobin O_2 dissociation curve (Fig. 2.10). The P_{50} (i.e. the partial pressure of O_2 at which haemoglobin is half saturated with O_2) of normal blood is 26.6 mmHg. With increased affinity for O_2, the curve shifts to the left (i.e. the P_{50} falls) while with decreased affinity for O_2, the curve shifts to the right (i.e. the P_{50} rises).

Normally, *in vivo*, O_2 exchange operates between 95% saturation (arterial blood) with a mean arterial O_2 tension of 95 mmHg and 70% saturation (venous blood) with a mean venous O_2 tension of 40 mmHg (Fig. 2.10).

The normal position of the curve depends on the concentration of 2,3-DPG, H^+ ions and CO_2 in the red cell and on the structure of the haemoglobin molecule. High concentrations of 2,3-DPG, H^+ or CO_2, and the presence of sickle haemoglobin (Hb S), shift the curve to the right (oxygen is given up more easily) whereas fetal haemoglobin (Hb F) – which is unable to bind 2,3-DPG – and certain rare abnormal haemoglobins associated with polycythaemia shift the curve to the left because they give up O_2 less readily than normal.

Methaemoglobinaemia

This is a clinical state in which circulating haemoglobin is present with iron in the oxidized (Fe^{3+}) instead of the usual Fe^{2+} state. It may arise because of a hereditary deficiency of methaemoglobin reductase deficiency or inheritance of a structurally abnormal haemoglobin (Hb M). Hb Ms contain an amino acid substitution affecting the haem pocket of the globin chain. Toxic methaemoglobinaemia (and/or sulphaemoglobinaemia) occurs when a drug or other toxic substance oxidizes haemoglobin. In all these states, the patient is likely to show cyanosis.

The red cell

In order to carry haemoglobin into close contact with the tissues and for successful gaseous exchange, the red cell, 8 μm in diameter, must be able: to pass repeatedly through the microcirculation whose minimum diameter is 3.5 μm, to maintain haemoglobin in a reduced (ferrous) state and to maintain osmotic equilibrium despite the high concentration of protein (haemoglobin) in the cell. A single journey round the body takes 20 seconds and its total journey throughout its 120-day lifespan has been estimated to be 480 km (300 miles). To fulfil these functions, the cell is a flexible biconcave disc with an ability to generate energy as adenosine triphosphate (ATP) by the anaerobic glycolytic (Embden–Meyerhof) pathway (Fig. 2.11) and to

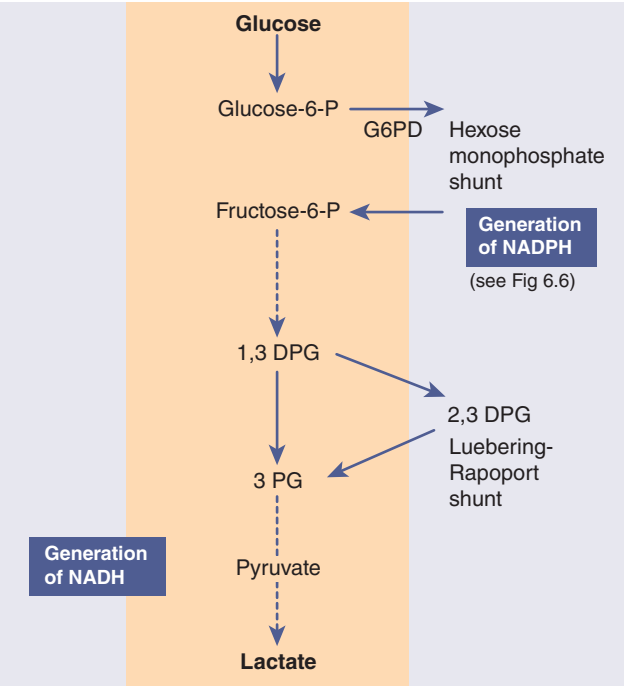


Figure 2.11 The Embden–Meyerhof glycolytic pathway. The Luebering–Rapoport shunt regulates the concentration of 2,3-diphosphoglycerate (2,3-DPG) in the red cell. ATP, adenosine triphosphate; NAD, NADH, nicotinamide adenine dinucleotide; PG, phosphoglycerate.

generate reducing power as nicotinamide adenine dinucleotide (NADH) by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt (see Fig. 6.6).

Red cell metabolism

Embden–Meyerhof pathway

In this series of biochemical reactions, glucose that enters the red cell from plasma by facilitated transfer is metabolized to lactate (Fig. 2.11). For each molecule of glucose used, two molecules of ATP and thus two high-energy phosphate bonds are generated. This ATP provides energy for maintenance of red cell volume, shape and flexibility.

The Embden–Meyerhof pathway also generates NADH, which is needed by the enzyme methaemoglobin reductase to reduce functionally dead methaemoglobin containing ferric iron (produced by oxidation of approximately 3% of haemoglobin each day) to functionally active, reduced haemoglobin containing ferrous ions. The Luebering–Rapoport shunt, or side arm, of this pathway (Fig. 2.11) generates 2,3-DPG, important in the regulation of haemoglobin's oxygen affinity (Fig. 2.9).

Hexose monophosphate (pentose phosphate) shunt

Approximately 10% of glycolysis occurs by this oxidative pathway in which glucose-6-phosphate is converted to 6-phosphogluconate and so to ribulose-5-phosphate (see Fig. 6.6). NADPH is generated and is linked with glutathione which maintains sulphydril (SH) groups intact in the cell, including those in haemoglobin and the red cell membrane. In one of the most common inherited abnormalities of red cells, glucose-6-phosphate dehydrogenase (G6PD) deficiency, the red cells are extremely susceptible to oxidant stress (see p. 66).

Red cell membrane

The red cell membrane comprises a lipid bilayer, integral membrane proteins and a membrane skeleton (Fig. 2.12). Approximately 50% of the membrane is protein, 20% phospholipids, 20% cholesterol molecules and up to 10% is carbohydrate. Carbohydrates occur only on the external surface while proteins are either peripheral or integral, penetrating the lipid bilayer. Several red cell proteins have been numbered according to their mobility on polyacrylamide gel electrophoresis (PAGE), e.g. band 3, proteins 4.1, 4.2 (Fig. 2.12).