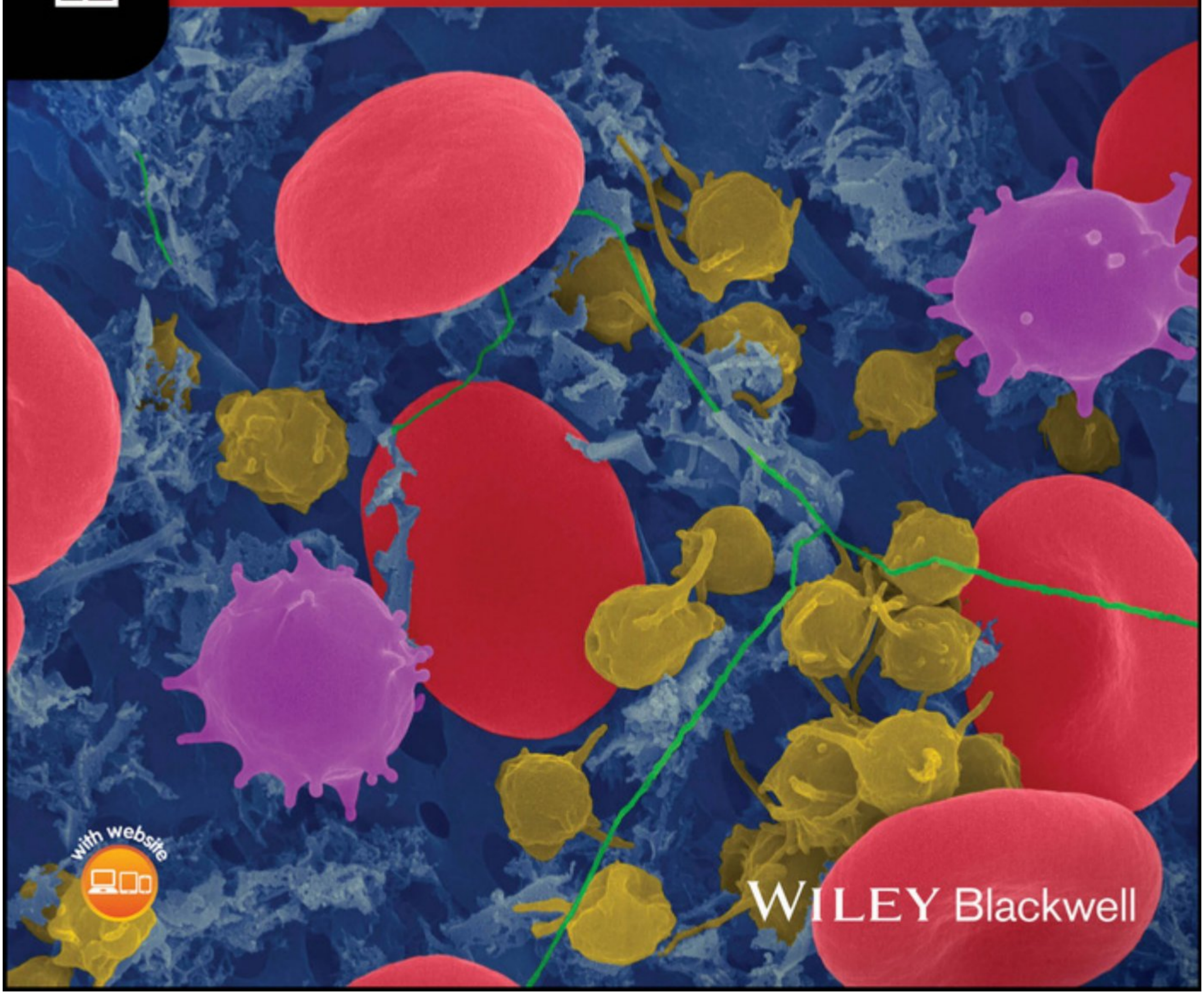
ESSENTIALS

HOFFBRAND'S ESSENTIAL HAEMATOLOGY

A. VICTOR HOFFBRAND

WITH PRATIMA CHOWDARY, GRAHAM COLLINS AND JUSTIN LOKE

NINTH EDITION



WILEY Blackwell

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Hoffbrand's Essential Haematology

Ninth Edition

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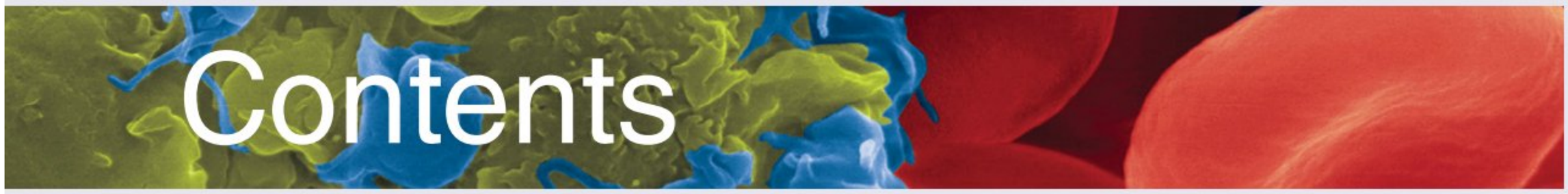
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Preface to the Ninth Edition

Advances in the understanding the pathogenesis of blood diseases and improvements in their treatment have continued apace in the 5 years since the eighth edition of *Hoffbrand's Essential Haematology* was published. Gene mutations are increasingly used to define and classify inherited and acquired haematological diseases, as a guide to therapy and to predict prognosis. Mutations underlying many rarer blood diseases have been identified, allowing appropriate panels of DNA probes to be established, facilitating the diagnosis of future cases. Many more drugs directed against specific sites in the cell signalling pathways have been approved.

The past five years have also seen substantial advances in immunological treatment for malignant haematological diseases. Mono- and bi-specific antibodies are increasingly incorporated into frontline therapy as well into treatment of relapsed disease. Chimeric antigen receptor (CAR)-T cells are challenging stem cell transplantation for potential cure for relapsed B-cell lymphoid neoplasms.

New drugs have also been introduced for treatment of benign (now termed in the United States 'classical') haematological diseases. These include mitapivat for pyruvate kinase deficiency, sutimlimab for cold agglutinin disease, luspatercept for anaemia in thalassaemia and myelodysplasia and pegcetacoplan for paroxysmal nocturnal haemoglobinuria. Drugs inhibiting prolyl hydroxylase in the hypoxia-inducible factor pathway are being developed to treat anaemia. They are already in illegal use for 'doping' of athletes to enhance their performance.

The fifth edition of the World Health Organisation (2022) Classification of the Haemato-lymphoid Tumours has been incorporated throughout this new edition and is given as an Appendix. The International Consensus Classification (ICC) of Myeloid Neoplasms, Acute Leukaemias and Mature Lymphoid Neoplasms was also published in 2022. It is beyond the scope of this book to compare and contrast the WHO and ICC classifications. Reference to the ICC classification are given in the Appendix.

David Steensma, the remarkable co-author of HEH8 stepped down for this new edition when he was appointed Global Head of Haematology at Novartis Institute for Biological Research. For the first time for *Essential Haematology*, there will be co-authorship by a specialist in the coagulation field. Professor Pratima Chowdary, Director of the Katherine Dormandy Haemophilia and Thrombosis Centre at the Royal Free Hospital, London, has ensured that the major section of the book dealing with bleeding and thrombotic disorders is authoritative and up to date. Graham Collins, Associate Professor of Haematology, Oxford Haematology and Cancer Centre has had the monumental task of updating the sections of HEH dealing with the lymphoid malignancies. Dr Justin Loke, AACR-CRUK Transatlantic Fellow, University of Birmingham, UK, now at the Dana-Farber Cancer Institute, Boston, USA, has undertaken the parallel task of updating the chapters dealing with the myeloid malignancies.

We are grateful to Dr Connor Sweeney, Professor Ashutosh Wechelaker and Professor Irene Roberts for their expert contributions to chapters 17 (acute lymphoblastic leukaemia), 23 (amyloid) and 34 (pregnancy and neonatal haematology), respectively. We are also grateful to Professor Barbara Bain who kindly checked the validity of our accompanying MCQs and to Dr Kirollos Kamel for his valuable contributions to chapters 30 and 31 (thrombosis and its management). We thank our publishers Wiley-Blackwell and especially Sophie Bradwell, Mandy Collison, Neelukiran Sekar and Kimberly Monroe-Hill for their unstinting help and support at all stages of production of this new edition. We also thank Jane Fallows for producing again such beautiful, clear diagrams.

Essential Haematology began life in 1980 as a textbook for medical students. We hope medical and other undergraduate students will continue to use it and share our excitement about one of the most advanced fields in medicine. With the vast expansion of knowledge of blood and its diseases over the past 44 years, the book has inevitably expanded. It is now also suitable for those beginning a career in haematology, for clinical and non-clinical scientists and nurses with an interest in blood and its diseases and for those working in closely related fields.

A. Victor Hoffbrand
London, 2024

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About the Companion Website

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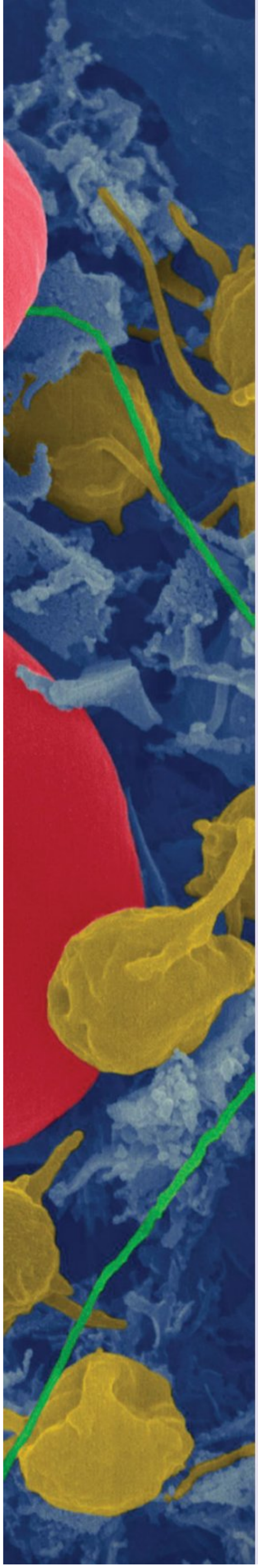
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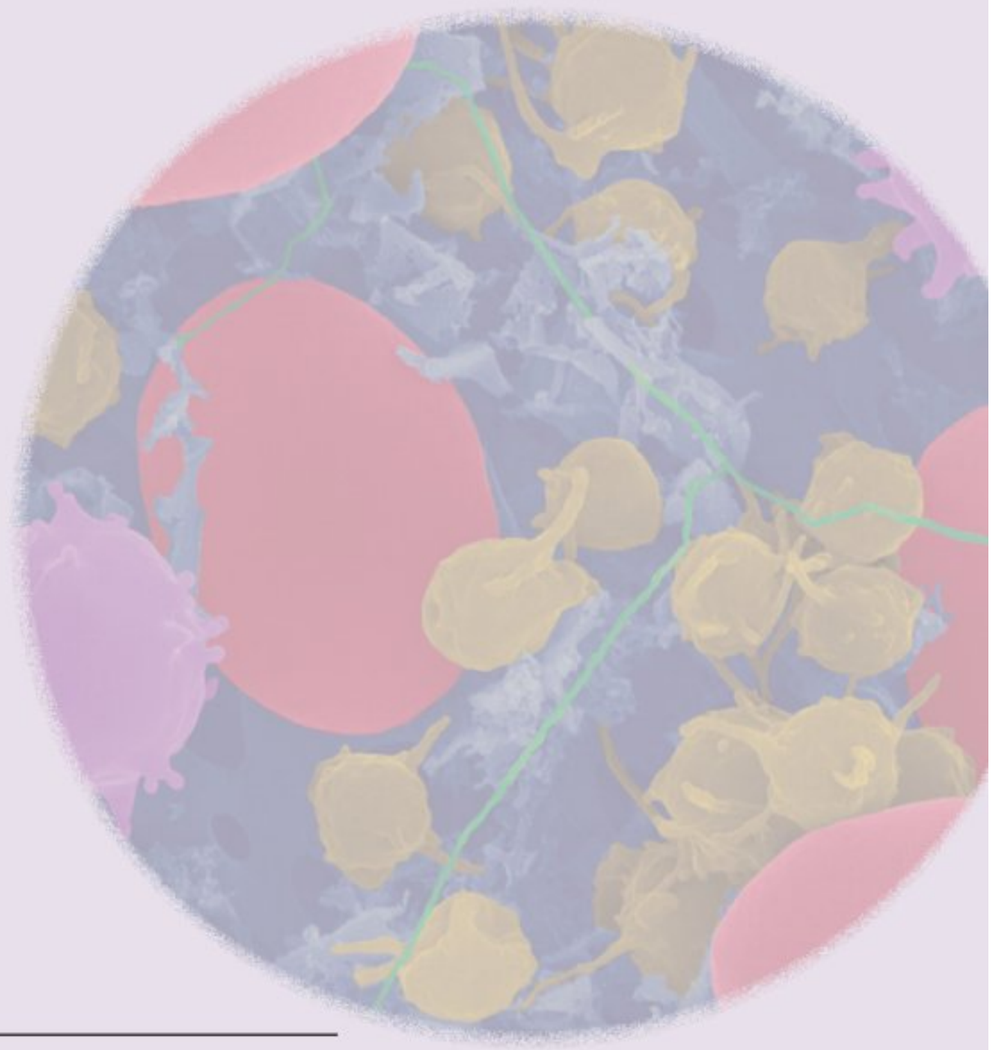


CHAPTER 1

Haemopoiesis

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- Haemopoietic stem and progenitor cells 2
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- The regulation of haemopoiesis 5
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This chapter deals 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 embryonic yolk sac is a transient site of primitive haemopoiesis. Definitive haemopoiesis derives from a population of stem cells first observed in the aorta–gonads–mesonephros (AGM) region of the developing embryo. These common precursors of endothelial and haemopoietic cells are called haemangioblasts and seed the liver, spleen and bone marrow.

From 6 weeks until 6–7 months of foetal 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 foetal haemopoiesis. The bone marrow takes over as the most important site from 6 to 7 months of foetal life. During normal childhood and adult life, the marrow is the only source of new red cells, granulocytes, monocytes and platelets. 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 and beyond there is progressive replacement of marrow throughout the long bones with fat cells, 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 active haemopoietic areas, approximately 50% of the marrow consists of fat in the middle-aged adult (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, in certain disease states, the liver and spleen can resume their foetal haemopoietic role ('extramedullary haemopoiesis').

Table 1.1 Dominant sites of haemopoiesis at different stages of development.

Foetus	0–2 months (yolk sac)
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	Bone marrow (practically all bones); dwindling contribution from liver/spleen that ceases in the first few months of life
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

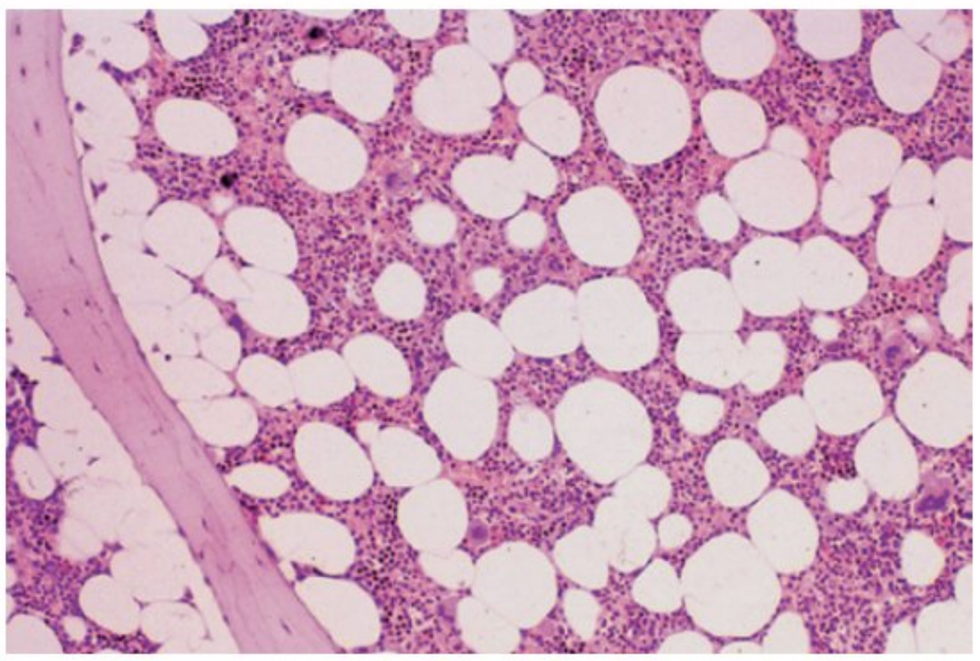


Figure 1.1 Normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% fat.

Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotent stem cell that can self-renew by asymmetrical cell division but also gives rise to the precursor of the separate cell lineages. The stem cells are able to repopulate a bone marrow from which all stem cells have been eliminated by lethal irradiation or chemotherapy. Self-renewal and repopulating ability define the **haemopoietic stem cell** (HSC). HSCs are rare perhaps 1 in every 20 million nucleated cells in bone marrow. Newer DNA sequencing techniques suggest that a typical adult has approximately 50 000 HSCs.

HSCs are heterogeneous, with some able to repopulate a bone marrow for more than 16 weeks, called **long-term HSCs**, while others, although able to produce all haemopoietic cell types, engraft only transiently for a few weeks and are called **short-term HSCs**. Although the exact cell surface marker phenotype of the HSC is still unknown, on immunological testing these cells are positive for the markers cluster of differentiation 34 (CD34), CD49f and CD90 and negative for CD38 and CD45RA and for cell lineage-defining markers (Lin). Morphologically, HSCs have the appearance of small- or medium-sized lymphocytes.

Cell differentiation occurs from the stem cells 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. Stem cells and very early progenitors are assayed by culture on bone marrow stroma as long-term culture-initiating cells, whereas later progenitors are generally assayed in semi-solid media. As examples, in the erythroid series progenitors can be identified in special cultures as burst-forming units (BFU-E, describing the 'burst' with which they form in culture) and

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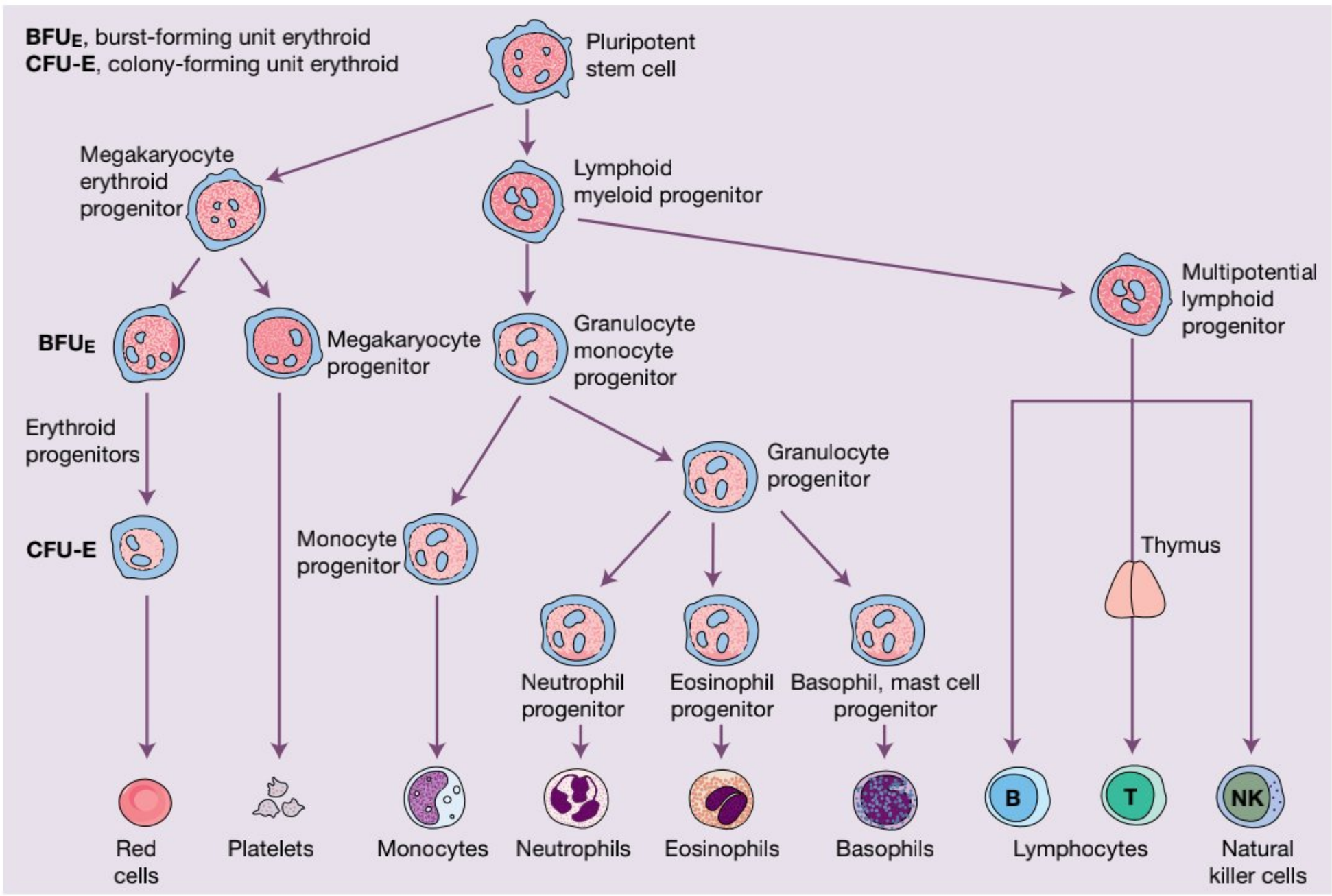


Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cells (haemopoietic stem cells, HSC) and the cell lines that arise from them. A megakaryocytic/erythroid progenitor (MkEP) and a mixed lymphoid/myeloid progenitor are formed from the pluripotent stem cells. Each gives rise to more differentiated progenitors. BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid.

colony-forming units (CFU-E; Fig 1.2); the mixed granulocyte/monocyte progenitor is identified as a colony-forming unit-granulocyte/monocyte (CFU-GM) in culture. Megakaryocytes derive from a megakaryocyte progenitor, itself derived from an earlier mixed erythroid–megakaryocyte progenitor.

In the haemopoietic hierarchy, the pluripotent stem cell gives rise to a **mixed erythroid and megakaryocyte progenitor**, which then divides into separate erythroid and megakaryocyte progenitors. The pluripotent stem cell also gives rise to a **mixed lymphoid, granulocyte and monocyte progenitor**, which divides into a progenitor of granulocytes and monocytes and a mixed lymphoid progenitor, from which B- and T-cell lymphocytes and natural killer (NK) cells develop (Fig. 1.2). The spleen, lymph nodes and thymus are secondary sites of lymphocyte production (Chapter 9).

As the stem cell has the capability for **self-renewal** (Fig. 1.3), the 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 (the 'Hayflick limit'), with progressive telomere shortening with each division affecting viability.

Under normal conditions most HSCs are dormant, with at most only a few percent active in cell cycle on any given day. Any given HSC enters the cell cycle approximately once every 3 months to 3 years in humans. By contrast, progenitor cells are much more numerous and highly proliferative. With ageing, 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 exonic coding mutations by age 60 years (1.3 per decade). These, either passengers without oncogenic potential or drivers that cause clonal expansion, may be present in neoplasms arising from these stem cells (Chapters 11, 16).

The progenitor and precursor cells are capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises. The development

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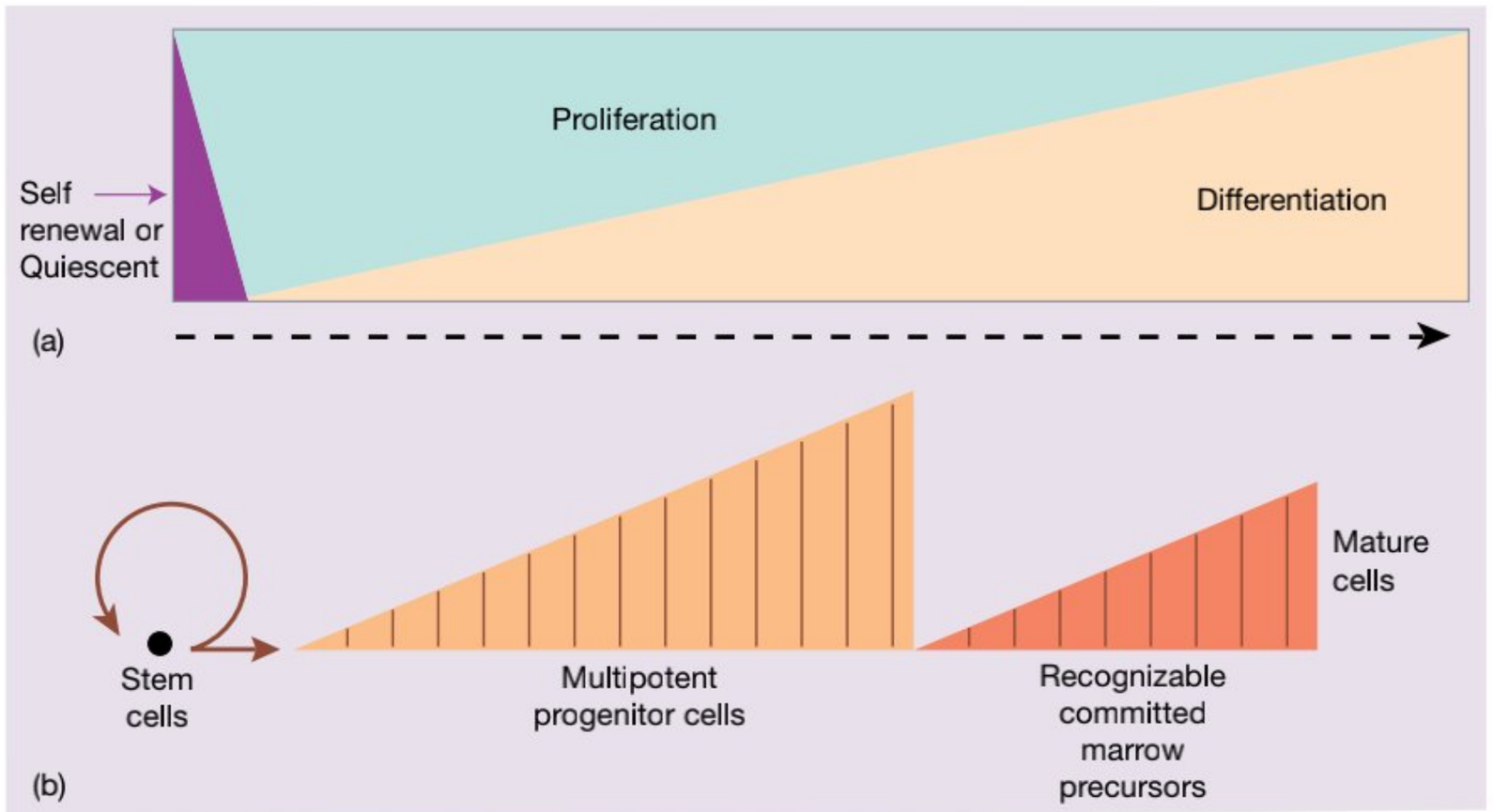


Figure 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

of the mature cells (red cells, granulocytes, monocytes, megakaryocytes and lymphocytes) is considered further in other sections of this book.

Bone marrow stroma and niches

The bone marrow forms a suitable environment for stem cell survival, self-renewal and formation of differentiated progenitor cells. It is composed of various types of stromal cells and a microvascular network (Fig. 1.4). **The stromal cells include adipocytes, fibroblasts, macrophages, megakaryocytes, osteoblasts, osteoclasts, endothelial cells and mesenchymal stem cells (which have the capacity to self-renew and differentiate into osteocytes, adipocytes and chondrocytes).** The stromal cells secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronic acid and chondroitin derivatives) to form an extracellular matrix.

The HSCs reside in two types of niche. These provide some of the growth factors, adhesion molecules and cytokines which support stem cells, maintaining their viability and reproduction, e.g. stem cell factor (SCF) expressed by stromal and endothelial cells binds to its receptor, KIT (CD117), on stem cells. The niches are either vascular, including arterioles and sinusoids that converge on a central vein, or endosteal with osteoblasts and osteoclasts closely associated with bone. Sympathetic nerves and non-myelinated Schwann cells are important regulators of stem cell quiescence or release.

Haemopoietic stem cells (as well as mesenchymal stem cells) traffic around the body. They 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 for HSCs by the administration of growth factors such as granulocyte colony-stimulating factor (G-CSF). The reverse process, stem cell homing, depends on a chemokine gradient in which stromal-derived factor 1 (SDF-1), which binds to its receptor CXCR4 on HSC, is critical.

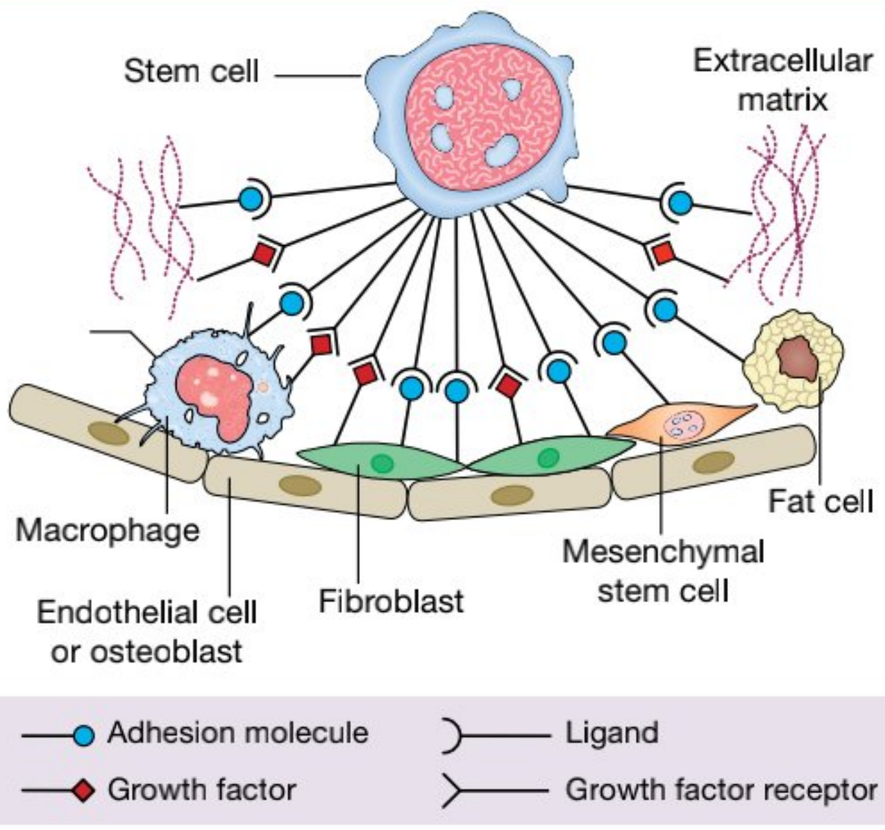


Figure 1.4 Haemopoiesis occurs in a suitable microenvironment ('niche') provided by a stromal matrix on which stem cells grow and divide. The niche may be vascular (lined by endothelium) or endosteal (lined by osteoblasts). There are specific recognition and adhesion sites; extracellular glycoproteins,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

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The regulation of haemopoiesis

Transcription factors

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 commit them to discrete cell lineages.

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families (Fig. 1.5). 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 the assembly of the transcription complex at a gene promoter. The transcription factors interact, so that reinforcement of one transcription programme may suppress that of another lineage

Which cell lineage is selected for differentiation depends on both chance and the external signals received by progenitor cells. Examples of transcription factors involved in haemopoiesis include RUNX1, GATA2 and MT2A in the earliest stages; GATA1, GATA2 and FOG1 in erythropoiesis and megakaryocytic differentiation; PU.1 and the CEBP family in granulopoiesis; PAX5 in B lymphocyte and NOTCH1 in T lymphocyte development. The transcription factors induce synthesis of proteins specific to a cell lineage. For example, GATA1 binds to specific motifs on the erythroid genes for globin and haem synthesis and so activates these genes. Mutation, deletion or translocation of transcription factor genes underlies many cases of haematological neoplasms (Chapter 11).

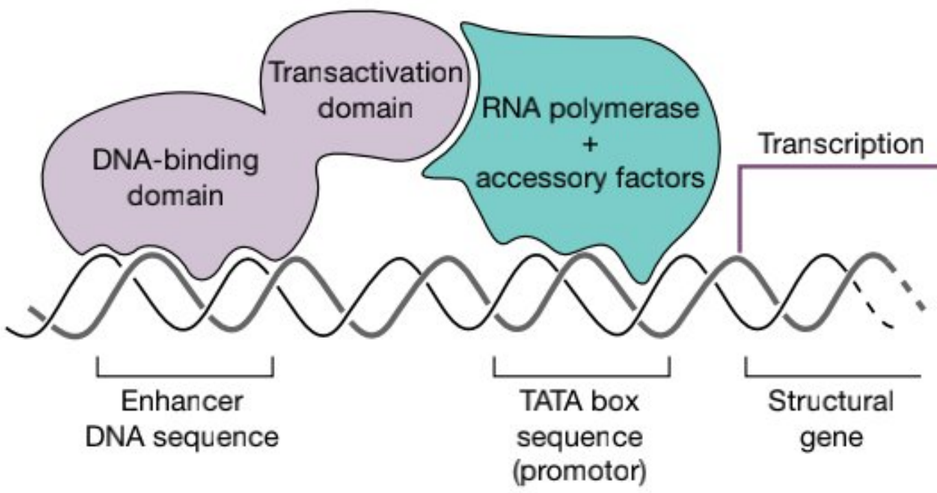


Figure 1.5 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box. 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. Transcription factors work in combination to both activate and repress the expression of a large number of genes.

Haemopoietic growth factors

The haemopoietic growth factors are a group of glycoproteins 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, e.g. SCF, or circulate in plasma, e.g. G-CSF or erythropoietin (EPO)**. 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 and maturation, prevent apoptosis and affect the function of mature cells (Fig. 1.6).

The growth factors 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 EPO, 90% of which is synthesized in the kidney, and thrombopoietin (TPO), made largely in**

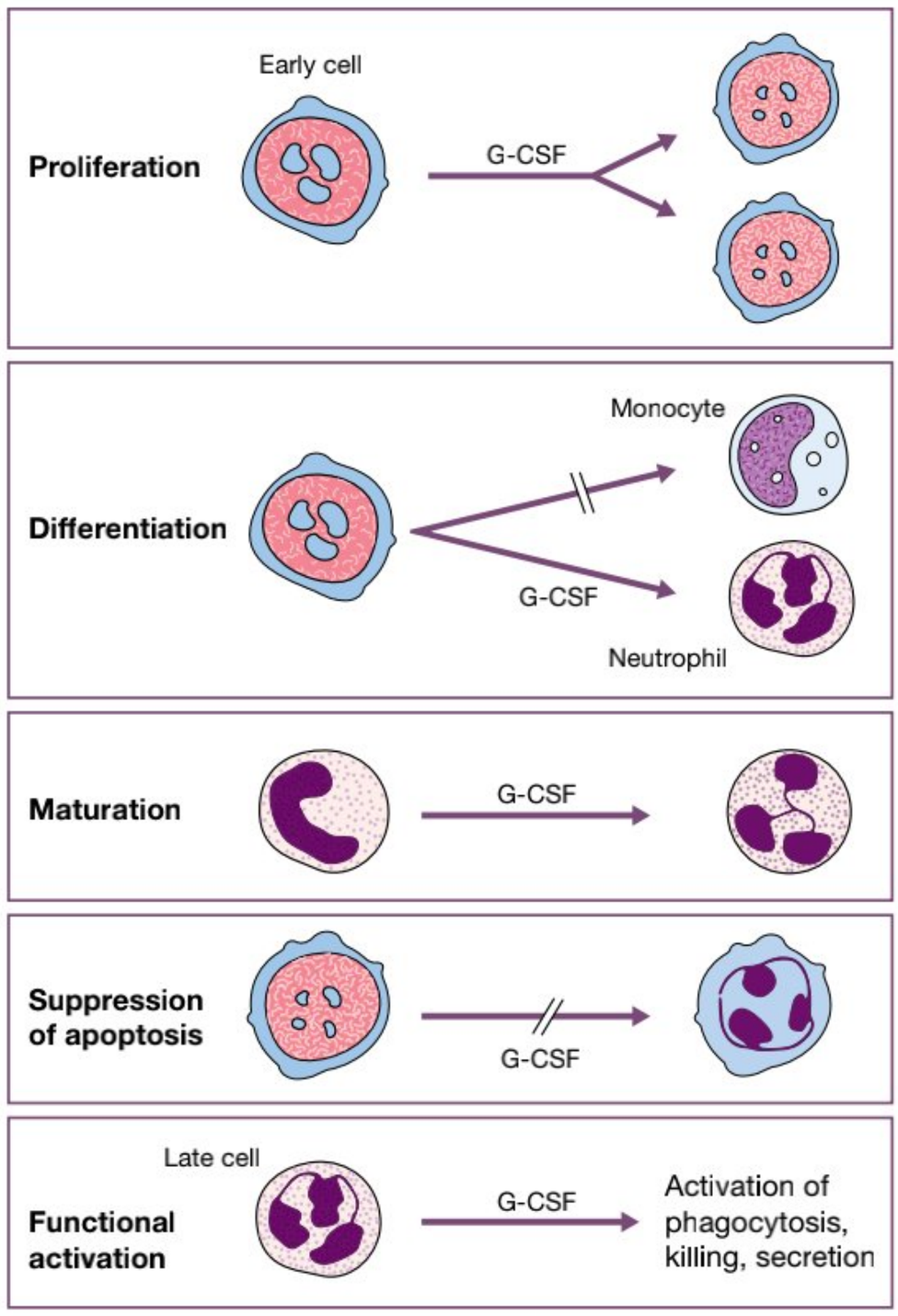


Figure 1.6 Growth factors may stimulate the proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF) for an early myeloid progenitor and a mature neutrophil.

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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, prevention of apoptosis, functional activation

Table 1.3 Haemopoietic growth factors (see also Fig. 1.7).

Act on stromal cells
IL-1, TNF

Act on pluripotential stem cells
SCF, TPO, FLT3-, NOTCH1

Act on multipotent lymphoid/myeloid progenitor cells
IL-3, IL-7, SCF, FLT3-L, TPO, GM-CSF

Act on lineage-committed progenitor cells
Granulocyte/monocyte production: IL-3, GM-CSF, G-CSF, M-CSF, IL-5 (eosinophil CSF)
Mast cell production: KIT-ligand
Red cell production:
IL-3, EPO
Platelet production: IL-3, TPO
Lymphocyte/NK cell production: IL-1, IL-2, IL-4, IL-7, IL-10, other ILs

CSF, colony-stimulating factor; EPO, erythropoietin; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage/monocyte colony-stimulating factor; NK, natural killer; SCF, stem cell factor (also known as TAL1); TNF, tumour necrosis factor; TPO, thrombopoietin.

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, TPO.NOTCH1 and FLT3 ligand act locally on the pluripotential stem cells and on myeloid/lymphoid progenitors (Fig. 1.7). Interleukin-3 (IL-3) has widespread activity on lymphoid/myeloid and megakaryocyte/erythroid progenitors. Granulocyte–macrophage colony-stimulating factor (GM-CSF), G-CSF and macrophage colony-stimulating factor (M-CSF) enhance neutrophil and macrophage/monocyte production, IL-5 eosinophil, KIT mast cell, TPO platelet and EPO red cell production. These lineage-specific growth factors also enhance the effects of SCF, FLT3-L and IL-3 on the survival and differentiation of early haemopoietic cells. Interleukin-7 is involved at all stages of lymphocyte production, and various other interleukins and toll-like receptor ligands (not shown) direct B and T lymphocyte and NK cell production (Fig. 1.7).

These factors maintain a pool of haemopoietic stem and progenitor cells on which later-acting factors, EPO, G-CSF, M-CSF, IL-5 and TPO, 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 (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 (p. 313).

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target cells. Many receptors, such as the EPO receptor (EPO-R) and GM-CSF-R, are from the **haemopoietin 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 (signal transducer and activator of transcription) pathway, the mitogen-activated protein (MAP) kinase and the phosphatidylinositol 3-kinase (PI3K) pathways (Fig. 1.8; see also Fig 9.4, 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. 1.8). 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 then phosphorylate members of the 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 the transcription of specific genes. A model for the control of gene expression by a transcription factor is shown in Fig. 1.5. The clinical importance of this pathway is revealed for example by the finding of an activating mutation of the *JAK2* gene as a cause of polycythaemia vera and related myeloproliferative neoplasms (p. 195).

JAK can also activate the MAPK pathway, which is regulated by RAS and controls proliferation. PI3 kinases phosphorylate

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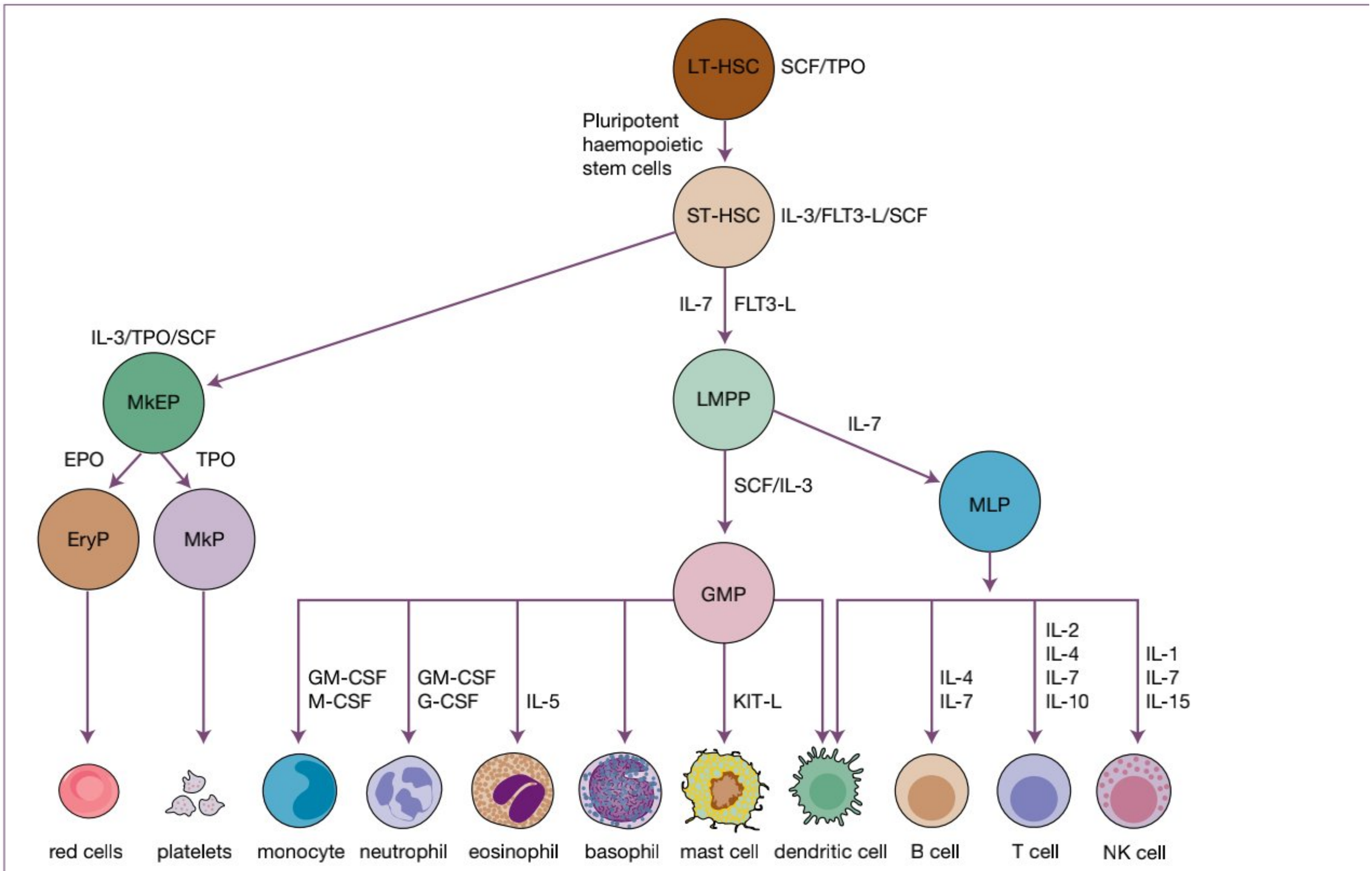


Figure 1.7 The role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; EryP, erythroid progenitor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GMP, granulocyte–macrophage progenitor; HSC, haemopoietic stem cells; IL, interleukin; LMPP, lymphoid-primed multipotential progenitor; LT, long-term; M-CSF, macrophage/monocyte colony-stimulating factor; MkEP, megakaryocyte–erythroid progenitor; MkP, megakaryocyte progenitor; MLP, multipotential lymphoid progenitor; NK, natural killer; PSC, pluripotential stem cell; SCF, stem cell factor; ST, short-term; TLR, toll-like receptor; TPO, thrombopoietin. Source: Adapted from A.V. Hoffbrand *et al*. (2019) *Color Atlas of Clinical Hematology: Molecular and Cellular Basis of Disease,* 5th edn. Reproduced with permission of John Wiley & Sons.

inositol lipids, which have a wide range of downstream effects, including activation of AKT. This results in a block of apoptosis and other actions (Figs. 1.8, 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, FLT3L 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

Cell adhesion molecules (CAMs) are glycoprotein molecules which mediate the attachment of cells to each other, to the extracellular matrix and play roles in cell-cell synapse formation. They typically are composed of three domains: intracellular, transmembrane and extracellular. They are divided into four large families: integrins, immunoglobulin super family, selectins and cadherins. They function as 'molecular glue' maintaining tissue structure and function. The integrins are particularly

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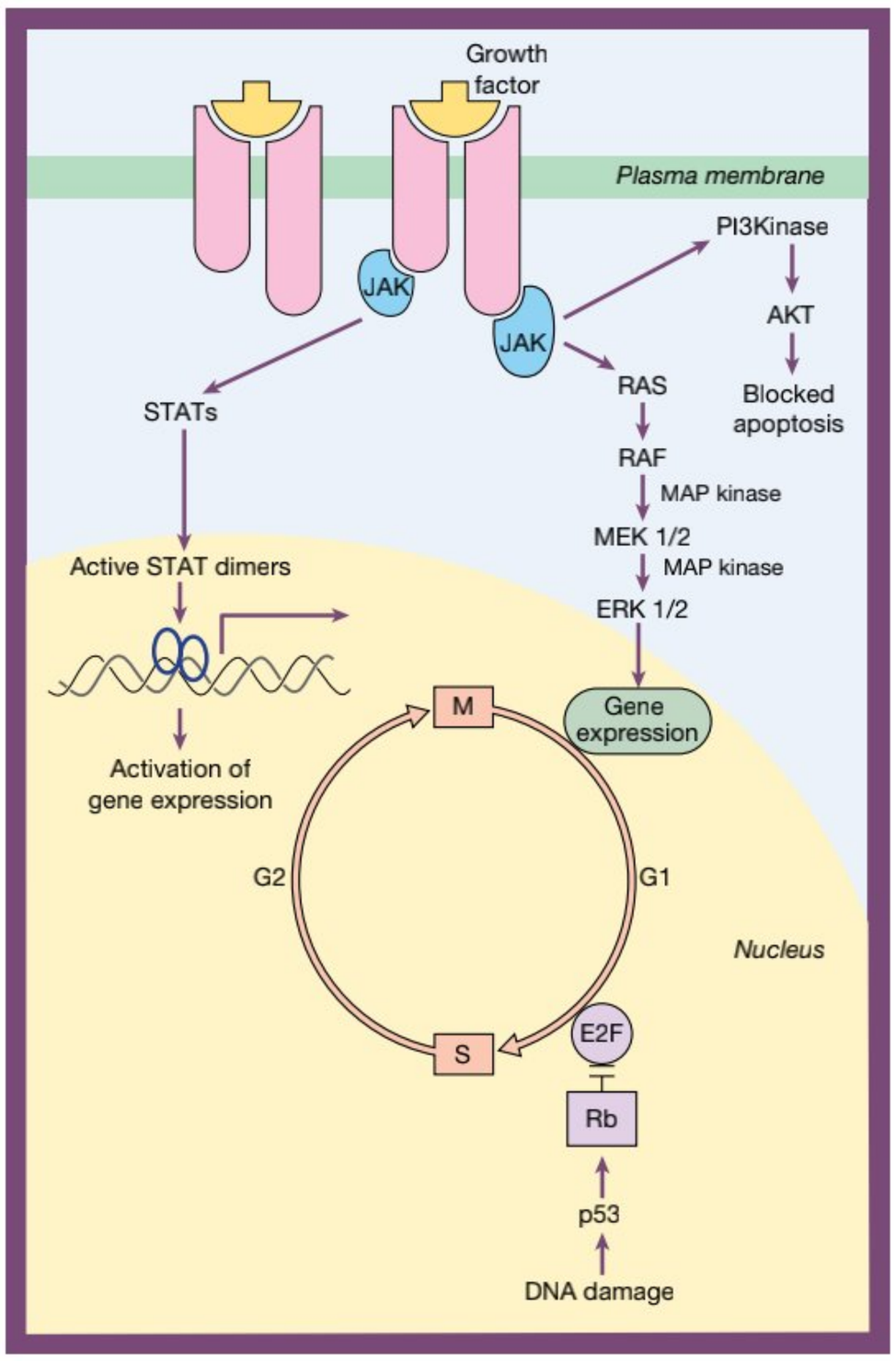


Figure 1.8 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and phosphatidyl-inositol 3-kinase (PI3K) pathways (see also Fig. 15.2), which leads to transcriptional activation 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 stimulate 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).

important in linking the extracellular environment including collagen, fibronectin and fibrinogen to intracellular signalling pathways. The selectins which include E (endothelial)-selectin, L (leucocyte)-selectin and P (platelet)-selectin are particularly important in the immune system in helping white cells in trafficking and homing.

In the bone marrow CAMs attach haemopoietic precursors, leucocytes and platelets to various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The CAMs on the surface of leucocytes and platelets are termed receptors and these interact with proteins termed ligands on the surface of target cells, e.g. endothelium. The molecules are important in the development and maintenance of inflammatory as well as immune responses, and in platelet–vessel wall and leucocyte–vessel wall interactions. Glycoprotein IIb/IIIa, for example, is a CAM, also called integrin IIβ/IIIα and involved in platelet adhesion to vessel walls and to each other (Chapter 26).

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 to specific visceral organs or bone or of 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. Attempts to treat cancer and other diseases with drugs which inhibit specific adhesion molecules have so far been unsuccessful.

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. 1.8). The M phase is further partitioned into classical **mitosis**, in which nuclear division is accomplished, and **cytokinesis**, in which cell fission occurs.

The 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.

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 phosphorylate downstream protein targets, and **cyclins**, which bind to Cdk 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 (p. 279).

Epigenetics

Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence (Fig. 16.1).

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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 of DNA bases. The methylation of cytosine residues to methylcytosine results in inhibition of gene transcription. The DNA methyltransferase genes *DNMT3A* and *B* are involved in this methylation. *TET1, 2, 3* and *IDH1* and *IDH2* are involved in the hydroxylation and breakdown of methylcytosine and restoration of gene expression (Fig. 16.1). These genes are frequently mutated in the myeloid malignancies, especially myelodysplastic syndromes and acute myeloid leukaemia (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 inter-nucleosomal 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 activation 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 encoded by the *TP53* gene on chromosome 17 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. 1.8). The cellular level of p53 is controlled by a second protein, MDM2. Following death, apoptotic cells display molecules that lead to their ingestion by macrophages. Loss of TP53 is a major mechanism by which malignant cells evade controls that would induce cell death.

As well as molecules that mediate apoptosis, there are several intracellular proteins that protect cells from apoptosis.

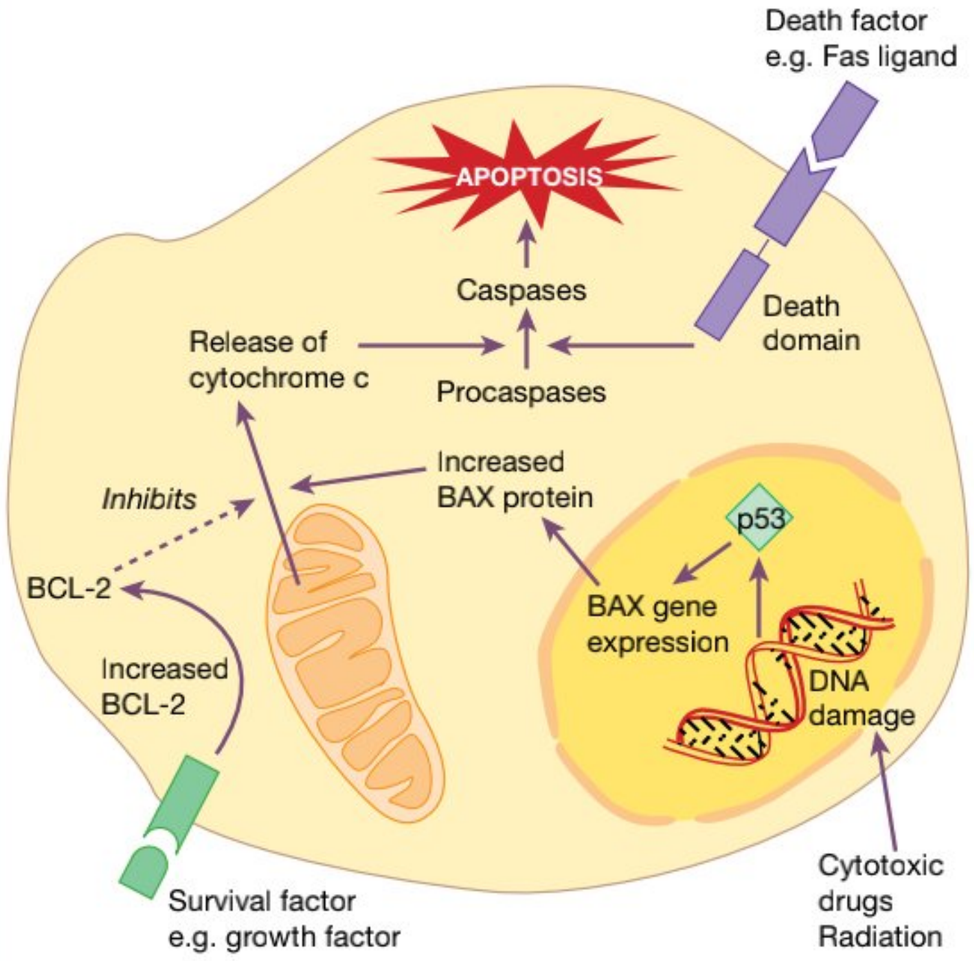


Figure 1.9 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death 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.

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 *BCL2* gene to the immunoglobulin heavy chain locus in the t(14;18) translocation in follicular lymphoma (p. xxx). Overexpression of the BCL-2 protein makes the malignant B cells less susceptible to apoptosis. The drug venetoclax which inhibits BCL-2 is now widely used to treat both myeloid and lymphoid malignant diseases. 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(11;14) and t(15;17), also result in inhibition of apoptosis (Chapter 11). In addition, genes encoding

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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 ischemia, chemical trauma or hyperthermia. The cells swell and 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. Haemopoietic stem cells give rise to mixed and then single lineage progenitor and precursor cells which, after multiple cell divisions and differentiation, form red cells, granulocytes (neutrophils, eosinophils and basophils), monocytes, platelets, B and T lymphocytes and natural killer (NK) cells.
- Haemopoietic 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 osteoblastic or endothelial niches formed by stromal cells. They also circulate in the blood.
- Growth factors attach to specific cell surface receptors and produce a cascade of phosphorylation events in the cell nucleus.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families. They carry the message to those genes that are to be 'switched on or off', to stimulate cell division, differentiation or functional activity or to suppress apoptosis.
- Adhesion molecules are a large family of glycoproteins that mediate the attachment of marrow precursors and mature leucocytes and platelets to extracellular matrix, to 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 (cytosine) methylation are two important examples relevant to haemopoiesis and haematological malignancies.
- 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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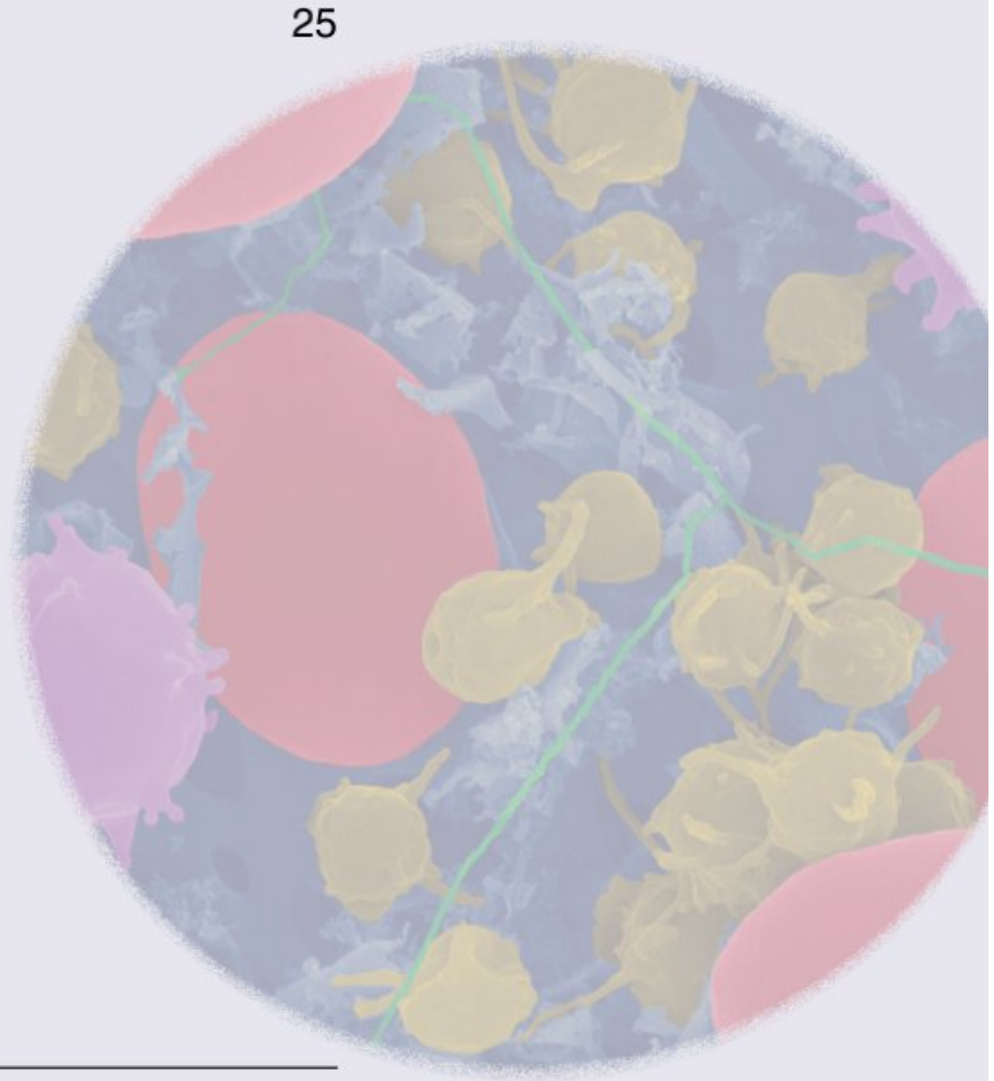


CHAPTER 2

Erythropoiesis and general aspects of anaemia

Key topics

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Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

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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 (erythrocytes)**, specialized for the 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 life span, whereas the smallest cells, **platelets** involved in haemostasis, circulate for only 10 days. **The white cells are made up of four main types of phagocyte: neutrophils, eosinophils, basophils and monocytes**, which protect against bacterial and fungal infections (Chapter 8); and of **lymphocytes**, which include **B cells**, involved in antibody production, **T cells** (CD4 helper and CD8 suppressor), concerned with the immune response and in protection against viruses and foreign cells, and **natural killer (NK) cells**, a subset of CD8 T cells (Chapter 9). White cells have a wide range of life span (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). The counter also enumerates the different types of white cell by flow cytometry and detects abnormal cells.

Erythropoiesis

We each make approximately 10^{12} new erythrocytes each day by the complex and finely regulated process of erythropoiesis. This progresses from the stem cell through progenitor cells, the erythroid and megakaryocyte colony-forming unit (CFU_{MkE}), burst-forming unit erythroid (BFU_E) and erythroid CFU (CFU-E; Fig. 1.2) to the first recognizable erythrocyte precursor in the bone marrow, the pronormoblast (Fig. 2.2). 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. These also contain

Table 2.1 The blood cells.

Cell	Diameter (μm)	Life span in blood	Number	Function
Red cells 	6–8	120 d	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 d	$140–400 \times 10^9$/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 	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
Natural killer cells 	10 (resting) 10–20 (active)	Hours or days	0.1–0.4	Protection against virus-infected and neoplastic cells

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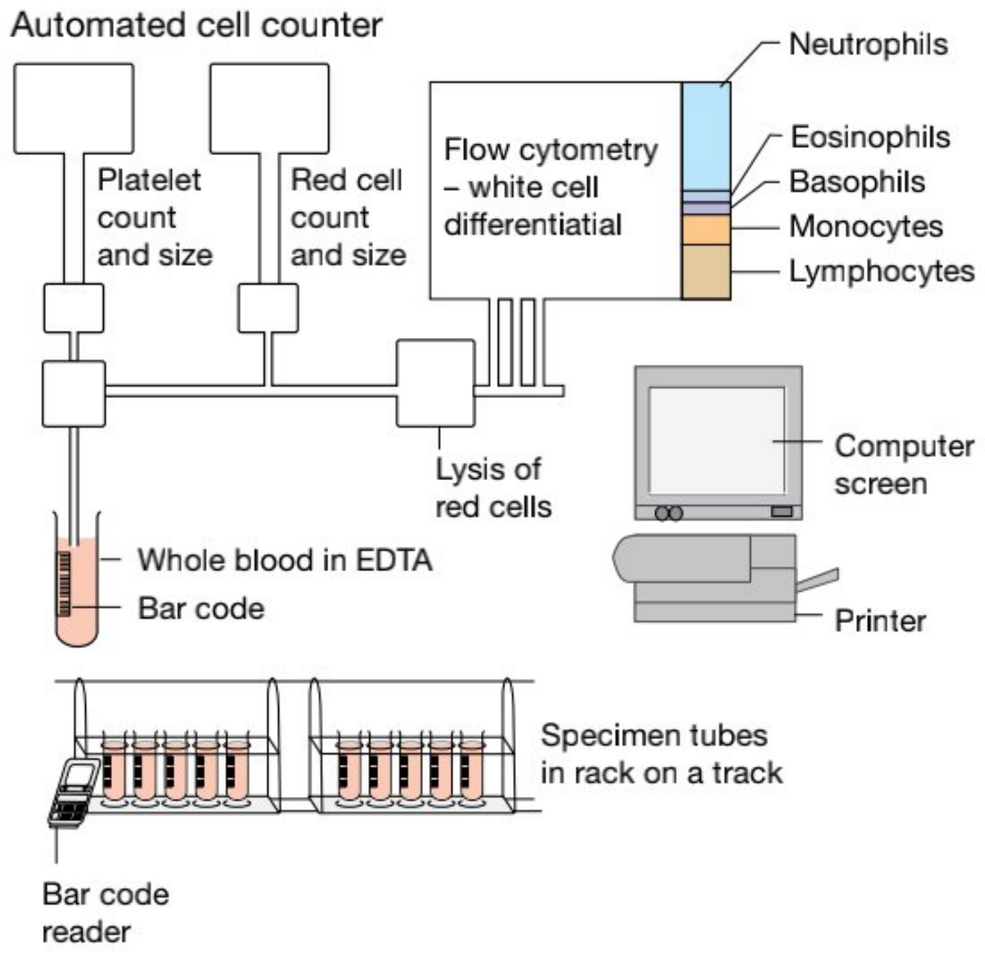


Figure 2.1 Automated blood cell counter. Source: A.B. Mehta, A.V. Hoffbrand (2014) *Haematology at a Glance*, 4th edn. Reproduced with permission of John Wiley & Sons.

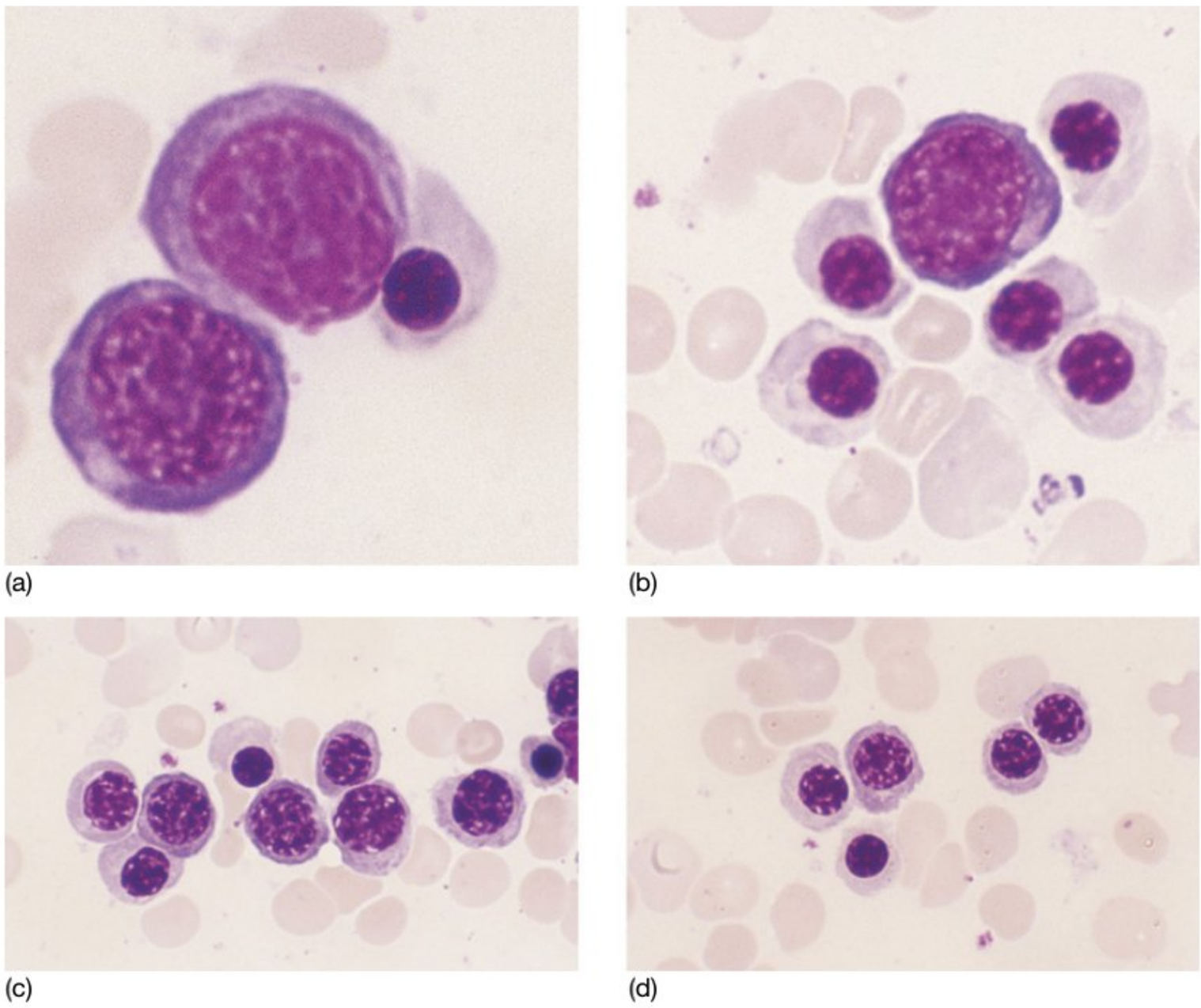


Figure 2.2 Erythroblasts (normoblasts) at varying stages of development. The earlier cells are larger, with more basophilic cytoplasm and a more open nuclear chromatin pattern **(a, b)**. The cytoplasm of the later cells is paler blue and more eosinophilic as a result of haemoglobin formation **(c, d)**.

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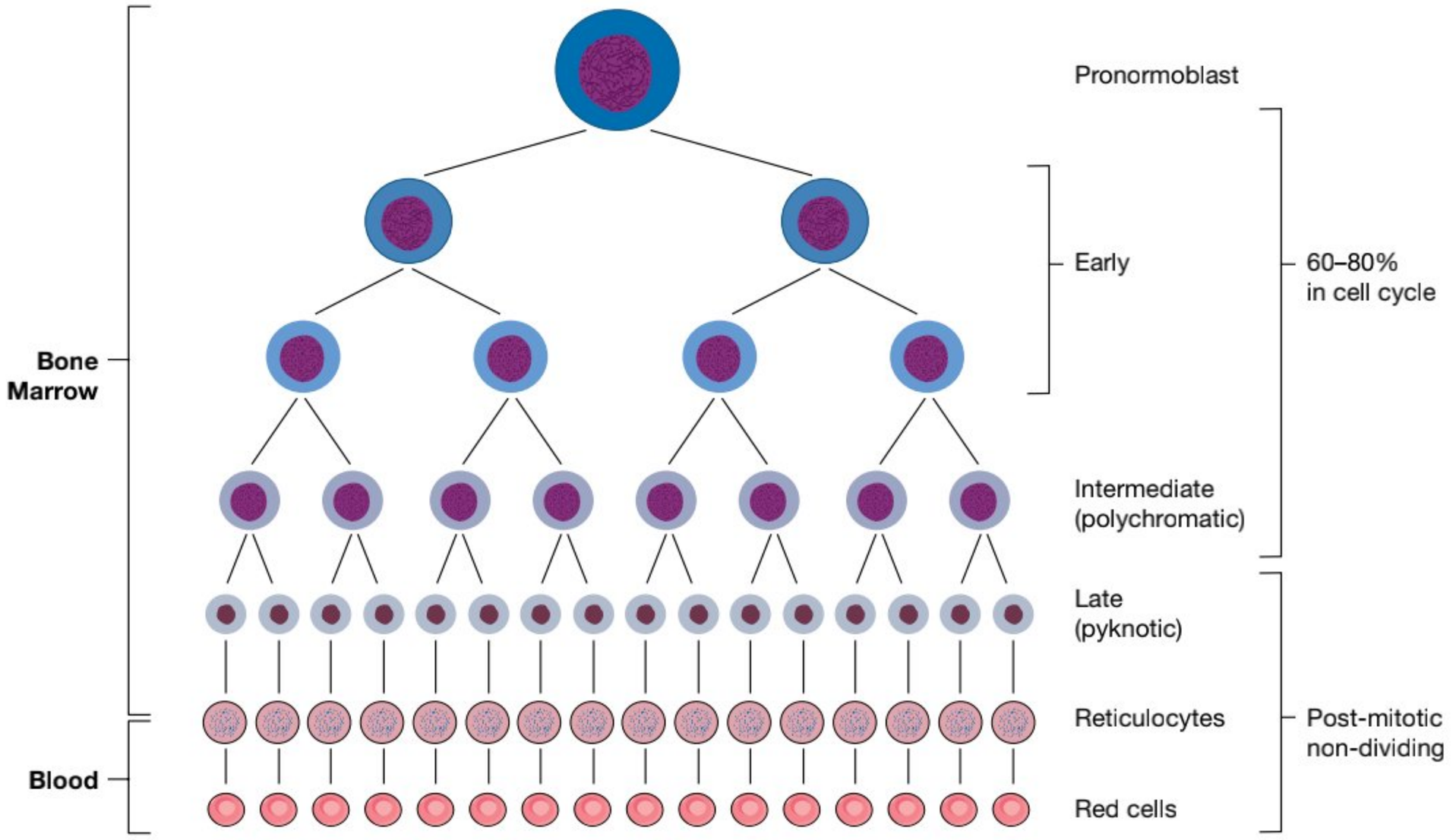
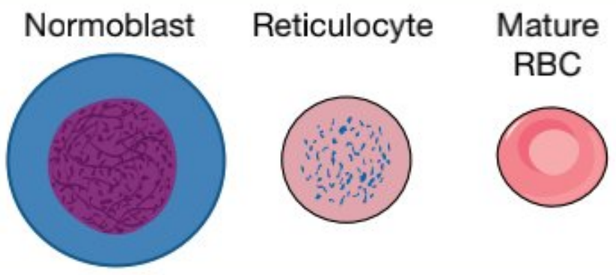


Figure 2.3 The amplification and maturation sequence in the development of mature red cells from the pronormoblast.

progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm also 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. This still contains some ribosomal RNA and so is still able to synthesize haemoglobin (Fig. 2.4).



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).

The **reticulocyte** is slightly larger than a mature red cell. It 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 (Fig. 2.4). One pronormoblast usually gives rise to 16 mature red cells (Fig. 2.3). 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, a heavily glycosylated polypeptide. Ninety percent 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. The stimulus to erythropoietin production is the oxygen (O_2) tension in the tissues of the kidney (Fig. 2.5a). Erythropoietin production increases with decreased O_2 delivery to the kidney. This is caused most frequently by anaemia, but also occurs when haemoglobin for some metabolic or structural reason is unable to give up O_2 normally, when atmospheric O_2 is low or with defective cardiac or pulmonary function or damage to the renal circulation.

Hypoxia induces stabilization of the hypoxia-inducible factor (HIF-1α) which then forms a dimer with HIFβ, the dimer stimulating erythropoietin production. The dimer also

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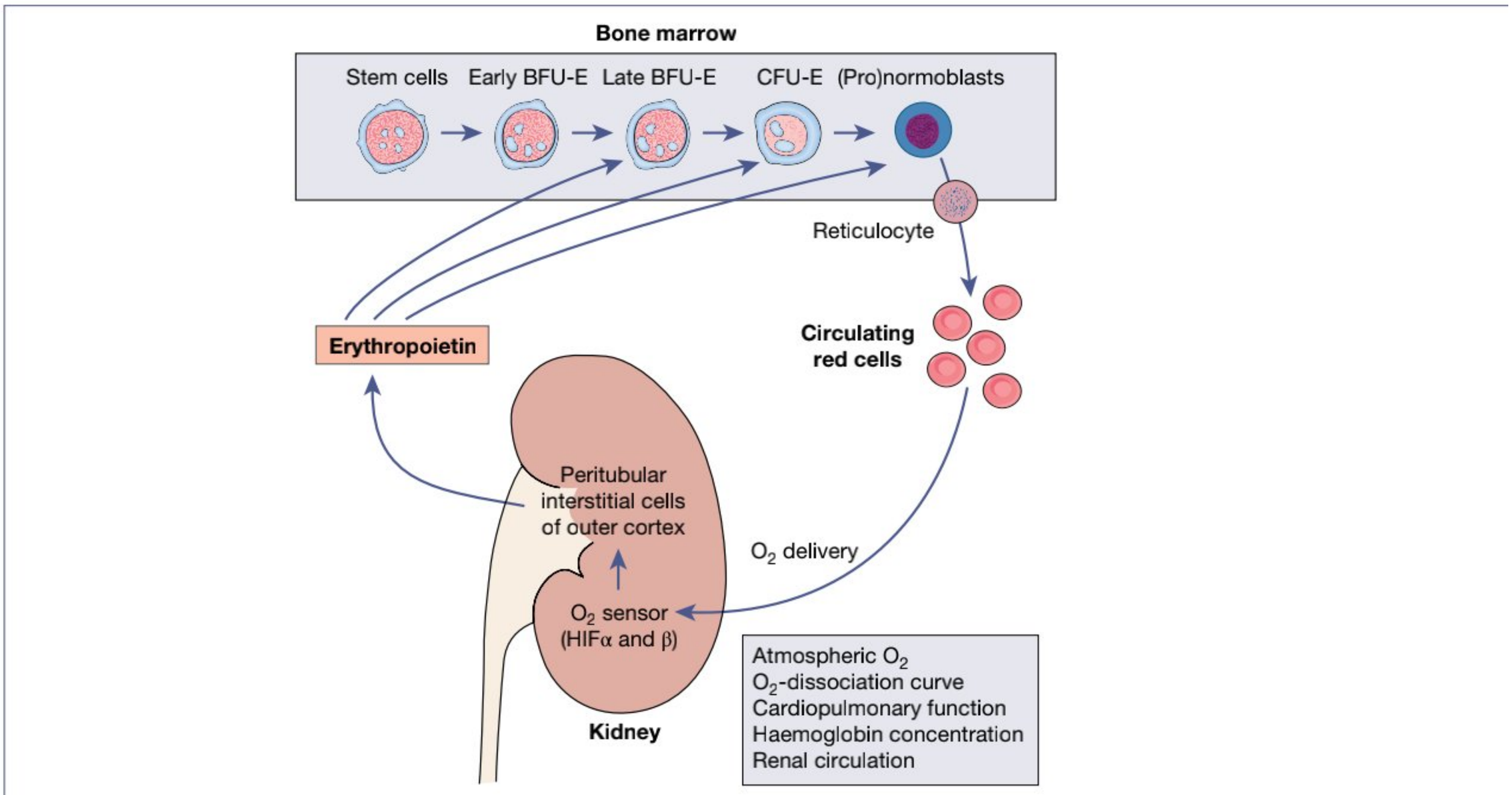


Figure 2.5a The production of erythropoietin by the kidney in response to its oxygen (O_2) supply. Erythropoietin stimulates erythropoiesis and so increases O_2 delivery. BFU_E, erythroid burst-forming unit; CFU-E, erythroid colony-forming unit.

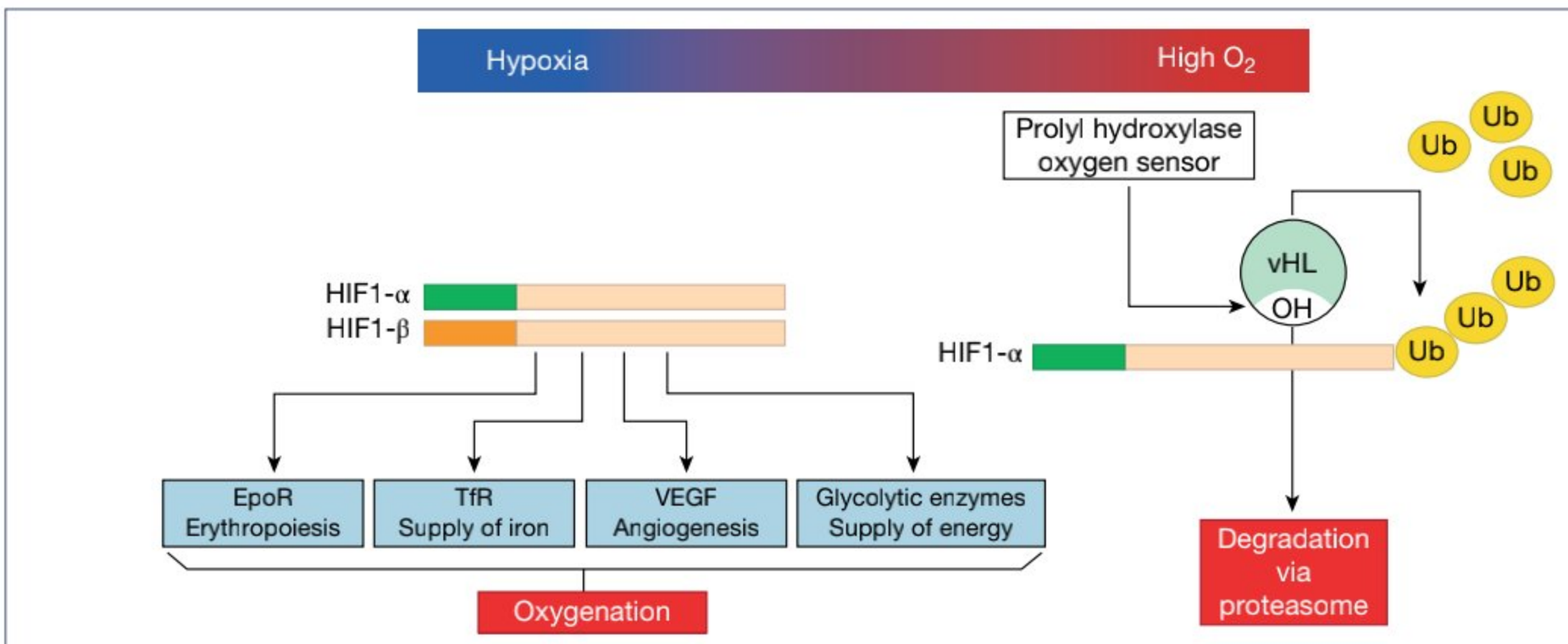


Figure 2.5b The oxygen sensor: hypoxia stabilises hypoxia inducible factor (HIF)α which then forms a dimer with HIFβ, which stimulates erythropoietin production. PHD2 (prolyl hydroxylase), the oxygen sensor, uses molecular oxygen to hydroxylate HIF-1α. This hydroxylation allows von Hippel-Lindau (vHL) binding to HIFα and stimulates its breakdown by ubiquitination. Source: D.R. Higgs *et al*. In A.V. Hoffbrand *et al*. (eds) (2016) *Postgraduate Haematology*, 7th edn. Reproduced with permission of John Wiley & Sons.

stimulates new vessel formation, glycolytic enzyme and transferrin receptor synthesis and increased iron absorption by reducing hepcidin synthesis. Prolyl hydroxylase (PHD2) is a key oxygen sensor. It uses molecular oxygen to hydroxylate HIFα. Hydroxylation allows the von Hippel-Lindau (vHL) protein to break down HIFα by ubiquitination (Fig. 2.5b). Mutations in the genes *vHL, PHD2* and *HIF2α* are rare causes of congenital polycythaemia (Chapter 15). Daprodustat,

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roxadustat and vadadustat which inhibit PDH2 and raise endogenous erythropoietin production are in clinical trials for treating the anaemia of chronic renal failure and as a result of chemotherapy for cancer.

Erythropoietin stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoiesis.

The transcription factor GATA2 is involved in initiating erythroid differentiation from pluripotential stem cells. Subsequently the transcription factors GATA1 and FOG1 are activated by erythropoietin receptor stimulation and are important in enhancing expression of erythroid-specific genes, e.g. of globin, haem biosynthetic and red cell membrane proteins, and also enhancing expression of anti-apoptotic genes and of the transferrin receptor1 (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 (Chapter 7).

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 or if a tumour-secreting erythropoietin is present, but low in severe renal disease or polycythaemia vera (Fig. 2.6).

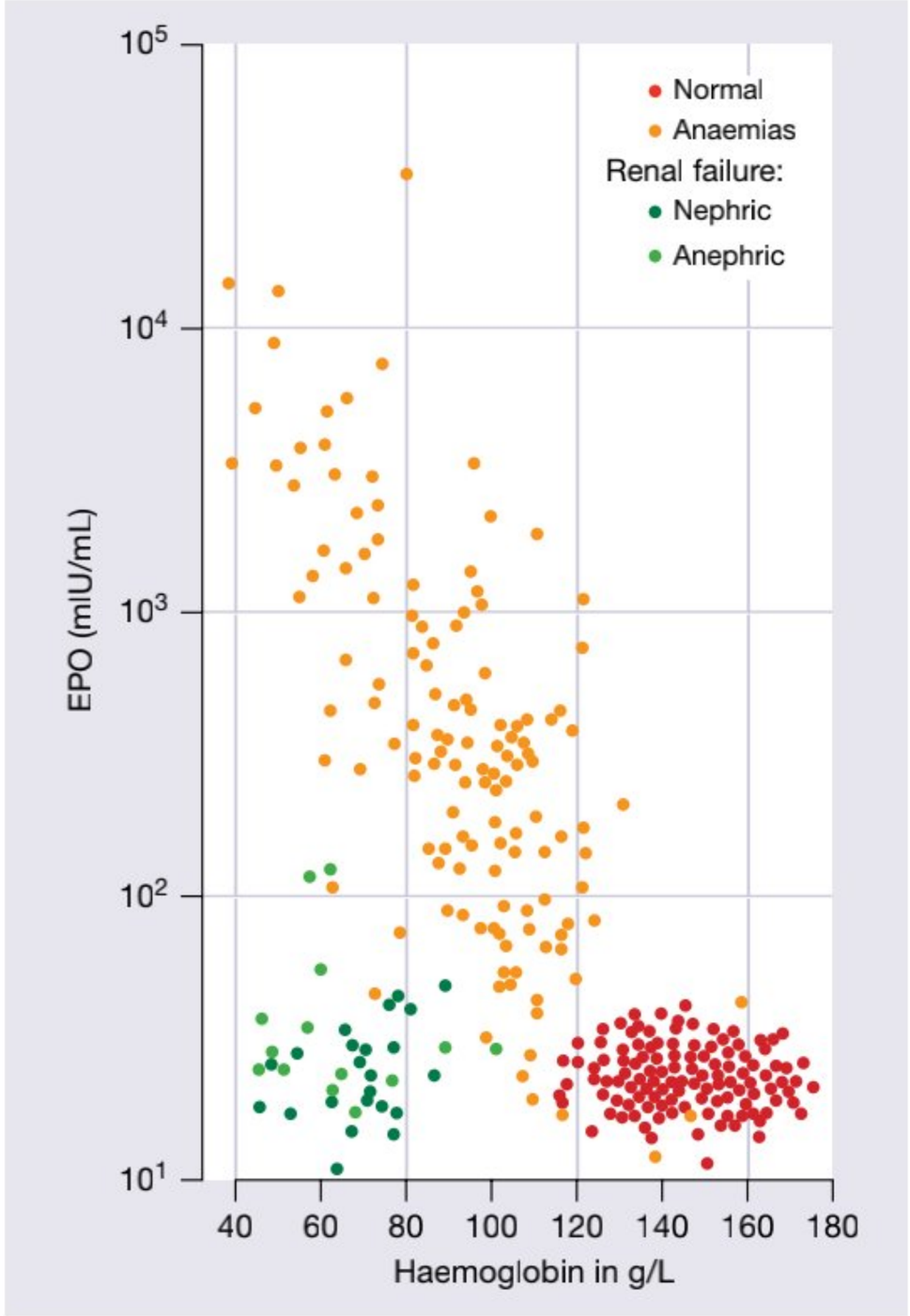


Figure 2.6 The relation between the concentration of erythropoietin (EPO) in plasma and haemoglobin concentration. Anaemias in this figure exclude conditions shown to be associated with impaired production of EPO. Source: Modified from M. Pippard *et al*. (1992) *Br. J. Haematol*. 82: 445. Reproduced 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, 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 indications 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. Erythropoietin has been associated with progression of some tumours which express EPO receptors and so with reduced survival. It is only indicated as an alternative to blood transfusion in cancer patients with symptomatic anaemia where the benefits outweigh the risks of tumour progression and of venous thrombosis. Prolyl hydroxylase inhibitors (see above) are undergoing trials for treating anaemia in cancer patients.

Table 2.2 Clinical indications (in selected subjects) for 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

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

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Haemoglobin

Haemoglobin synthesis

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 foetus and adult is discussed in Chapter 7.

Haem synthesis occurs largely in 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 synthase (ALAS) (Fig. 2.7). Pyridoxal phosphate (vitamin B_6) is a coenzyme for this reaction. The main sources of succinyl CoA are glutamine and glucose, which are converted to alpha-ketoglutarate, a succinate precursor inside the erythroid cells. Ultimately, protoporphyrin combines with iron in the ferrous (Fe^{2+}) state to form haem (Fig. 2.8). 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). An enzyme eIF2alpha kinase, also known as haem-regulated inhibitor (HRI), senses intracellular haem concentration. If this is low as in iron deficiency, HRI phosphorylates its substrate eIF2alpha which then reduces globin synthesis by inhibiting its mRNA translation.

An adjacent gene *Nprl3* shares some enhancers with the α-globin gene and so the control of expression of the two genes is coupled. *Nprl3* provides negative regulation of mTORC1, a critical controller of cellular metabolism. Nprl3 is essential for optimal erythropoiesis and for responding to fluctuating nutrient (including iron) and growth factor concentrations.

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

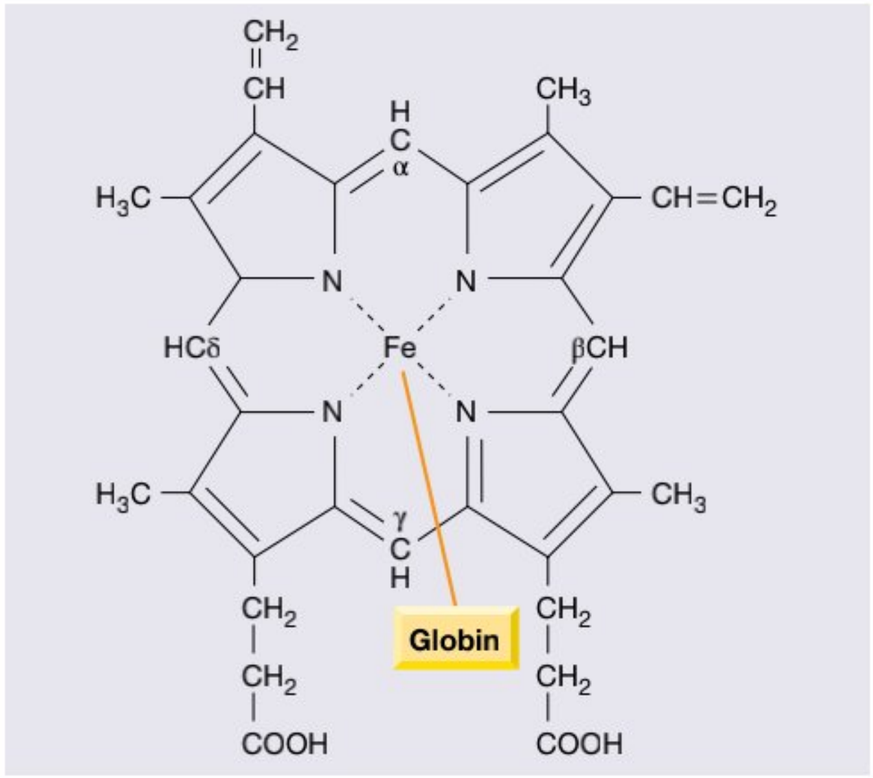


Figure 2.8 The structure of haem.

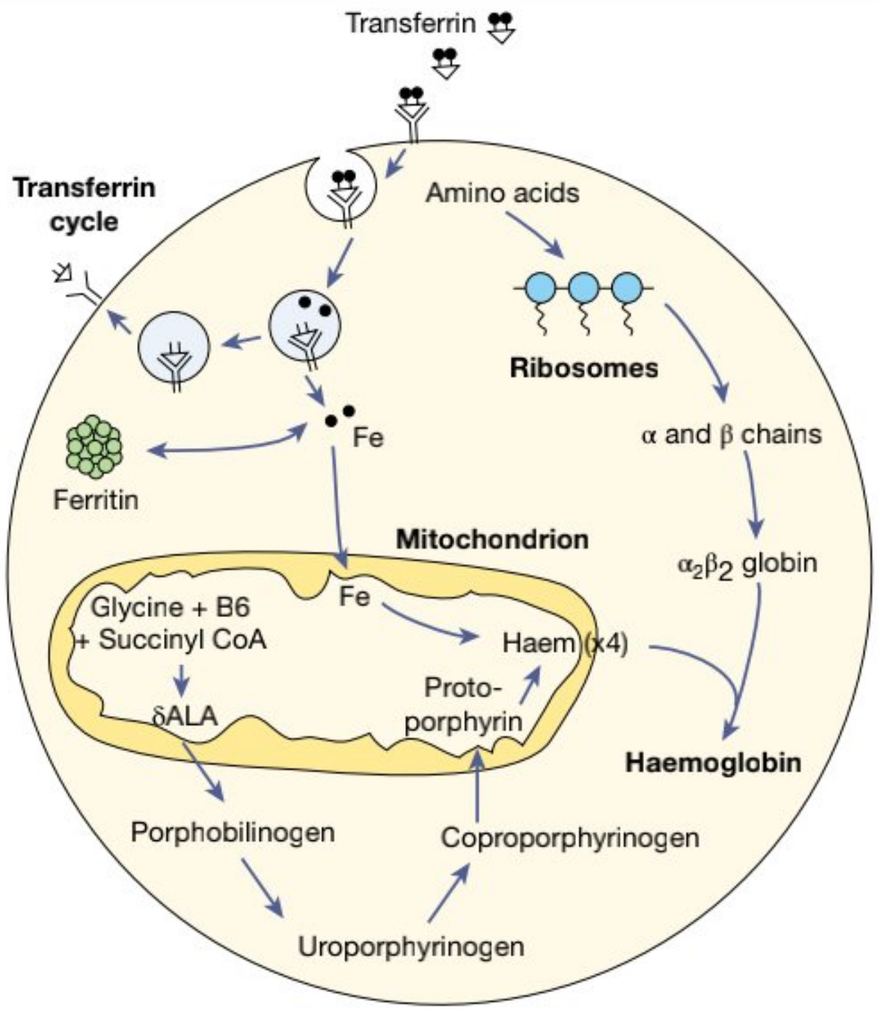


Figure 2.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main sites of protoporphyrin synthesis, iron (Fe) is supplied from circulating transferrin and globin chains are synthesized on ribosomes. δ-ALA, δ-aminolaevulinic acid; CoA, coenzyme A.

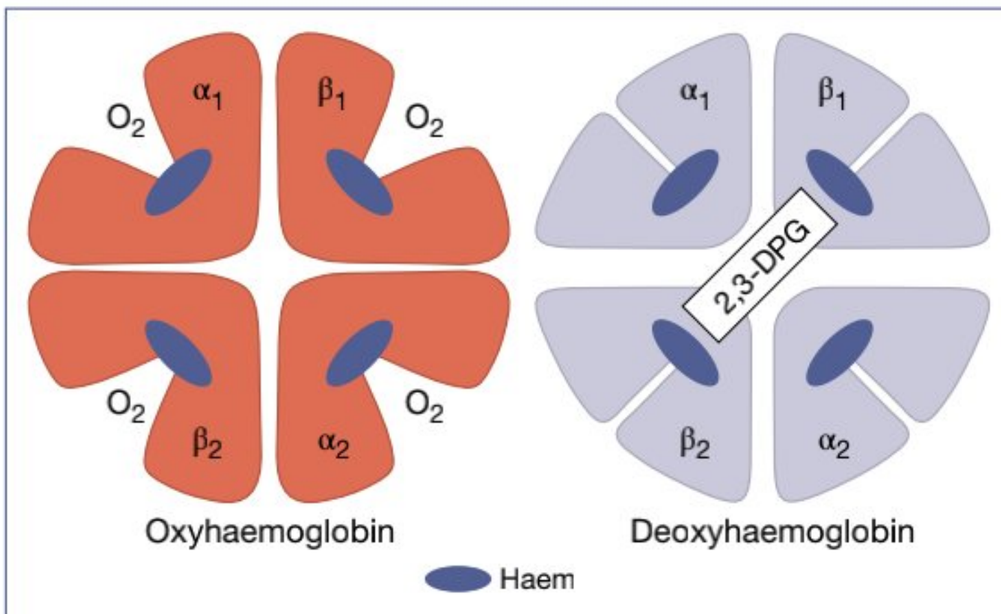


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A); 2,3-DPG, 2,3-diphosphoglycerate.

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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} (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 (the P_{50} falls), while with decreased affinity for O_2, the curve shifts to the right (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 of CO_2, and the presence of sickle haemoglobin (Hb S), shift the curve to the right (oxygen is given up more easily), whereas foetal 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.

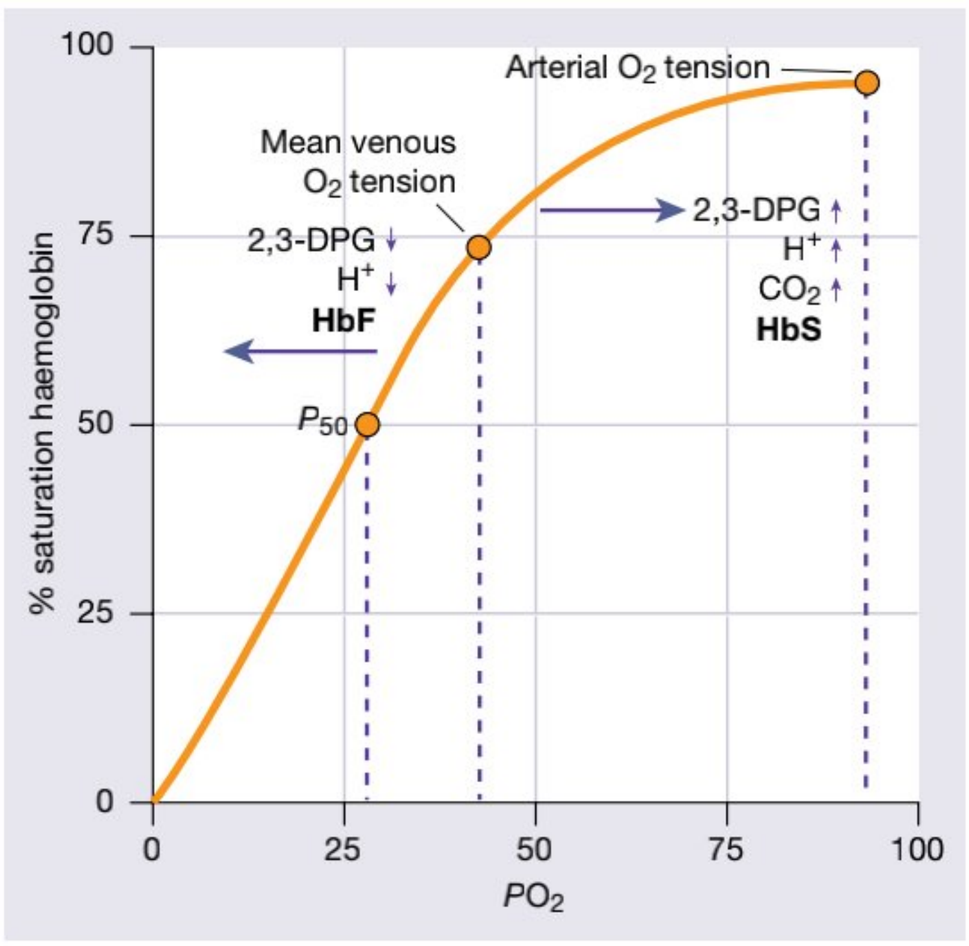


Figure 2.10 The haemoglobin oxygen (O2) dissociation curve. 2,3-DPG, 2,3-diphosphoglycerate.

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 the enzyme methaemoglobin reductase 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 pass repeatedly through the microcirculation, whose minimum diameter is 3.5 µm. It must maintain haemoglobin in a reduced (ferrous) state and 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 life span 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 generate reducing power both as nicotinamide adenine dinucleotide (NADH) by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt (Fig. 2.11, 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 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 haemoglobin containing ferrous ions. The Rapoport–Luebering shunt, or side-arm, of this pathway (Fig. 2.11) generates 2,3-DPG, important in the regulation of haemoglobin's oxygen affinity (Fig. 2.10).

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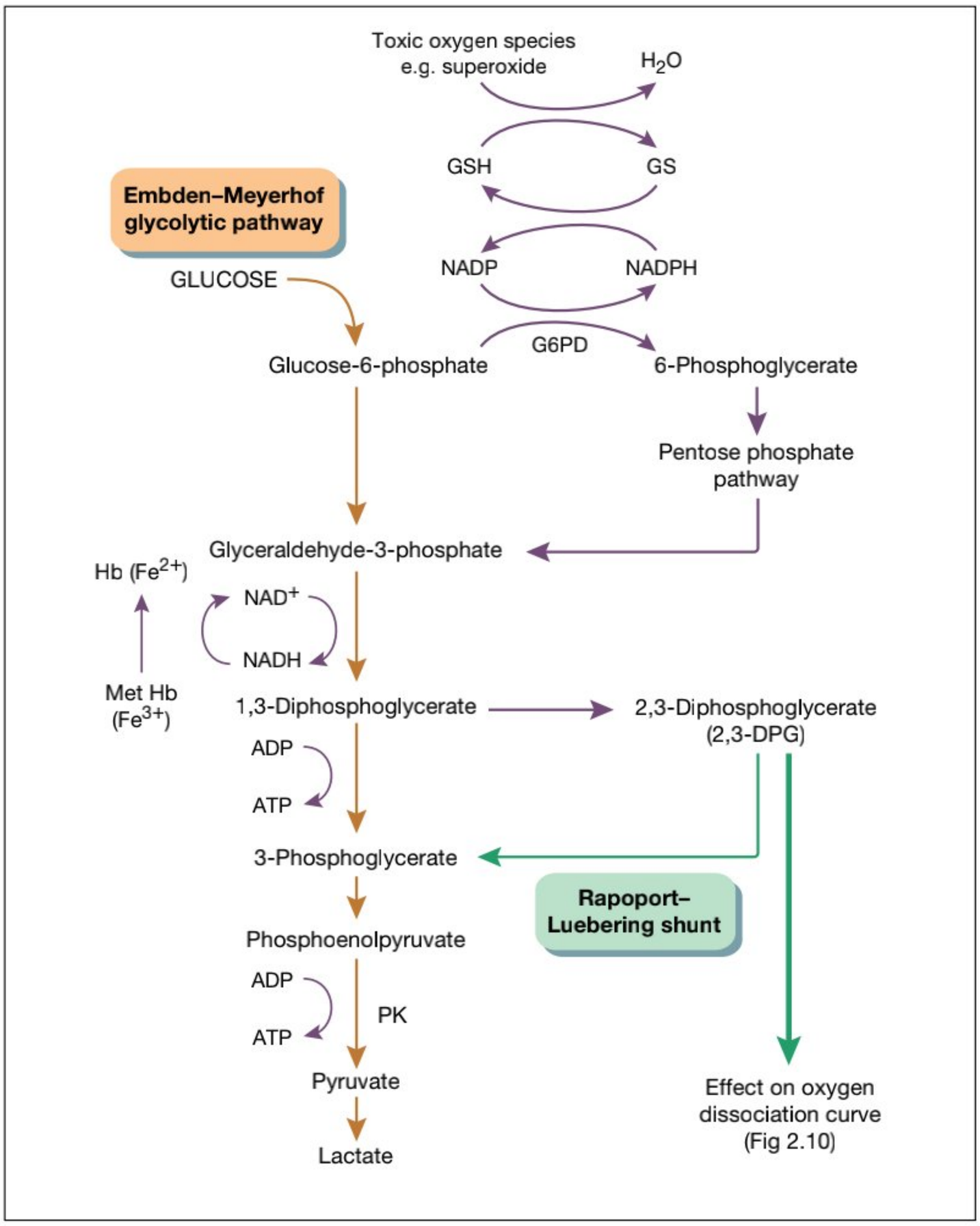


Figure 2.11 The anaerobic Embden–Meyerhof pathway generates energy as ATP and reducing power as NADH. The pentose-phosphate shunt pathway generates additional reducing power as NADPH. Further down the main pathway the Rapoport–Luebering shunt generates 2,3 DPG which effects oxygen binding and release by haemoglobin (Fig 2.10). GS, glutathione; GSH, reduced glutathione; G6PD, glucose-phosphate dehydrogenase; PK, pyruvate kinase; NAD, NADP, ADP, ATP see text.

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 a pentose-5-phosphate (Fig. 2.11, 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 in 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 (Chapter 6).

Red cell membrane

The red cell membrane comprises a lipid bilayer, membrane integral 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 integral, penetrating the lipid bilayer, or form a skeleton on the inner surface of the membrane. 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).

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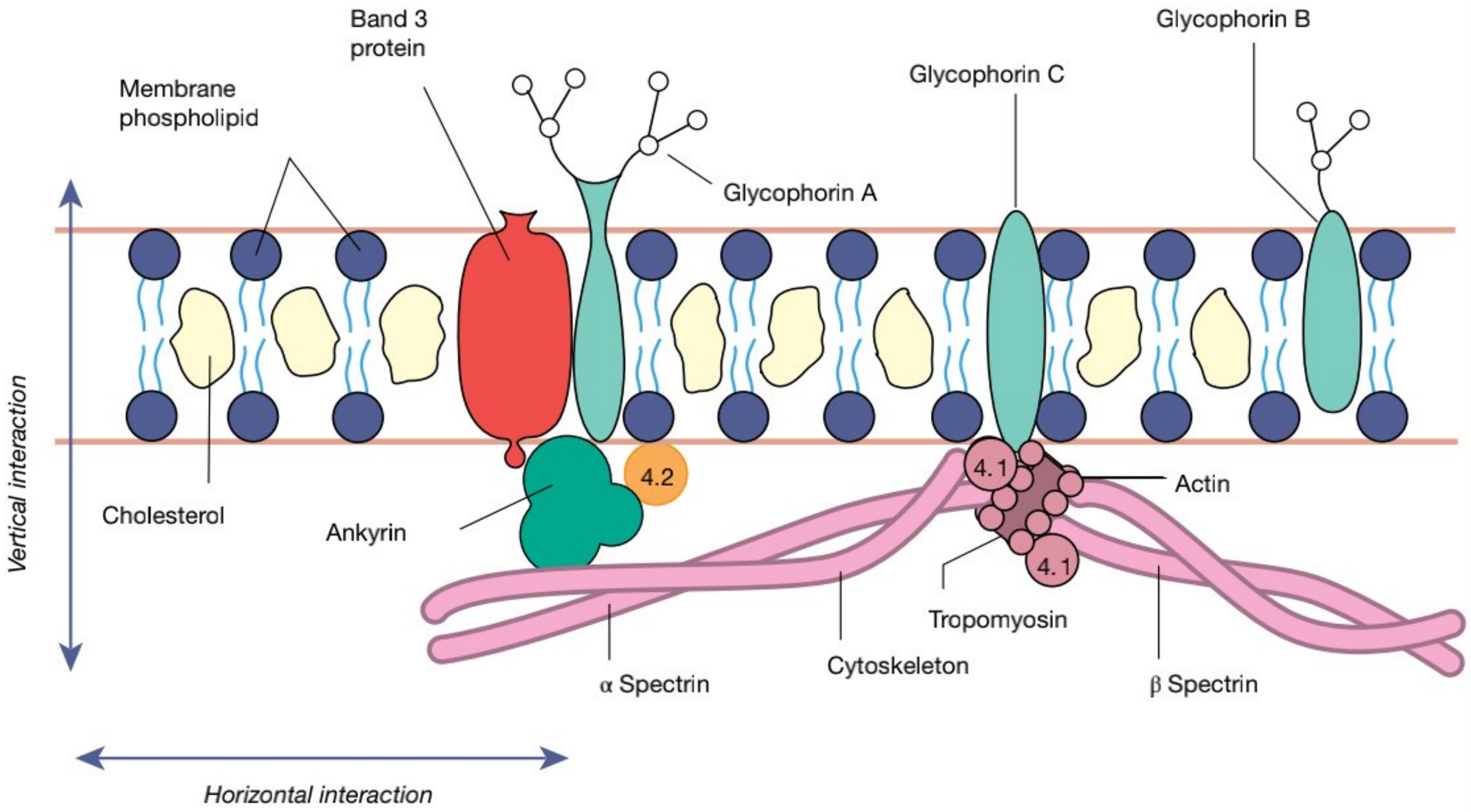


Figure 2.12 The structure of the red cell membrane. Some of the penetrating and integral proteins carry carbohydrate antigens; other antigens are attached directly to the lipid layer.

The membrane skeleton is formed by structural proteins that include α and β spectrin, ankyrin, protein 4.1 and actin. These proteins form a horizontal lattice important in maintaining the biconcave shape. Spectrin is the most abundant and consists of two chains, α and β, wound around each other to form heterodimers, which then self-associate head to head to form tetramers. These tetramers are linked at the tail end to actin and attached there to protein band 4.1. At the head end, the β spectrin chains attach to ankyrin, which connects them to band 3, the transmembrane protein that acts as an anion channel ('vertical connection'; Fig. 2.12). Protein 4.2 enhances this interaction.

Defects of the membrane proteins explain some of the abnormalities of shape of the red cell membrane, e.g. hereditary spherocytosis and elliptocytosis (Chapter 6), while alterations in lipid composition because of congenital or acquired abnormalities in plasma cholesterol or phospholipid may be associated with other membrane abnormalities (Fig. 2.16).

Anaemia

Anaemia is defined as a reduction in the haemoglobin concentration of the blood below normal for age and sex (Table 2.4). Although normal values can vary between laboratories, typical values would be less than 135 g/L in adult males and less than 115 g/L in adult females (Fig. 2.13). The World Health Organisation (WHO) defines anaemia as a haemoglobin level < 130 g/L for adult males, < 120 g/L for adult non-pregnant females and <110 g/L from the age of 6–59 months. Newborn infants have a high haemoglobin level; 140 g/L is taken as the lower limit at birth (Fig. 2.13). Anaemia in pregnancy and neonates is discussed in Chapter 34.

Alterations in total circulating plasma volume as well as in total circulating haemoglobin mass determine the haemoglobin concentration. Reduction in plasma volume as in dehydration may mask anaemia or even cause apparent (pseudo) polycythaemia (Chapter 15). Conversely, an increase in plasma volume as with splenomegaly or pregnancy may cause anaemia even with a normal total circulating red cell and haemoglobin mass.

After acute major blood loss, anaemia is not immediately apparent because the total blood volume is reduced. It takes up to a day for the plasma volume to be replaced and so for the degree of anaemia to become apparent. Regeneration of red cells and haemoglobin mass takes substantially longer. The initial clinical features of major blood loss are therefore a result of reduction in blood volume rather than of anaemia.

Global incidence

On the basis of the WHO definitions, anaemia was estimated in 2010 to occur in about 33% of the global population. Prevalence was greater in females than males at all ages and most frequent in children less than 5 years old. Anaemia was most frequent in

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Table 2.4 Normal values for blood cells and haematinics.

	Males	Females
Haemoglobin (g/L)	135.0–175.0	115.0–155.0
Red cells (erythrocytes) ($\times10^{12}$/L)	4.5–6.5	3.9–5.6
PCV (haematocrit) (%)	40–52	36–48
Mean cell volume (MCV) (fL)	80–95	
Mean cell haemoglobin (MCH) (pg)	27–34	
Reticulocyte count ($\times10^9$/L)	50–150	
White cells (leucocytes)		
Total ($\times10^9$/L)	4.0–11.0	
Neutrophils ($\times10^9$/L)	1.8–7.5 (Caucasians 1.5–7.5 (Africa and Middle-East)	
Lymphocytes ($\times10^9$/L)	1.5–3.5	
Monocytes ($\times10^9$/L)	0.2–0.8	
Eosinophils ($\times10^9$/L)	0.04–0.44	
Basophils ($\times10^9$/L)	0.01–0.1	
Platelets ($\times10^9$/L)	150–400	
Serum iron (μmol/L)	10–30	
Total iron-binding capacity (μmol/L)	40–75 (2.0–4.0 g/L as transferrin)	
Serum ferritin* (μg/L)	40–340	14–150
Serum vitamin B_{12}* (ng/L)	160–925 (20–680 pmol/L)	
Serum folate* (μg/L)	3.0–15.0 (4–30 nmol/L)	
Red cell folate** (μg/L)	160–640 (360–1460 nmol/L)	

PCV, packed cell volume.
* Normal ranges differ between laboratories.
** Normal ranges differ between different laboratories.

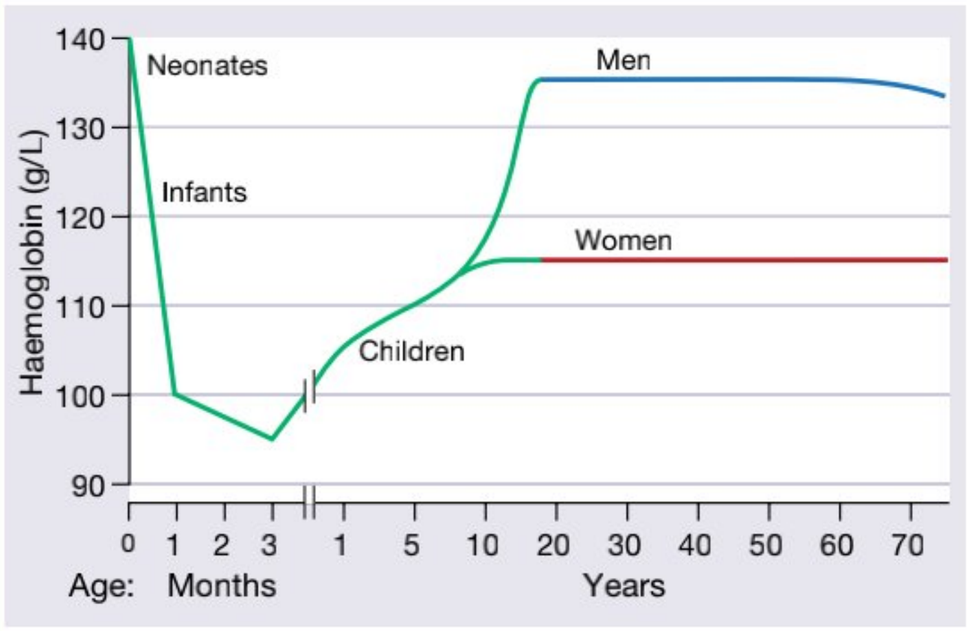


Figure 2.13 The lower limit of blood haemoglobin concentration in healthy men, women and children of various ages.

South Asia, and in Central, West and East Sub-Saharan Africa. The main causes are iron deficiency (caused by life-long poor diet combined with menstruation and/or repeated pregnancies, hookworm, schistosomiasis), the anaemia of inflammation (Chapter 3), sickle cell diseases, thalassaemia (Chapter 7), malaria (Chapter 32).

Clinical features of anaemia

The major adaptations to anaemia are in the cardiovascular system with increased cardiac stroke volume and tachycardia and in the haemoglobin O_2 dissociation curve. In some patients with quite severe anaemia, there may be no symptoms or signs, whereas others with mild anaemia may be severely incapacitated. The presence or absence of clinical features depends on:

1 ***Speed of onset*** Rapidly progressive anaemia causes more symptoms than anaemia of slow onset. This is because there is less time for adaptation in the cardiovascular system and in the O_2 dissociation curve of haemoglobin.
2 ***Severity*** Mild anaemia often produces no symptoms or signs, but these are usually present when the haemoglobin is less than 90 g/L. Even severe anaemia (haemoglobin concentration as low as 60 g/L) may produce remarkably few symptoms, when there is very gradual onset in young subjects who are otherwise healthy.
3 ***Age*** The elderly tolerate anaemia less well than the young because normal cardiovascular compensation is impaired.
4 ***Haemoglobin O_2 dissociation curve*** Anaemia, in general, is associated with a rise in 2,3-DPG in the red cells and a shift in the O_2 dissociation curve to the right, so that oxygen is given up more readily to tissues. This adaptation, which takes days to occur, is particularly marked in some anaemias that either raise 2,3-DPG directly, e.g. pyruvate kinase deficiency (Chapter 6), or that are associated with a low-affinity haemoglobin, e.g. Hb S (Fig. 2.10).

Symptoms

If the patient does have symptoms, these are usually shortness of breath, particularly on exertion, weakness, lethargy, palpitation and headaches. In older subjects, symptoms of cardiac failure, angina pectoris, intermittent claudication or confusion may be present. Visual disturbances because of retinal haemorrhages may complicate very severe anaemia, particularly of rapid onset (Fig. 2.14).

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Figure 2.14 Retinal haemorrhages in a patient with severe anaemia (haemoglobin 25 g/L) caused by severe haemorrhage.

Signs

These may be divided into general and specific. General signs include pallor of mucous membranes or nail beds, which occurs if the haemoglobin level is less than 90 g/L (Fig. 2.15). Conversely, skin colour is not a reliable sign. A hyperdynamic circulation may be present with tachycardia, a bounding pulse, cardiomegaly and a systolic flow murmur. Particularly in the elderly, features of congestive heart failure may be present.

Specific signs are associated with particular types of anaemia, e.g. koilonychia (spoon nails) with iron deficiency, jaundice with haemolytic or megaloblastic anaemias, leg ulcers with sickle cell and other haemolytic anaemias, or bone deformities with thalassaemia major.

The association of features of anaemia with excess infections or spontaneous bruising suggests that neutropenia or thrombocytopenia may be present, possibly as a result of bone marrow failure.

Classification of anaemia

Red cell indices

The most useful classification is based on the red cell indices, especially MCV. This divides the anaemia into microcytic, normocytic and macrocytic (Table 2.5). As well as suggesting the nature of the primary defect, this classification may also indicate an underlying abnormality before overt anaemia has developed.

In two common physiological situations, the mean corpuscular volume (MCV) may be outside the normal adult range. In the newborn for a few weeks, the MCV is high, but in infancy it is low, e.g. 70 fL at 1 year of age and rises slowly throughout childhood to the normal adult range. In normal pregnancy there is a slight rise in MCV, even in the absence of other causes of macrocytosis, e.g. folate deficiency.

Other laboratory findings

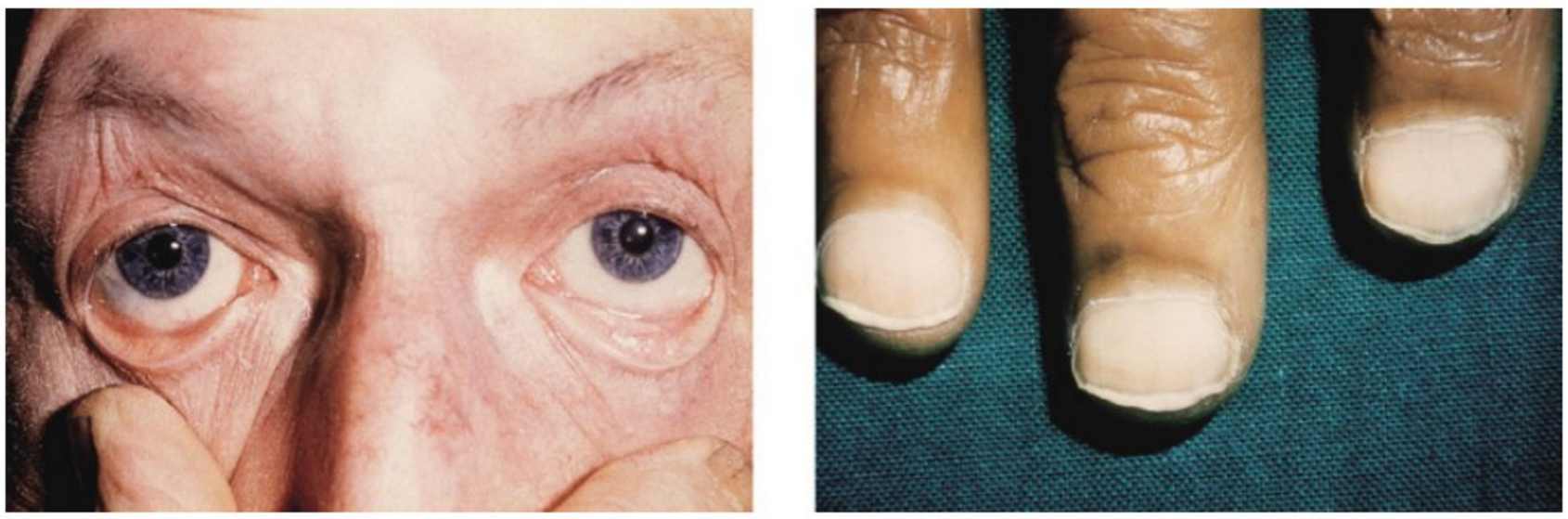
Although the red cell indices will indicate the type of anaemia, further useful information can be obtained from the initial blood sample.

Leucocyte and platelet counts

Measurement of these helps to distinguish 'pure' anaemia from 'pancytopenia' (subnormal levels of red cells, neutrophils and platelets), which suggests a more general marrow defect or destruction of cells, e.g. hypersplenism. In anaemias caused by haemolysis or haemorrhage, the neutrophil and platelet counts are often raised; in infections and leukaemias, the leucocyte count is also often raised, and there may be abnormal leucocytes or neutrophil precursors present.

Reticulocyte count

The normal percentage is 0.5–2.5%, and the absolute count $50–150 \times 10^9$/L (Table 2.4). This should rise in anaemia because of erythropoietin increase and be higher the more



(a) (b)

Figure 2.15 Pallor of the conjunctival mucosa **(a)** and of the nail bed **(b)** in two patients with severe anaemia (haemoglobin 60 g/L).

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Table 2.5 Classification of anaemia.

Microcytic, hypochromic	Normocytic, normochromic	Macrocytic
MCV <80 fL	MCV 80–95 fL	MCV >95 fL
MCH <27 pg	MCH ≥27 pg	Megaloblastic: vitamin B_{12} or folate deficiency. Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anaemia, etc. (Table 5.10)
Iron deficiency	Many haemolytic anaemias	
Thalassaemia Anaemia of chronic disease (some cases) Lead poisoning Sideroblastic anaemia (some cases)	Anaemia of chronic disease (some cases)	
	After acute blood loss	
	Renal disease	
	Mixed deficiencies	
	Bone marrow failure e.g. post-chemotherapy, infiltration by carcinoma, etc.	

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

severe the anaemia. This is particularly so when there has been time for erythroid hyperplasia to develop in the marrow as in chronic haemolysis. After an acute major haemorrhage, there is an erythropoietin response in 6 hours, and the reticulocyte count rises within 2–3 days, reaches a maximum in 6–10 days and remains raised until the haemoglobin returns to the normal level. If the reticulocyte count is not raised in an anaemic patient, this suggests impaired marrow function, lack of erythropoietin (renal disease) or lack of erythropoietin stimulus (Table 2.6).

Table 2.6 Factors impairing the normal reticulocyte response to anaemia.

Marrow diseases, e.g. hypoplasia, infiltration by carcinoma, lymphoma, myeloma, acute leukaemia, tuberculosis
Deficiency of iron, vitamin B_{12} or folate
Lack of erythropoietin, e.g. renal disease
Reduced tissue O_2 consumption, e.g. myxoedema, protein deficiency
Ineffective erythropoiesis, e.g. thalassaemia major, megaloblastic anaemia, myelodysplasia, myelofibrosis
Chronic inflammatory or malignant disease

Blood film

It is important to examine the blood film in all cases of anaemia. Abnormal red cell morphology (Fig. 2.16) or red cell inclusions (Fig. 2.17) may suggest a particular diagnosis. During the blood film examination, white cell abnormalities are sought, platelet number and morphology assessed and the presence of abnormal cells, e.g. normoblasts, granulocyte precursors or blast cells, is noted.

Bone marrow examination

This is needed when the cause of anaemia or other abnormality of the blood cells cannot be diagnosed from the blood count, film and other blood tests alone. It may be performed by aspiration or trephine biopsy (Fig. 2.18). For bone marrow aspiration, a needle is inserted into the marrow cavity and a liquid sample of marrow is sucked into a syringe. This sample is then spread on a slide for microscopy and stained by the usual Romanowsky technique. The detail of the developing cells can be examined, e.g. normoblastic or megaloblastic and the proportion of the different cell lines assessed (myeloid: erythroid ratio), the proportion of granulocyte precursors to red cell precursors in the bone marrow, normally 2.5 : 1 to 12 : 1. The presence of cells foreign to the marrow, e.g. secondary carcinoma, can also be observed. The cellularity of the marrow can be viewed provided fragments are obtained. An iron stain is performed routinely so that the amount of iron in reticuloendothelial stores (macrophages) and as fine

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	Red cell abnormality	Causes		Red cell abnormality	Causes
	Normal			Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
	Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia		Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
	Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy		Elliptocyte	Hereditary elliptocytosis
	Stomatocyte	Liver disease, alcoholism		Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
	Pencil cell	Iron deficiency		Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
	Echinocyte	Liver disease, post-splenectomy. storage artefact		Sickle cell	Sickle cell anaemia
	Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure		Microcyte	Iron deficiency, haemoglobinopathy

Figure 2.16 Some of the more frequent variations in size (anisocytosis) and shape (poikilocytosis) that may be found in different anaemias. DIC, disseminated intravascular coagulopathy; G6PD, glucose-6-phosphate dehydrogenase; HUS, haemolytic-uraemic syndrome; TTP, thrombotic thrombocytopenic purpura.

granules ('siderotic' granules) in the developing erythroblasts can be assessed (Fig. 3.10).

An aspirate sample may also be used for a number of other specialized investigations (Table 2.7).

A trephine biopsy provides a solid core of bone including marrow and is examined as a histological specimen after fixation in formalin, decalcification and sectioning. Usually immunohistology is performed, depending on the diagnosis suspected (Chapter 11). A trephine biopsy specimen is less valuable than aspirate when individual cell detail is to be examined, but provides a panoramic view of the marrow, from which overall marrow architecture, cellularity and presence of fibrosis or abnormal infiltrates can, with immunohistology if needed, be reliably determined.

Ineffective erythropoiesis

Erythropoiesis is not entirely efficient since approximately 10–15% of developing erythroblasts die within the marrow without producing mature cells. This is termed ineffective erythropoiesis and it is substantially increased in a number of chronic anaemias (Fig. 2.19). The serum unconjugated bilirubin (derived from breaking down haemoglobin) and lactate dehydrogenase (LDH, derived from breaking down cells) are usually raised when ineffective erythropoiesis is marked. The reticulocyte count is low in relation to the degree of anaemia and to the proportion of erythroblasts in the marrow.

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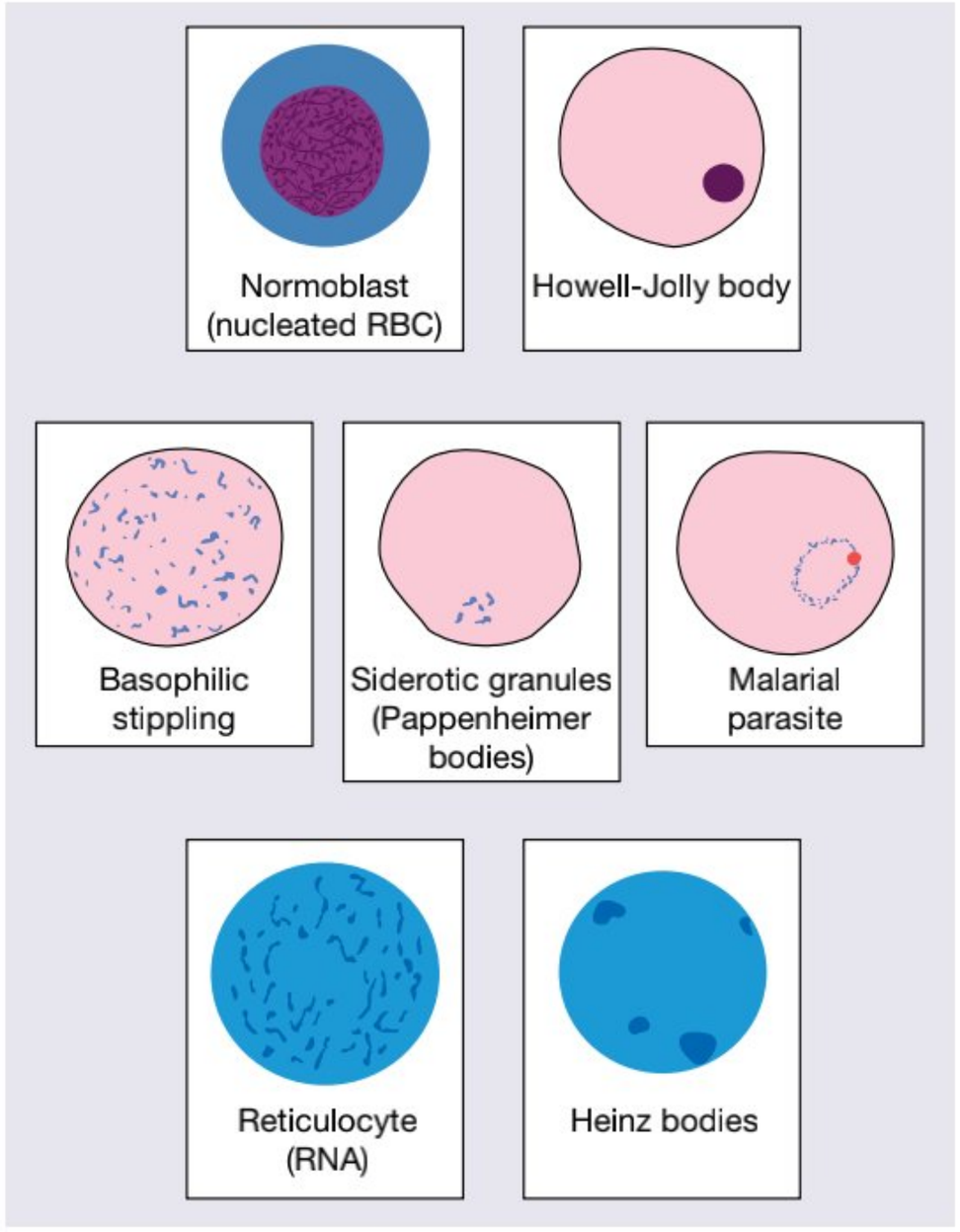


Figure 2.17 Red blood cell (RBC) inclusions which may be seen in the peripheral blood film in various conditions. The reticulocyte RNA and Heinz bodies are only demonstrated by supravital staining, e.g., with new methylene blue. Heinz bodies are oxidized denatured haemoglobin. Siderotic granules (Pappenheimer bodies) contain iron. They are purple on conventional staining, but blue with Perls' stain. The Howell–Jolly body is a DNA remnant. Basophilic stippling is denatured RNA.

Assessment of erythropoiesis

Total erythropoiesis and the amount of erythropoiesis that is effective in producing circulating red cells can be assessed by examining the bone marrow, haemoglobin level and reticulocyte count.

Total erythropoiesis is assessed from the marrow cellularity and the myeloid: erythroid ratio. This ratio falls and may be reversed when total erythropoiesis is selectively increased.

Effective erythropoiesis is assessed by the reticulocyte count. This is raised in proportion to the degree of anaemia when erythropoiesis is effective, but is low when there is ineffective erythropoiesis or an abnormality preventing normal marrow response (Table 2.6).

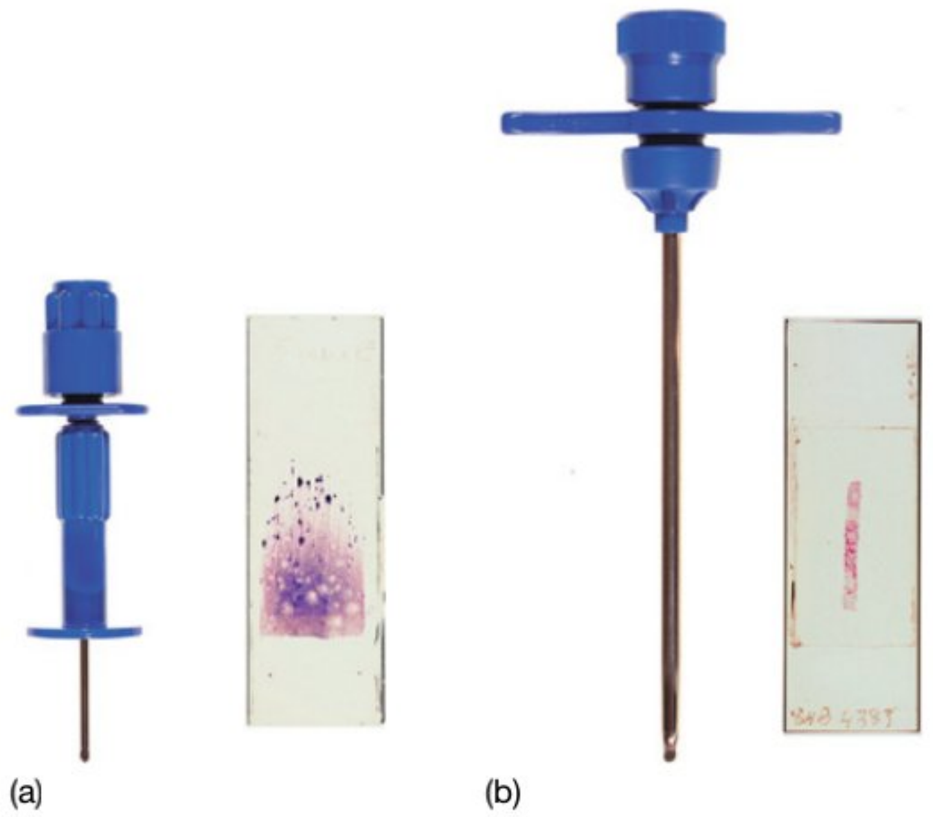


Figure 2.18 **(a)** The bone marrow aspiration needle and a smear made from a bone marrow aspirate. **(b)** The bone marrow trephine biopsy needle and a normal trephine biopsy section.

Table 2.7 Indications for bone marrow aspiration and trephine biopsy.

	Aspiration	Trephine biopsy
Site	Posterior iliac crest (sternum if obese; tibia in infants)	Posterior iliac crest
Stains	Romanowsky; Perls' reaction (for iron)	Haematoxylin and eosin; reticulin (silver stain)
Result available	1–2 h	1–7 d (according to decalcification method)
Indications	Investigation of unexplained anaemia, neutropenia, thrombocytopenia, suspicion of leukaemia, myeloproliferative disorders, myelodysplasia, aplastic anaemia, lymphoma, myeloma, amyloid, secondary carcinoma, cases of splenomegaly or pyrexia of undetermined cause	
Special tests	Flow cytometry, cytogenetics, FISH and molecular tests including DNA or RNA analysis for gene abnormalities. Consider microbiological culture, cytochemical markers and progenitor cell culture	Immunohistology

FISH, fluorescence *in situ* hybridization.

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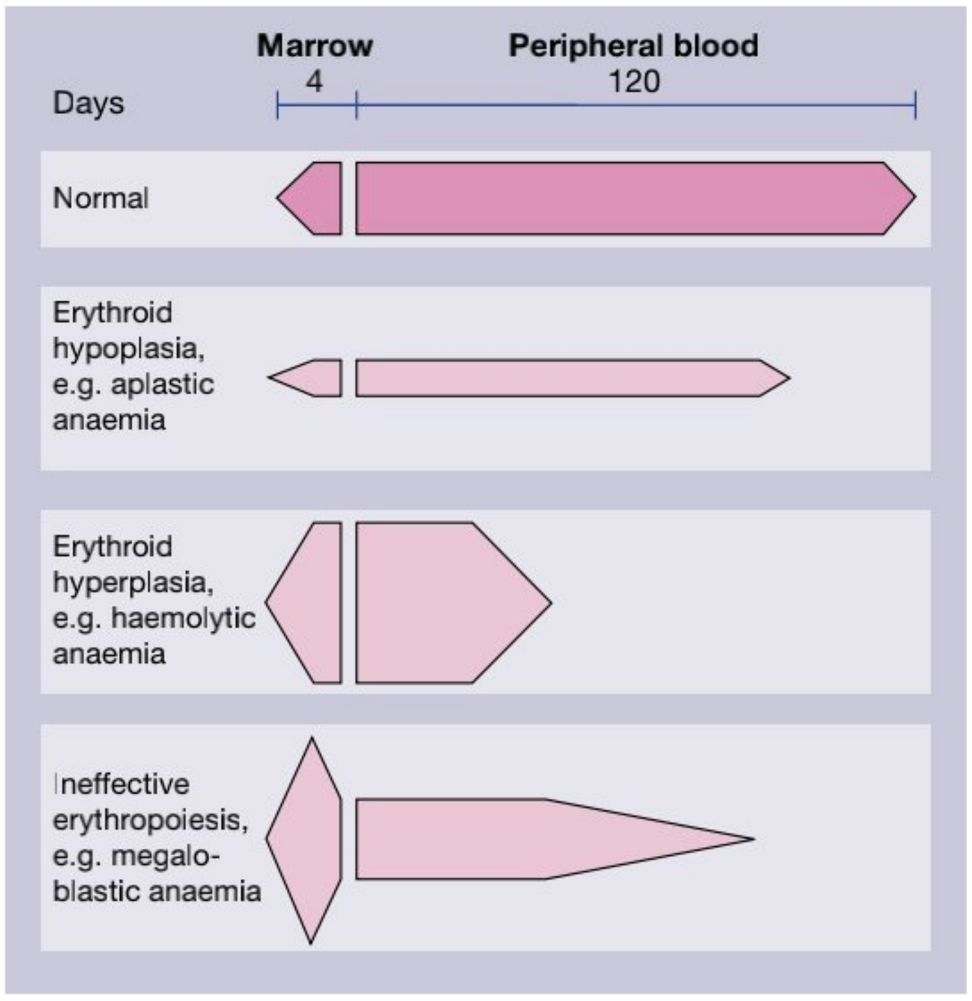


Figure 2.19 The relative proportions of marrow erythroblastic activity, circulating red cell mass and red cell lifespan in normal subjects and in three types of anaemia.

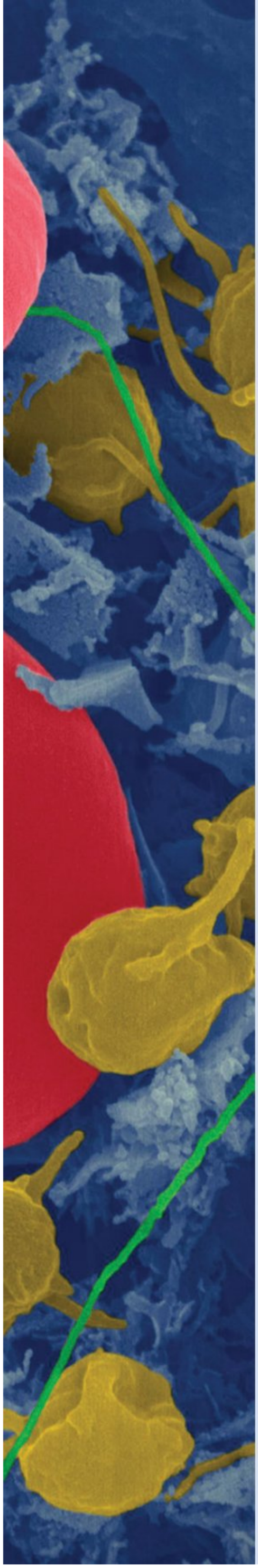
SUMMARY

- Erythropoiesis (red cell production) is regulated by erythropoietin, which is secreted by the kidney in response to hypoxia. Erythropoiesis occurs from mixed progenitor cells through a series of nucleated red cell precursors (normoblasts) to a reticulocyte stage, containing RNA but not DNA.
- Various short- or long-acting preparations of erythropoietin are used clinically to treat anaemia in renal failure and other diseases.
- Haemoglobin is the main protein in red cells. It consists of four polypeptide (globin) chains, in adults dominantly 2α and 2β, each containing an iron atom bound to protoporphyrin to form haem.
- The red cell has two biochemical pathways for metabolizing glucose, the Embden–Meyerhof pathway, which generates ATP, needed for maintenance of red cell shape and flexibility, and NADH, which prevents oxidation of haemoglobin; and the hexose monophosphate pathway, which generates NADPH, important for maintaining glutathione, which protects haemoglobin and proteins in the red cell membrane from oxidation.
- The Luebering–Rapoport shunt, or side arm, of the Embden–Meyerhof pathway generates 2,3-DPG, important in the regulation of haemoglobin's oxygen affinity
- The red cell membrane consists of a lipid bilayer, proteins which form a membrane skeleton and are integral to the membrane, and carbohydrate surface antigens.
- Anaemia is defined as a haemoglobin level in blood below the normal level for age and sex. It is classified according to the size of the red cells (MCV) into macrocytic, normocytic and microcytic. The reticulocyte count, morphology of the red cells and changes in the white cell and/or platelet count also help in the diagnosis of the cause of anaemia.
- The general clinical features of anaemia include fatigue, headaches, shortness of breath on exertion, pallor of mucous membranes and tachycardia.
- Other features relate to particular types of anaemia, e.g. jaundice, glossitis, leg ulcers, bone changes.
- Bone marrow examination by aspiration or trephine biopsy may be important in the investigation of anaemia as well as of many other haematological diseases. Special tests, e.g. immunology by flow cytometry or immunohistology, cytogenetics or molecular genetics, can be performed on the cells or core of bone obtained.



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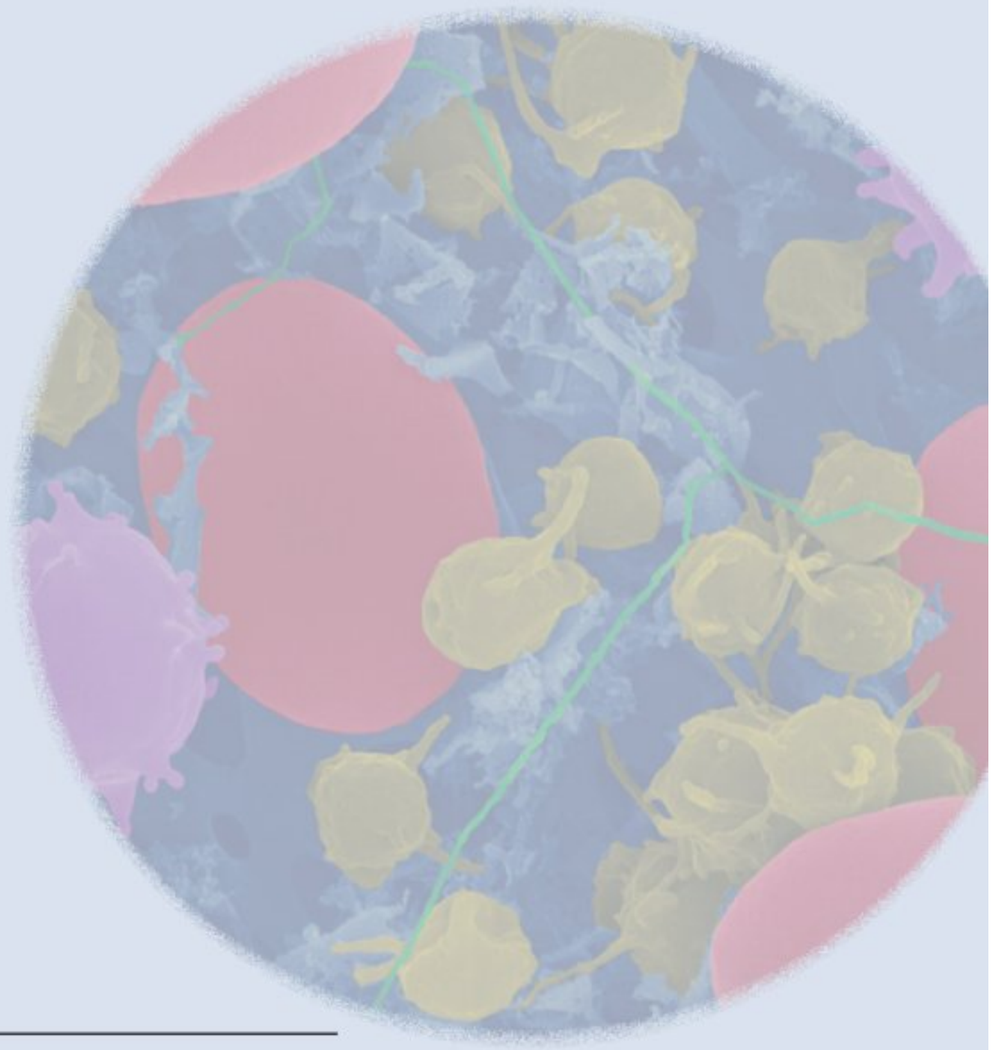


CHAPTER 3

Hypochromic anaemias

Key topics

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Iron is one of the most common elements in the Earth's crust, yet iron deficiency is the most common cause of anaemia, affecting about 500 million people worldwide. It is particularly frequent in low-income populations, such as in sub-Saharan Africa or South Asia, where the diet is frequently of poor quality and parasites, e.g. hookworm or schistosomiasis, which cause iron loss due to haemorrhage, may be present. Moreover, the body has limited ability to absorb iron. **Iron deficiency is the major cause of a microcytic, hypochromic anaemia, in which the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are both reduced and the blood film shows small (microcytic) and pale (hypochromic) red cells**. This appearance is caused by a defect in haemoglobin synthesis. The major differential diagnosis of a microcytic, hypochromic anaemia is between iron deficiency, the anaemia of chronic disease, which are both dealt with in this chapter, and thalassaemia, which is considered in Chapter 7 (Fig. 3.1).

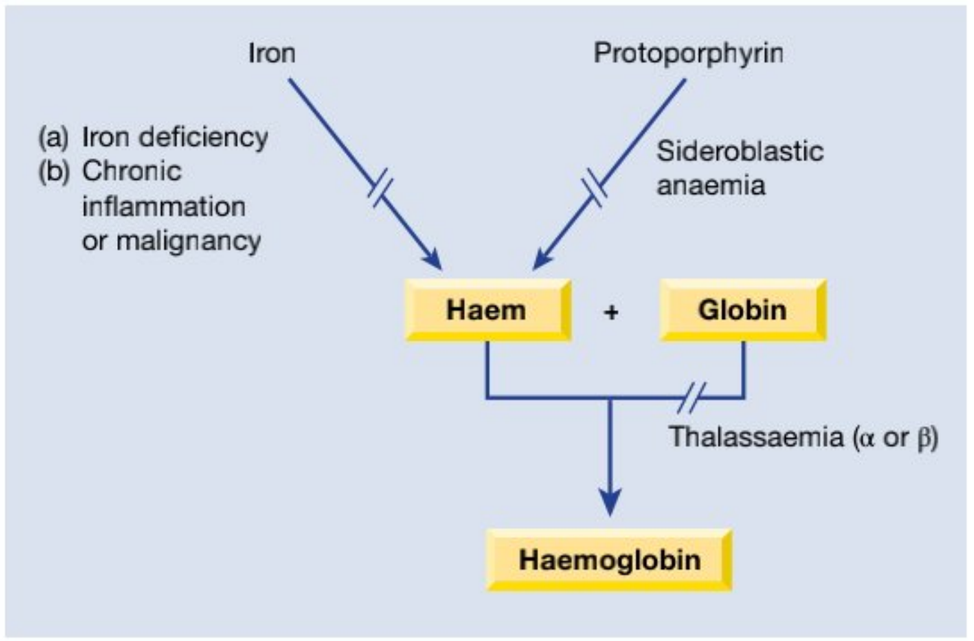


Figure 3.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic disease), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α- or β-thalassaemia). Lead also inhibits haem and globin synthesis.

Nutritional and metabolic aspects of iron

Body iron distribution and transport

The transport and storage of iron are largely mediated by three proteins: transferrin, transferrin receptor 1 (TFR1) and ferritin.

Each transferrin molecule can contain up to two atoms of iron in the ferric form. Transferrin delivers iron to tissues that have transferrin receptors (TFR1s), especially erythroblasts in the bone marrow, which incorporate the iron into haemoglobin (Figs. 2.7 and 3.2). The transferrin is then reutilized. At the end of their life, red cells are broken down in the macrophages of the reticuloendothelial system and the iron released from haemoglobin enters the plasma and provides most of the iron attached to transferrin. Only a small proportion of plasma transferrin iron comes from dietary iron, absorbed each day through the duodenum. Iron in excess of that needed for haemoglobin synthesis is also released from erythroblasts and erythrocytes to plasma transferrin. There is a diurnal variation in serum iron, highest in the mornings or noon and then falling to lowest levels in the evening.

Some iron is stored in the macrophages as **ferritin and haemosiderin**, the amount varying widely according to overall body iron status. **Ferritin** is a water-soluble protein–iron complex. It is made up of an outer protein shell, apoferritin, consisting of 22 subunits and an iron–phosphate–hydroxide core. It contains up to 20% of its weight as iron and is not visible by light microscopy. Specialized intracellular carrier proteins transfer iron to ferritin and from ferritin to phagolysosomes for its degradation and release of its iron.

Haemosiderin is an insoluble protein–iron complex of varying composition containing approximately 37% iron by weight. It is derived from partial lysosomal digestion of ferritin molecules and is visible in macrophages and other cells by light microscopy after staining by Perls' (Prussian blue) reaction (Fig. 3.10). Iron in ferritin and haemosiderin is in the ferric form. It is mobilized after reduction to the ferrous form. A copper-containing enzyme caeruloplasmin catalyses oxidation of the iron to the ferric form for binding to plasma transferrin.

Most body iron is in haemoglobin. Iron is also present in muscles as myoglobin and in most body cells in iron-containing enzymes, e.g. cytochromes or catalase (Table 3.1). This tissue iron

Table 3.1 The distribution of body iron.

Amount of iron in average adult	Male (g)	Female (g)	Percentage of total
Haemoglobin	2.4	1.7	65
Ferritin and haemosiderin	1.0 (0.3–1.5)	0.3 (0–1.0)	30
Myoglobin	0.15	0.12	3.5
Haem enzymes, e.g., cytochromes, catalase, peroxidases, flavoproteins	0.02	0.015	0.5
Transferrin-bound iron	0.004	0.003	0.1

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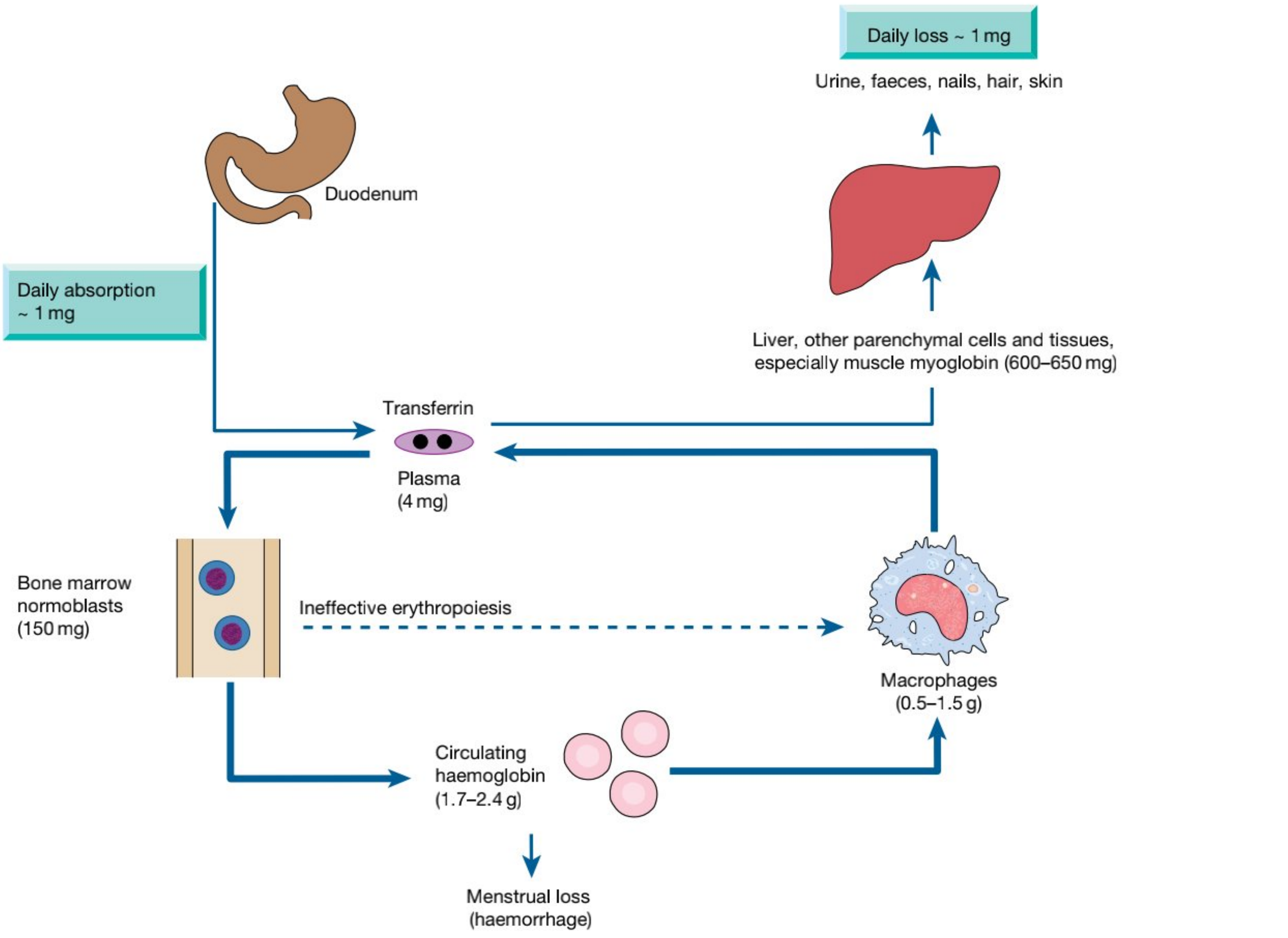


Figure 3.2 Daily iron cycle. Most of the iron in the body is contained in circulating haemoglobin and is reutilized for haemoglobin synthesis after the red cells die. Iron is transferred from macrophages to plasma transferrin and so to bone marrow erythroblasts. Iron absorption is normally just sufficient to make up for iron loss. The dashed line indicates ineffective erythropoiesis.

is less likely to become depleted than haemosiderin, ferritin and haemoglobin in states of iron deficiency, but some reduction of tissue haem-containing enzymes may also occur.

Regulation of ferritin and transferrin receptor 1 synthesis

The levels of ferritin, TFR1, δ-aminolaevulinic acid synthase (ALAS) and also of the divalent metal transporter 1 (DMT1), important in iron absorption, are linked to iron status, so that iron overload causes a rise in tissue ferritin and a fall in TFR1 and DMT1, whereas in iron deficiency ferritin and ALAS are low and TFR1 and DMT1 increased. This linkage arises through the binding of an iron regulatory protein (IRP) to iron response elements (IREs) on the ferritin, TFR1, ALAS and DMT1 mRNA molecules. Iron deficiency increases the ability of IRP to bind to the IREs, whereas iron overload reduces the binding. The site of IRP binding to the IREs determines whether the amount of the individual mRNAs and so protein produced is increased or decreased (Fig. 3.3). Upstream binding reduces translation, whereas downstream binding stabilizes the mRNA, increasing translation and so protein synthesis.

When plasma iron is raised and transferrin is saturated, the amount of iron transferred to parenchymal cells e.g. those of the liver, endocrine organs and heart is increased and this is the basis of the pathological changes associated with iron loading conditions. There may also be free iron in plasma (non-transferrin bound iron) which is toxic to different organs (Chapter 4).

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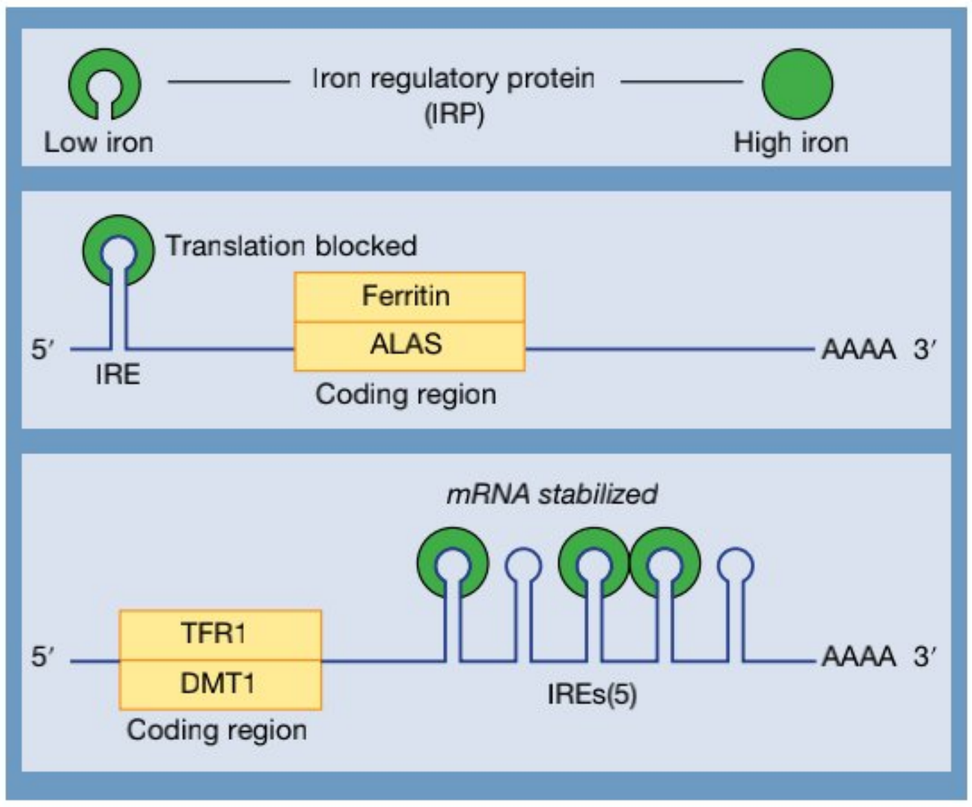


Figure 3.3 Regulation of transferrin receptor 1 (TFR1), δ-aminolaevulinic acid synthase (ALA-S), divalent metal transporter 1 (DMT1) and ferritin expression by iron regulatory protein (IRP) sensing of intracellular iron levels. IRPs are able to bind to stem-loop structures called iron response elements (IREs). IRP binding to the IRE within the 3′ untranslated region of TFR1 and DMT1 leads to stabilization of the mRNA and increased protein synthesis, whereas IRP binding to the IRE within the 5′ untranslated region of ferritin and ALA-S mRNA reduces translation. IRPs can exist in two states: at times of high iron levels the IRP binds iron and exhibits a reduced affinity for the IREs, whereas when iron levels are low the binding of IRPs to IREs is increased. In this way, synthesis of TfR, ALAS, DMT1 and ferritin is coordinated to physiological requirements.

Hepcidin

Hepcidin is a 25 amino acid polypeptide produced by liver cells. It is the major hormonal regulator of iron homeostasis (Fig. 3.4a). It inhibits iron release from macrophages, from intestinal epithelial cells and from other cells by its interaction with the transmembrane iron exporter, ferroportin. It accelerates degradation of ferroportin protein by lysosomes and so directly inhibits iron export from ferroportin. Raised hepcidin levels therefore profoundly affect iron metabolism by reducing its absorption and its release from macrophages and hepatocytes.

Control of hepcidin expression

The synthesis of hepcidin in the liver is stimulated or inhibited by several factors. These include iron status, plasma cytokines, erythropoiesis and hypoxia (Fig. 3.4a). Control of hepcidin synthesis and so of iron status is best considered by comparing the control in iron overload when, except in genetic haemochromatosis, plasma hepcidin levels are high with that in iron deficiency when plasma hepcidin levels are low (Fig. 3.4c).

In **iron overload**, diferric transferrin stimulates liver sinusoidal cells to secrete bone morphogenetic proteins (BMPs) (Fig. 3.4b). Among them BMP2 controls basal hepcidin levels while BMP6 is especially increased in iron overload. The BMPs bind to their receptors (BMPRs) on the hepatic cell membrane. Diferric transferrin also binds to and stabilizes the transferrin receptor 2 (TFR2) on the cell membrane. Binding of BMPs to BMPRs results in the formation of a signalling complex consisting of BMPRs and three proteins TFR2, hemojuvelin (HJV) and HFE. This complex stimulates hepcidin synthesis via the signalling proteins SMADs, which transfer the message to the cell nucleus. Diferric transferrin also binds to TFR1 to provide the cell with iron.

In **iron deficiency** there is little if any circulating diferric transferrin. This results in reduced synthesis of BMPs by the liver sinusoidal cells (Fig. 3.4c). Also in the absence of binding to diferric transferrin, TFR2 is degraded or shed from the cell membrane and HFE is deviated to bind to the unoccupied TFR1. Iron deficiency also stimulates the protease matriptase 2 (TMPRSS6) to cleave HJV from the cell surface. The result of all these reactions is failure to form the necessary complex to signal for hepcidin synthesis. Finally, iron deficiency stimulates a histone deacetylase which acts on the hepcidin locus to suppress the transcription of hepcidin.

Erythroblasts secrete **erythroferrone** and probably other proteins, which suppress BMP-mediated signalling for hepcidin secretion (Fig. 3.4a). This results in low plasma hepcidin levels in conditions with increased numbers of early erythroblasts in the marrow, e.g. conditions of ineffective erythropoiesis, such as thalassaemia major, so iron absorption is inappropriately increased despite the presence of iron overload due to transfusions. Hypoxia also suppresses hepcidin synthesis, whereas in inflammation interleukin 6 (IL-6) and other cytokines increase hepcidin synthesis (Fig. 3.4a). This results in lowering of plasma iron and helps to protect against infection by depriving bacteria of non-transferrin-bound iron in plasma.

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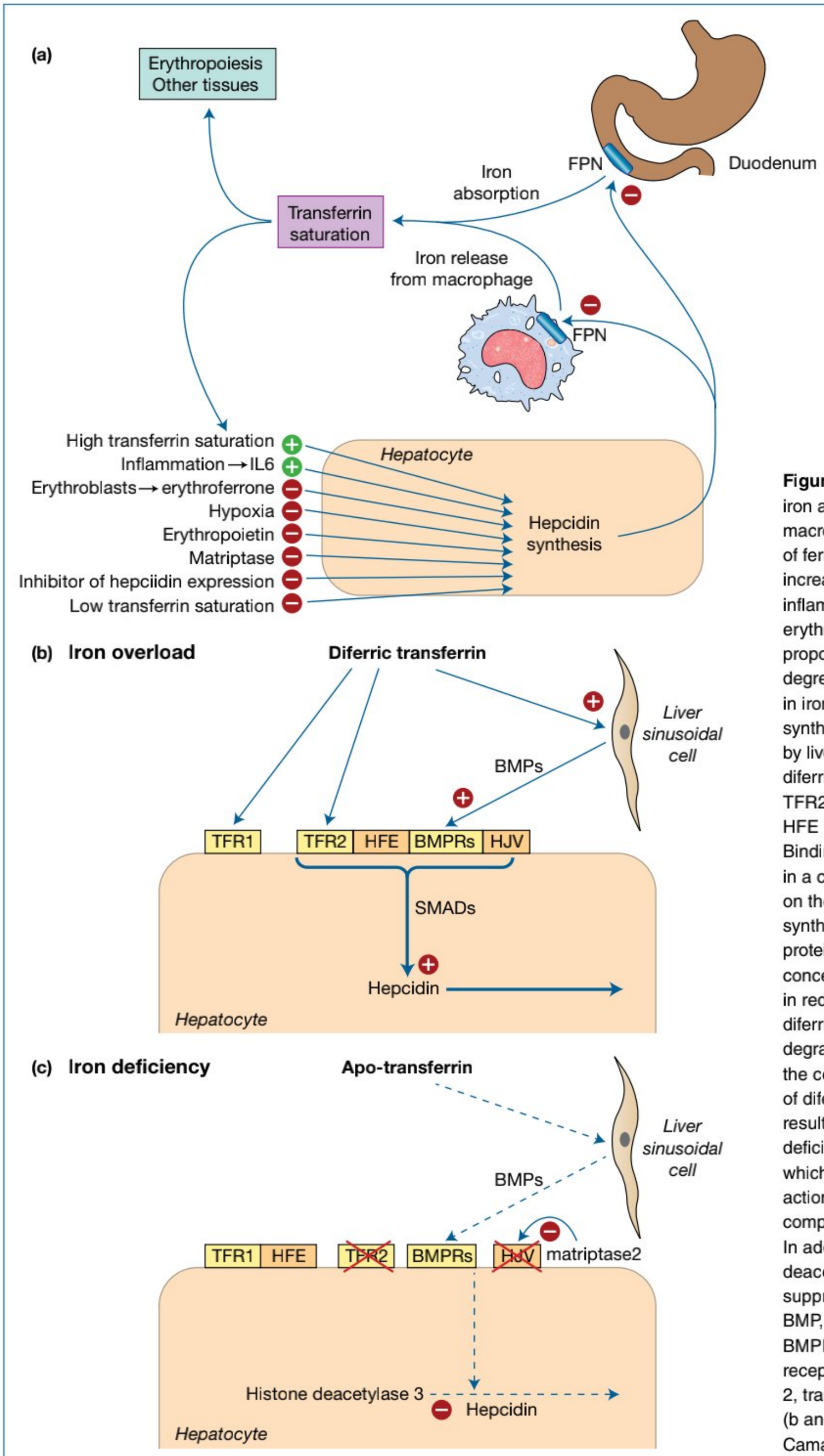


Figure 3.4 **(a)** Hepcidin reduces iron absorption and release from macrophages by stimulating degradation of ferroportin. Its synthesis is increased by diferric transferrin and by inflammation, but reduced by increased erythropoiesis and hypoxia. **(b)** The proposed mechanism by which the degree of transferrin saturation by iron in iron overload stimulates hepcidin synthesis (see also text). BMP synthesis by liver sinusoidal cells is stimulated by diferric transferrin, which also stabilizes TFR2 and by binding to TFR1 prevents HFE being deviated by attaching to TFR1. Binding of BMPs to BMPRs stimulates, in a complex with HFE, TFR2 and HJV on the hepatic cell membrane, hepcidin synthesis by signalling through SMAD proteins. **(c)** In iron deficiency low concentrations of diferric transferrin result in reduced BMP synthesis. Also lack of diferric iron to bind to TFR2 results in degradation or shedding of TFR2 from the cell membrane. Additionally, failure of diferric transferrin to bind to TFR1 results in HFE binding to TFR1. Iron deficiency also activates matriptase2, which cleaves HJV. The result of all these actions is failure to form the necessary complex to stimulate hepcidin synthesis. In addition in iron deficiency, a histone deacetylase 3 is activated and this suppresses transcription of hepcidin. BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; HJV, hemojuvelin; TFR1 and 2, transferrin receptor 1 and 2. Source: (b and c) Courtesy of Professor Clara Camaschella.

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Table 3.2 Iron absorption.

Factors favouring absorption	Factors reducing absorption
Inorganic iron	Haem iron
Ferrous form (Fe^{2+})	Ferric form (Fe^{3+})
Acids (hydrochloric acid, vitamin C)	Alkalis – antacids, pancreatic secretions
Solubilizing agents, e.g. sugars, amino acids	Precipitating agents, e.g. phytates, phosphates, tea
Reduced serum hepcidin e.g. iron deficiency	Increased serum hepcidin e.g. inflammation
Ineffective erythropoiesis (increased plasma erythroferrone)	Decreased erythropoiesis (decreased plasma erythroferrone)
Pregnancy	Iron overload (acquired)
Hereditary haemochromatosis	

Dietary iron

Iron is present in food as ferric hydroxides and as ferric–protein and haem–protein complexes. Both the iron content and the proportion of iron absorbed differ from food to food; in general meat, in particular liver, is a better source than vegetables, eggs or dairy foods. The average Western diet contains 10–15 mg iron daily, from which only 5–10% is normally absorbed. The proportion can be increased to 20–30% in iron deficiency or pregnancy (Table 3.2), but even in these situations most dietary iron remains unabsorbed.

Iron absorption

Organic dietary iron is partly absorbed as haem and partly broken down in the stomach and duodenum to inorganic iron. Absorption occurs through the duodenum. Haem is absorbed through a receptor on the apical membrane of the duodenal enterocyte. It is then broken down to release its iron. Inorganic iron absorption is favoured by factors such as acid and reducing agents that keep iron in the gut lumen in the Fe^{2+} rather than the Fe^{3+} state (Table 3.2). The protein DMT1 is involved in transfer of inorganic iron from the lumen of the gut across the enterocyte microvilli (Fig. 3.5). Ferroportin at the basolateral surface controls the exit of iron from the cell into portal plasma. The amount of iron absorbed is regulated according to the body's needs by changing the levels of DMT1 and ferroportin. For DMT1 this occurs by the IRP/IRE binding mechanism (Fig. 3.3) and for ferroportin by hepcidin (Fig. 3.4a). Ferroportin is also present in liver, heart, kidney, brain and placenta, where it is important in exporting iron.

Ferrireductase present at the enterocyte's apical surface converts iron from the Fe^{3+} to Fe^{2+} state and another enzyme, hephaestin (ferrioxidase), converts Fe^{2+} to Fe^{3+} at the basal surface prior to its binding to transferrin.

Iron requirements

The amount of iron required each day to compensate for losses from the body and for growth varies with age and sex; it is highest in pregnancy, adolescent and menstruating females (Table 3.3). Therefore these groups are particularly likely to develop iron deficiency if there is any additional iron loss or prolonged reduced dietary intake.

Table 3.3 Estimated daily iron requirements. Units are mg/day.

	Urine, sweat, faeces	Menses	Pregnancy	Growth	Total
Adult male	0.5–1				0.5–1
Postmenopausal female	0.5–1				0.5–1
Menstruating female*	0.5–1	0.5–1			1–2
Pregnant female*	0.5–1		1–2		1.5–3
Children (average)	0.5			0.6	1.1
Female (age 12–15)*	0.5–1	0.5–1		0.6	1.6–2.6

*These groups are more likely to develop iron deficiency.

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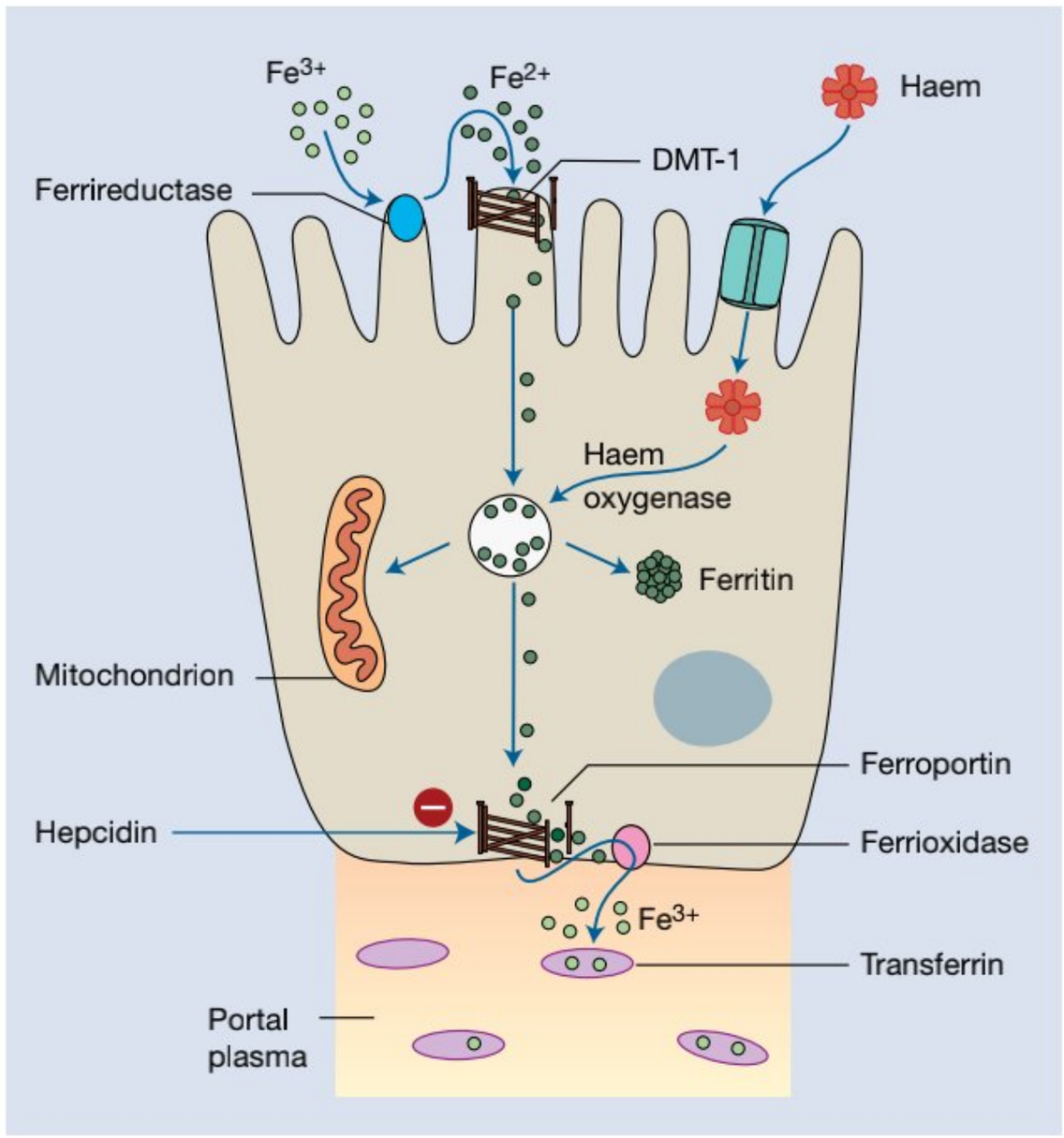


Figure 3.5 The regulation of iron absorption. Dietary ferric (Fe^{3+}) iron is reduced to Fe^{2+} by ferrireductase and its entry to the enterocyte is through the divalent cation binder DMT-1. Its export into portal plasma is controlled by ferroportin. It is oxidized by hephaestin (ferrioxidase) before binding to transferrin in plasma. Haem is absorbed after binding to its receptor protein.

Iron deficiency

Clinical features

When iron deficiency is developing, the reticuloendothelial stores (haemosiderin and ferritin) become completely depleted before anaemia occurs (Fig. 3.6). As the deficiency progresses, the individual may show the general symptoms and signs of anaemia (Chapter 2); and also painless glossitis, angular stomatitis, brittle, ridged or spoon nails (koilonychia; Fig. 3.7), hair loss and unusual dietary cravings (pica). Fatigue and depression are frequent and may occur even before anaemia is present. Oral or parenteral iron has been shown to reduce fatigue in iron-deficient (low serum ferritin) non-anaemic women. The cause of the epithelial cell changes may be related to reduction of tissue iron-containing enzymes. Neonatal iron deficiency is associated with cognitive and behavioural abnormalities, while in children it can cause irritability, poor cognitive function and a decline in psychomotor development.

Causes of iron deficiency

In developed countries, chronic blood loss, especially uterine or from the gastrointestinal tract, is the dominant cause of iron deficiency (Table 3.4) and dietary deficiency is rarely the sole cause. Five hundred millilitres of blood contain approximately 250 mg iron and, despite the increased absorption of iron at an early stage of iron deficiency, negative iron balance is usual in chronic blood loss.

	Normal	Latent iron deficiency	Iron deficiency anaemia
Red cell iron (peripheral film and indices)	Normal	Normal	Hypochromic, microcytic MCV↓ MCH↓
Iron stores (bone marrow macrophage iron)	++	0	0

Figure 3.6 The development of iron deficiency anaemia. Reticuloendothelial (macrophage) stores are lost completely before anaemia develops. MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

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(a) (b)

Figure 3.7 Iron deficiency anaemia. **(a)** Koilonychia: typical 'spoon' nails. **(b)** Angular cheilosis: fissuring and ulceration of the corner of the mouth.

Increased demands for iron during infancy, adolescence, pregnancy, lactation and in menstruating women account for the high risk of iron deficiency anaemia in these particular clinical groups. Newborn infants have a store of iron derived from delayed clamping of the cord and the breakdown of excess red cells. From 3 to 6 months, there is a tendency for a negative iron balance because of growth. From 6 months, supplemented formula milk and mixed feeding, particularly with iron-fortified foods, prevent iron deficiency.

In pregnancy increased iron is needed for an increased maternal red cell mass requiring approximately 600 mg iron, transfer of 300 mg of iron to the foetus and because of blood loss at delivery (Chapter 34). Menorrhagia (a loss of 80 mL or more of blood at each cycle) is difficult to assess clinically, although the loss of clots, the use of large numbers of pads or tampons or prolonged periods all suggest excessive loss.

It takes about 8 years for an adult male starting with normal iron stores to develop iron deficiency anaemia solely as a result of a poor diet or malabsorption resulting in no iron intake. In developed countries inadequate intake or malabsorption is only rarely the sole cause of iron deficiency anaemia. Gluten-induced enteropathy, partial or total gastrectomy and atrophic gastritis (often auto-immune and with *Helicobacter pylori* infection) may, however, predispose to iron deficiency.

Table 3.4 Causes of iron deficiency.

Chronic blood loss
Uterine
Gastrointestinal, e.g. peptic ulcer; oesophageal varices; aspirin (or other non-steroidal anti-inflammatory drugs) ingestion; gastrectomy; carcinoma of the stomach, caecum, colon or rectum; hookworm; schistosomiasis; angiodysplasia; inflammatory bowel disease; piles; diverticulosis
Rarely, haematuria, haemoglobinuria, pulmonary haemosiderosis, self-inflicted blood loss
Increased demands (see also Table 3.3)
Prematurity
Growth
Pregnancy
Erythropoietin therapy
Malabsorption
Gluten-induced enteropathy, gastrectomy, autoimmune gastritis, bariatric surgery, Helicobacter infection
Poor diet
A major factor in many developing countries, but rarely the sole cause in developed countries

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In developing countries, iron deficiency may occur as a result of a life-long poor diet, consisting mainly of cereals and vegetables. Hookworm or schistosomiasis may aggravate iron deficiency, as may repeated pregnancies, growth in children and menorrhagia in young females.

Laboratory findings

These are summarized and contrasted with those in other hypochromic anaemias in Table 3.7.

Red cell indices and blood film

Even before anaemia occurs, the red cell indices fall, and they fall progressively as the anaemia becomes more severe. The blood film usually shows hypochromic, microcytic red cells with occasional target cells and pencil-shaped poikilocytes (Fig. 3.8). Rarely the red cell indices may be normal. The reticulocyte count is low in relation to the degree of anaemia. When iron deficiency is associated with severe folate or vitamin B_{12} deficiency, a 'dimorphic' film occurs with a dual population of red cells of which one is macrocytic and the other microcytic and hypochromic; the indices may be normal. A dimorphic blood film is also seen in patients with iron deficiency anaemia who have received recent iron therapy and produced a population of new haemoglobinized normal-sized red cells (Fig. 3.9) and when the patient has been transfused. The platelet count is often moderately raised in iron deficiency, particularly when haemorrhage is continuing. Low iron promotes megakaryocyte commitment of megakaryocytic-erythroid bone marrow progenitors.

Bone marrow iron

Bone marrow examination is not needed to assess iron stores except in complicated cases. In iron deficiency anaemia, there is a *complete* absence of iron from stores (macrophages) and from developing erythroblasts (Fig. 3.10). The erythroblasts are small and have a ragged cytoplasm.

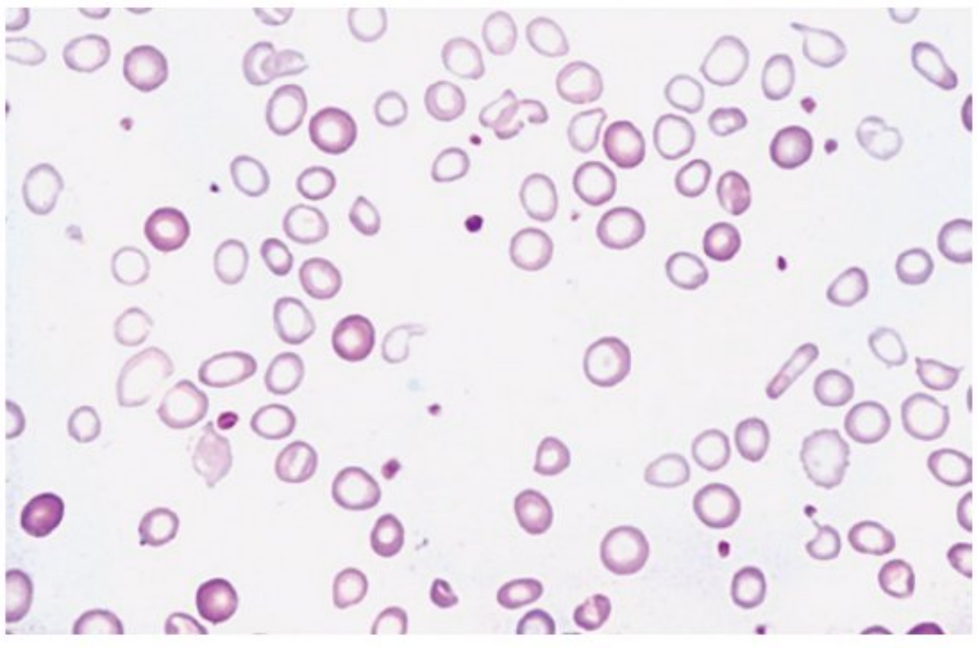


Figure 3.8 The peripheral blood film in severe iron deficiency anaemia. The cells are microcytic and hypochromic with occasional target cells.

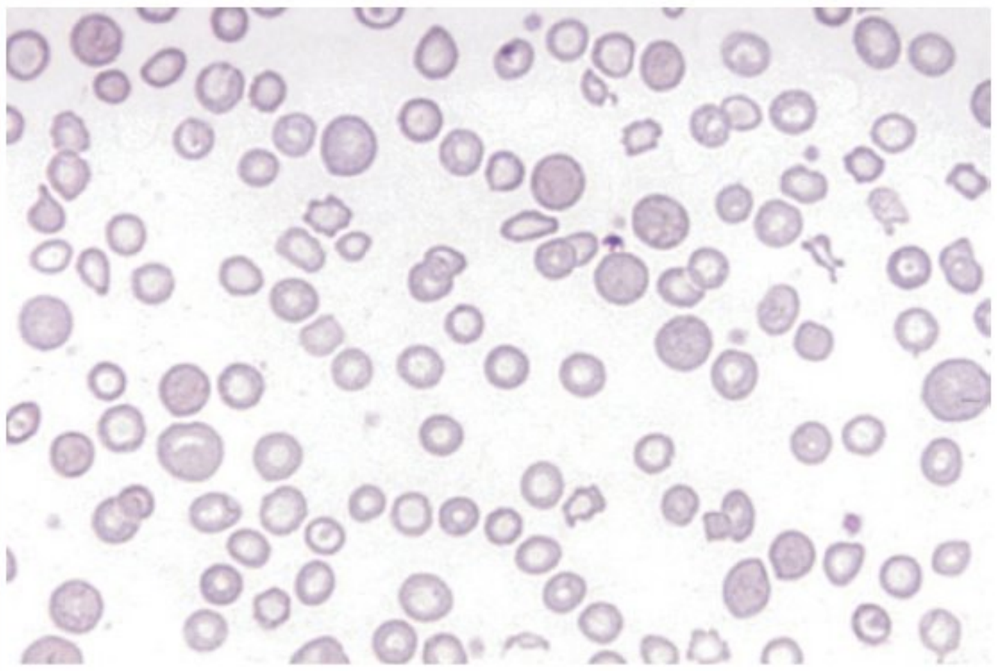


Figure 3.9 Dimorphic blood film in iron deficiency anaemia responding to iron therapy. Two populations of red cells are present: one microcytic and hypochromic, the other normocytic and well haemoglobinized.

Serum iron, total iron-binding capacity (TIBC) and transferrin saturation (TSAT)

The serum iron falls but this assessment alone is not useful. The total iron-binding capacity (TIBC) (or transferrin level) rises so that the TSAT (the ratio between serum iron and TIBC or transferrin expressed as a percentage) is <16% though some take <20% as abnormal (Fig. 3.11). This contrasts both with the anaemia of chronic disease (see below), when the serum iron and the TIBC are both reduced, and with other hypochromic anaemias where the serum iron is normal or even raised. Because of the diurnal fluctuations in serum iron, the TSAT may also fluctuate.

Serum ferritin

A small fraction of body ferritin circulates in the serum, the concentration being related to tissue, particularly reticuloendothelial, iron stores. The normal range in men is higher than in women (Fig. 3.11). In iron deficiency anaemia, the serum ferritin is very low <15 µg/L. A raised serum ferritin indicates iron overload, excess release of ferritin from damaged tissues or an acute phase response, e.g. in inflammation. The serum ferritin is normal or raised in the anaemia of chronic disorders so it cannot exclude iron deficiency in this setting. If an active inflammatory state is present, a serum ferritin <150 ug/L suggests iron deficiency may also be present.

i

Other tests

Plasma soluble transferrin receptors and red cell zinc protoporphyrin levels are raised in iron deficiency but neither are sufficiently specific or sensitive to be recommended for routine use. Assays of serum hepcidin have been developed and are likely to become available in the routine clinical laboratory.

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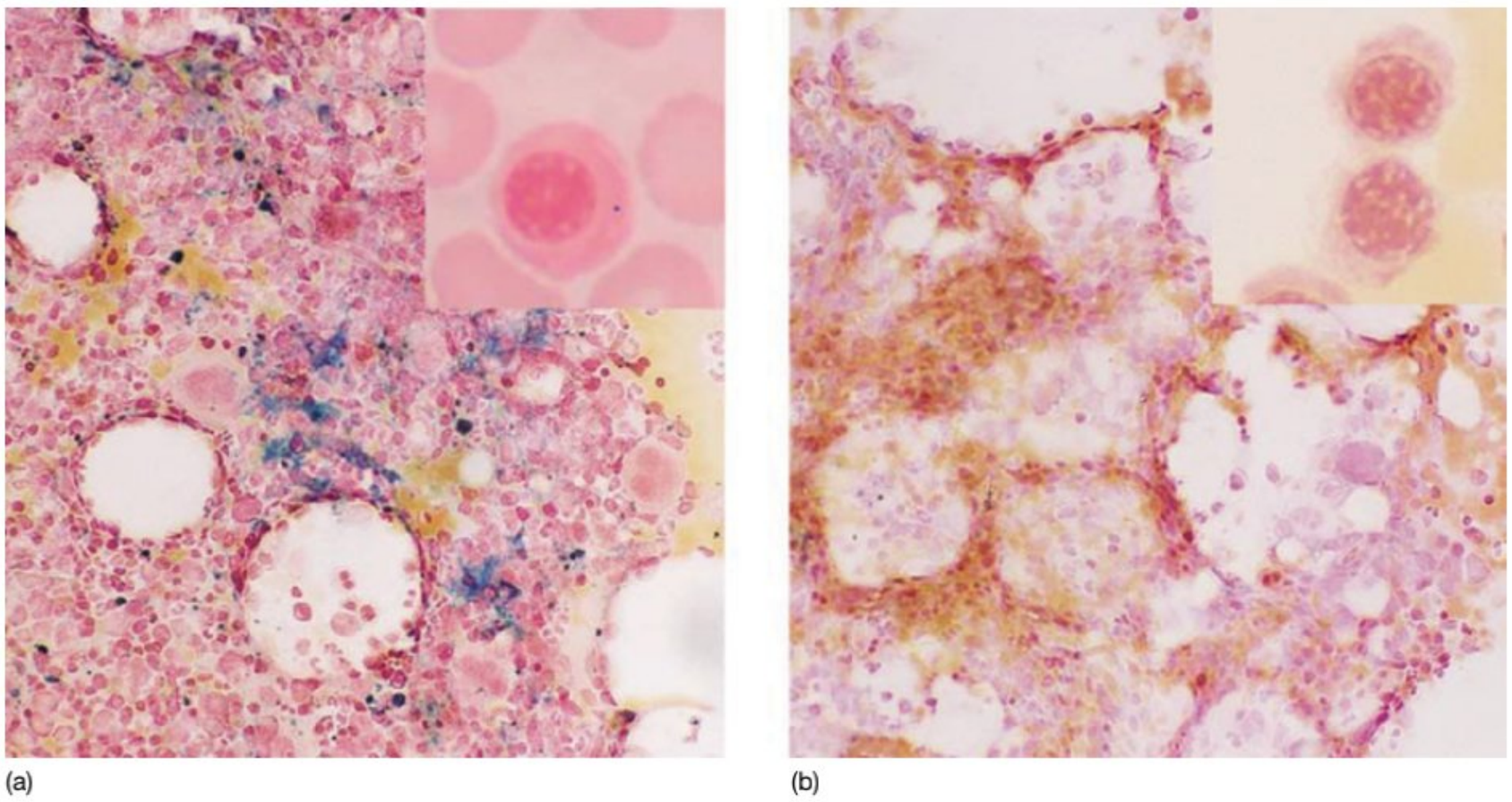


Figure 3.10 Bone marrow iron assessed by Perls' stain. **(a)** Normal iron stores indicated by blue staining in the macrophages. Inset: normal siderotic granule in erythroblast. **(b)** Absence of blue staining (absence of haemosiderin) in iron deficiency. Inset: absence of siderotic granules in erythroblasts.

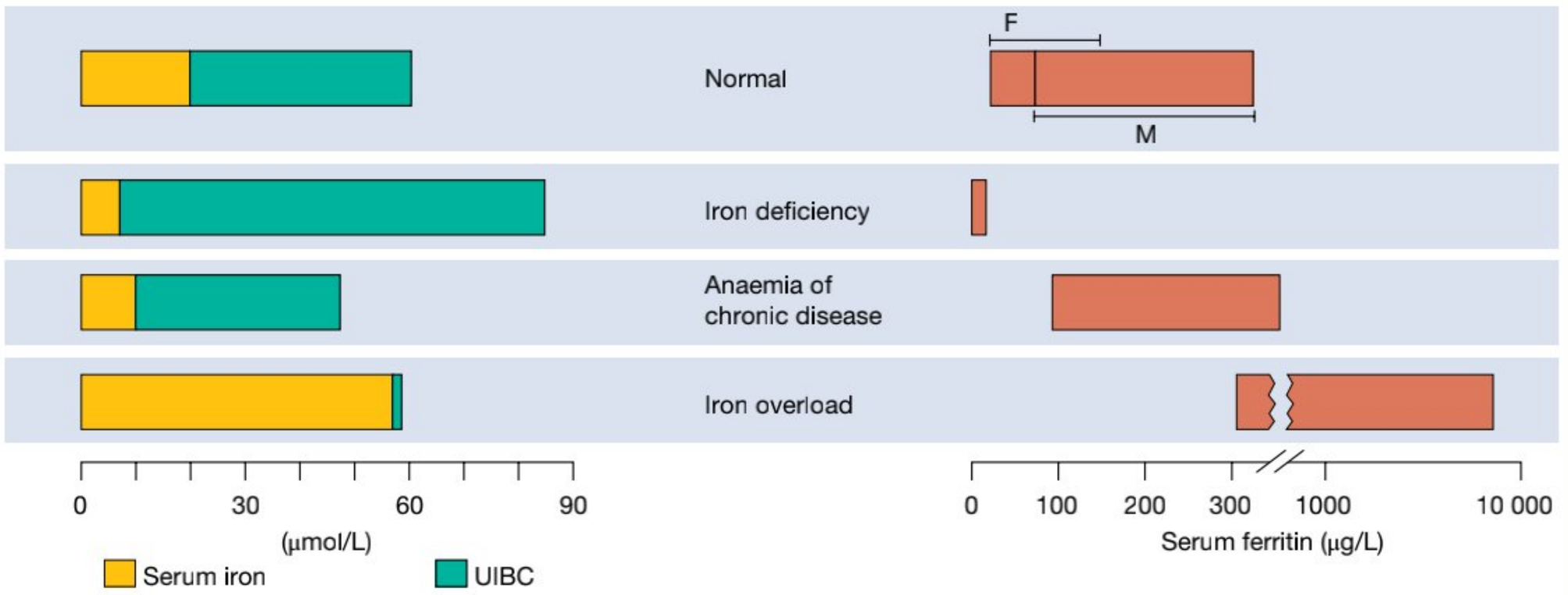


Figure 3.11 The serum iron, unsaturated serum iron-binding capacity (UIBC) and serum ferritin in normal subjects and in those with iron deficiency, anaemia of chronic disease and iron overload. The total iron-binding capacity (TIBC) is made up of the serum iron and the UIBC. In some laboratories, the transferrin content of serum is measured directly by immunodiffusion, rather than by its ability to bind iron, and is expressed in g/L. Normal serum contains 2–4 g/L transferrin (1 g/L transferrin = 20 μmol/L binding capacity). Normal ranges for serum iron are 10–30 μmol/L; for TIBC, 40–75 μmol/L; for serum ferritin, male, 40–340 μg/L; female, 14–150 μg/L.

Investigation of the cause of iron deficiency (Fig. 3.12)

In premenopausal women, menorrhagia and/or repeated pregnancies are the usual causes. If these are not present, other causes must be sought. In some patients with menorrhagia, a clotting or platelet abnormality, e.g. von Willebrand disease is present. In poor countries, the combination of prolonged inadequate intake of iron with expansion of blood volume with growth, and the onset of menstruation and repeated pregnancies in young women, is the major cause of iron deficiency in childhood, adolescence and fertile adult females. In men and postmenopausal women, gastrointestinal blood loss is the main cause of iron deficiency and the exact site is sought from the clinical history, physical and rectal examination, by occult blood tests, and by appropriate use of

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upper and lower gastrointestinal endoscopy and/or radiology, e.g. computed tomography (CT) of the pneumocolon, or virtual colonoscopy using the 3D colon system (Figs. 3.12 and 3.13). In difficult cases, a camera in a capsule can be swallowed, which relays pictures of the gastrointestinal tract electronically. Tests for parietal cell antibodies, *Helicobacter* infection and serum gastrin level may help to diagnose autoimmune gastritis. Tests for transglutaminase antibodies and duodenal biopsy to look for gluten-induced enteropathy may be needed. Hookworm and schistosomiasis ova are sought in stools of subjects from areas where these infestations are endemic. Serological tests for schistosomiasis can also be performed. Rarely, a coeliac axis angiogram is needed to demonstrate angiodysplasia.

If gastrointestinal blood loss is excluded, loss of iron in the urine as haematuria or haemosiderinuria (resulting from chronic intravascular haemolysis) is considered. A normal chest X-ray excludes the rare condition of pulmonary haemosiderosis. Rarely, patients bleed themselves, producing iron deficiency.

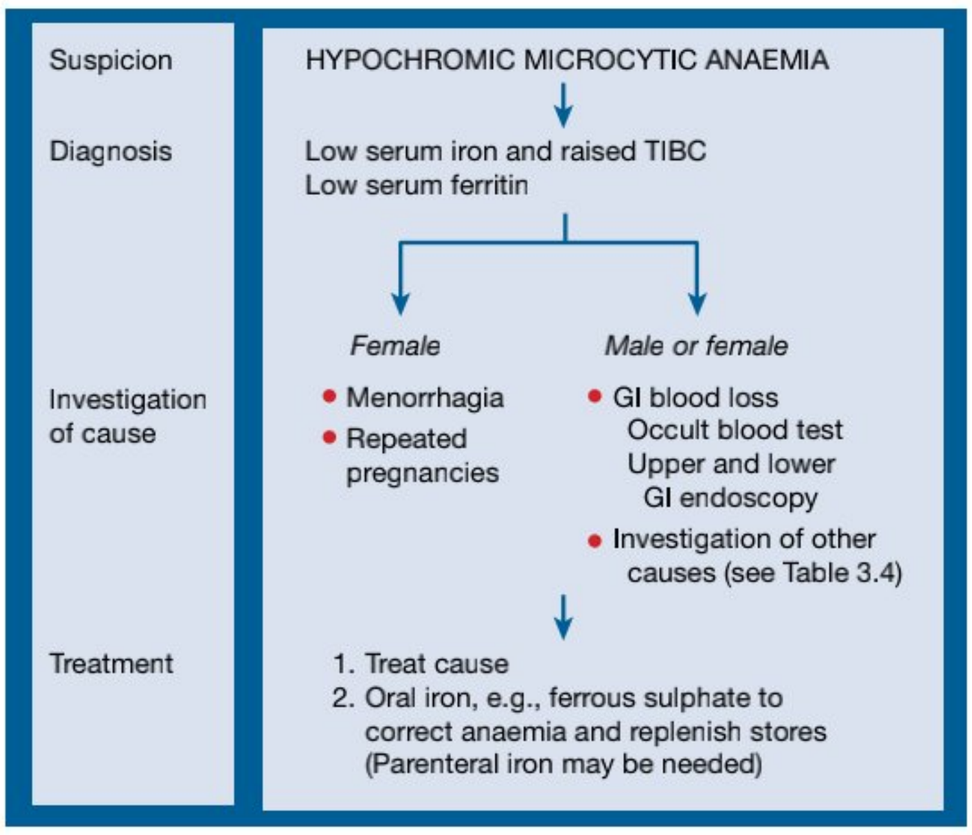


Figure 3.12 Investigation and management of iron deficiency anaemia. GI, gastrointestinal; TIBC, total iron-binding capacity.

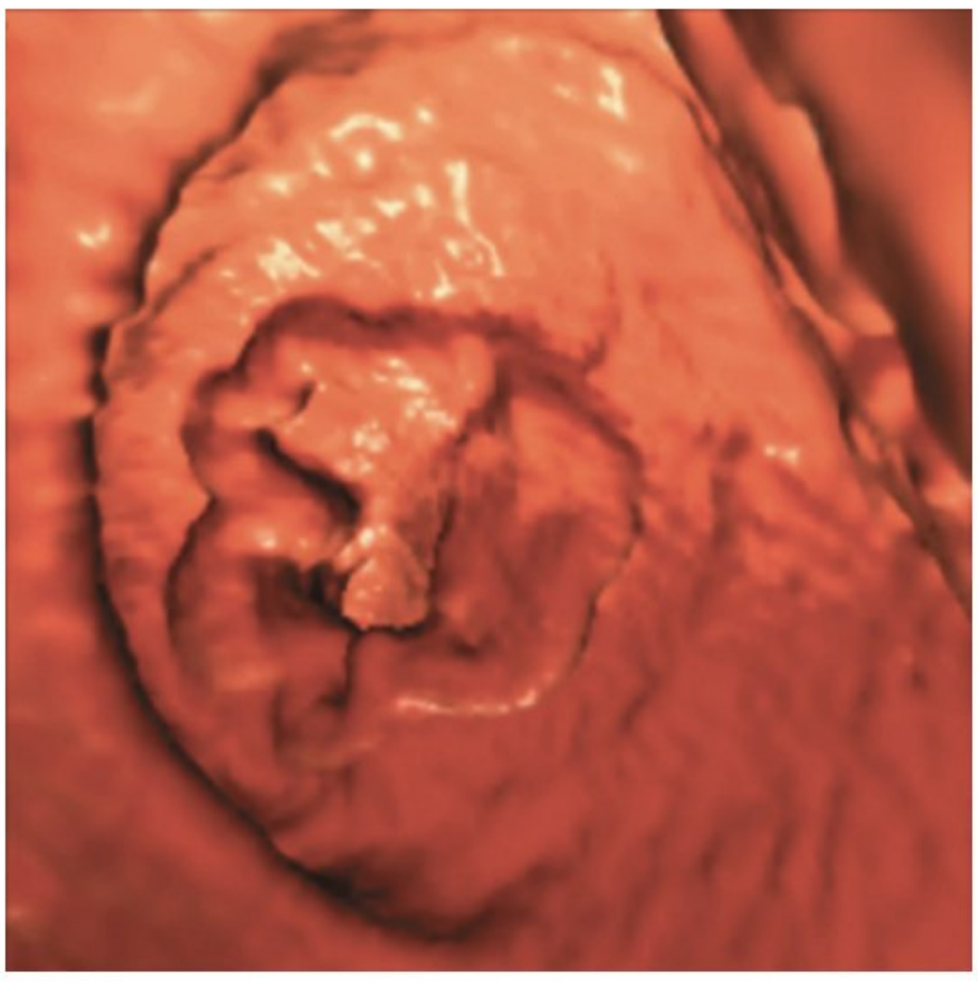


Figure 3.13 Virtual colonoscopy showing carcinoma of colon causing colonic obstruction and iron deficiency.

Treatment

The underlying cause is treated as far as possible. In addition, iron is given to correct the anaemia and replenish iron stores.

Oral iron

The best preparations are ferrous sulphate (200 mg) and ferrous fumarate (210 mg), which are cheap and contain 67 mg iron in each tablet. Iron is best absorbed if they are taken on an empty stomach. In the past doses two or three times daily were used to overcome limited absorption. Once daily doses are now advocated based on the rise in plasma hepcidin level (which blocks further iron absorption) within hours of a single oral dose of iron. For those without anaemia or only mildly anaemic, alternate day dosing may be used. For those with more severe anaemia, daily doses or even intravenous iron are needed to correct the anaemia more quickly. If side effects occur, e.g. nausea, abdominal pain, constipation or diarrhoea, these can be reduced by giving iron with food or by using a preparation with a lower iron content, e.g. ferrous gluconate, which contains only 37 mg per 300 mg tablet. An elixir of iron is available for children. Slow-release preparations should not be used.

Oral iron therapy should be given for long enough both to correct the anaemia and to replenish body iron stores, which usually means for at least up to 6 months. The haemoglobin should rise at the rate of approximately 20 g/L every 3 weeks. Failure of response to oral iron has several possible causes (Table 3.5). These should all be considered before parenteral

Table 3.5 Failure of response to oral iron.

Continuing haemorrhage
Failure to take tablets
Wrong diagnosis – especially thalassaemia trait, sideroblastic anaemia, IRIDA
Mixed deficiency – associated folate or vitamin B_{12} deficiency
Another cause for anaemia e.g. malignancy, inflammation
Malabsorption – see Table 3.4
Use of slow-release preparation

IRIDA, iron-refractory iron deficiency anaemia.

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Table 3.6 Intravenous iron preparations.

Trade name	Cosmofer (Europe) INFeD (USA)	Ferinject (Europe) Injectafer (USA)	Feraheme	Monofer, Diafer (Europe) Monoferric (USA)	Ferrlecit	Venofer (Europe only)
Carbohydrate	Low molecular weight dextran	Carboxymaltose	Ferumoxytol	Derisomaltoside	Gluconate in sucrose solution	Sucrose
Vial	50 mg/μL	50 mg/ml	30 mg/mL	100 mg/mL or 50 mg/mL	5 mL (12.5 mg/mL)	20 mg/mL
Total dose	Yes	Yes	Yes	Yes	No	No
Test dose required	Yes	No	No	No	No	No
Infusion time	1 h	15 min	15 min	20 min	60 min	15 min
Slow intravenous injection	Yes	Yes	No	Yes	Yes	Yes

iron is used. Iron fortification of the diet in infants in Africa reduces the incidence of anaemia, but increases susceptibility to malaria.

Parenteral iron

Many different preparations are available with varying licensing arrangements in different countries (Table 3.6). The dose may be calculated according to body weight and degree of anaemia, but it is more usual in order not to waste costly drugs to give 1000 mg as a single dose to moderately anaemic patients and 1500 mg divided into two doses, given at least 48 hours apart, to those more severely anaemic or of large body mass. All the preparations contain ferric (usually ferric hydroxide) iron. Iron dextran can be given in small doses by slow intravenous injection or by infusion as a total dose in one day. Ferric carboxymaltose and ferric isomaltoside may be given as a total dose in one day by intravenous infusion or by slow intravenous injections. Ferumoxytol is given only by intravenous infusion. Ferric hydroxide–sucrose, which releases iron from its sugar more rapidly than the other preparations, is administered by slow intravenous injection or infusion, to a maximum of 200 mg iron in each dose.

Rarely there may be hypersensitivity or anaphylactoid reactions to parenteral iron, especially in those with a previous reaction, multiple drug allergies and severe atopy. If the reaction is severe, it is treated with intravenous hydrocortisone and possibly adrenaline.

Parenteral iron is given when there are high iron requirements, as in gastrointestinal bleeding, severe menorrhagia, middle or late pregnancy (avoided in the first trimester), chronic haemodialysis and chronic renal failure with erythropoietin therapy, post-operative after major surgery. It is also given when oral iron is ineffective, e.g. iron malabsorption (Table 3.4), if IRIDA (see below) is present, or when oral iron causes intolerable side effects or is impractical, e.g. active inflammatory bowel disease. The haematological response to parenteral iron is no faster than to adequate dosage of oral iron, but the iron stores are replenished faster. Intravenous iron may increase functional capacity and quality of life in some patients with congestive heart failure, even in the absence of anaemia (see p. xxx). It may also be effective in restless leg syndrome. Early trials show it can reduce blood transfusion needs in chemotherapy-induced anaemia in cancer patients.

Anaemia of chronic disease (inflammation)

One of the most common anaemias occurs in patients with a variety of chronic inflammatory and malignant diseases (Table 3.7). With iron deficiency it accounts for about two-thirds of the world's anaemias. The characteristic features are:

1. Normochromic, normocytic or mildly hypochromic (MCV rarely <75 fL) indices and red cell morphology.
2. Mild and non-progressive anaemia (haemoglobin rarely <90 g/L) – the severity being related to the severity of the underlying disease.

Table 3.7 Causes of the anaemia of chronic disease.

Chronic inflammatory diseases
Infections, e.g. pulmonary abscess, tuberculosis, osteomyelitis, pneumonia, bacterial endocarditis
Non-infectious, e.g. rheumatoid arthritis, systemic lupus erythematosus and other connective tissue diseases, sarcoidosis, inflammatory bowel disease
Other chronic disorders
Congestive heart failure Chronic pulmonary disease Chronic renal disease Obesity Anaemia in the elderly Anaemia in critical illness (accelerated course)
Malignant diseases
Carcinoma, lymphoma, sarcoma

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Table 3.8 Laboratory diagnosis of a hypochromic anaemia.

	Iron deficiency	Chronic disease	Thalassaemia trait (α or β)	Sideroblastic anaemia	IRIDA
MCV/ MCH	Reduced in relation to severity of anaemia	Normal or mild reduction	Reduced; low for degree of anaemia	Usually low in congenital type but MCV usually raised in acquired type	Reduced in relation to severity of anaemia
Serum iron	Reduced	Reduced	Normal	Raised	Reduced
TIBC	Raised	Reduced	Normal	Normal	Reduced
Serum ferritin	Reduced	Normal or raised	Normal	Raised	Raised
Bone marrow iron stores	Absent	Present	Present	Present	Present
Erythroblast iron	Absent	Absent	Present	Ring forms	Absent
Haemoglobin electrophoresis	Normal	Normal	Hb A_2 raised in β form	Normal	Normal

Hb, haemoglobin; IRIDA, non-refractory iron deficiency anaemia; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; TIBC, total iron-binding capacity.

3 Both the serum iron and TIBC are reduced.
4 The serum ferritin and hepcidin levels are normal or raised.
5 Bone marrow storage (reticuloendothelial) iron is normal, but erythroblast iron is reduced (Table 3.8).
6 Raised plasma levels of hepcidin and inflammation-induced cytokines block intestinal iron absorption and cause iron retention in the reticuloendothelial cells.
7 Shortened reduced red cell life span, suppressed erythropoietin response to anaemia and inhibited erythroid cell differentiation.

The pathogenesis of this anaemia is related to decreased release of iron from macrophages to plasma, because of raised serum hepcidin levels stimulated by IL-6 and IL-1β and lipopolysaccharide, a mechanism to protect the host against bacteria which need iron to multiply. There is also an inadequate erythropoietin response to anaemia caused by the effects of cytokines such as IL-1, IL-6, IL-10 interferon-gamma and tumour necrosis factor. These cytokines directly damage red cells whose life span is also reduced both by activation of macrophages and by deposition of antibody and complement on their surface. Interferon-γ further impairs haemopoiesis by binding to thrombopoietin, inhibiting thrombopoietin binding to its receptor on marrow cells.

The anaemia is corrected by successful treatment of the underlying disease. It does not respond to iron therapy but diagnosis of the anaemia when there is coexisting iron deficiency may be difficult. If the serum ferritin is <100 ug/L or saturation of the TIBC <20%, co-existing iron deficiency is suspected. The combination of iron therapy and erythropoietin injections improve the anaemia in some cases. In many conditions, this anaemia is complicated by anaemia resulting from other causes, e.g. vitamin B_{12} or folate deficiency, renal failure, bone marrow failure, hypersplenism, endocrine abnormality or leucoerythroblastic anaemia. These are discussed in Chapter 32.

Measurement of serum hepcidin and drugs to inhibit hepcidin, both being developed, would be major advances in the diagnosis and treatment of the anaemia of chronic disease.

Iron refractory iron deficiency anaemia (IRIDA)

This rare autosomal recessive syndrome presents with an hypochromic microcytic anaemia. The serum iron is low with <5% saturation of the iron binding capacity (Table 3.8). It is caused by inherited mutations of matriptase 2, which allow uninhibited hepcidin secretion, or even more rarely mutations of *DMT1* genes (Figs. 3.4 and 3.5). There may be a haematological response to intravenous but usually not to oral iron.

Sideroblastic anaemia

This is an uncommon refractory anaemia defined by the presence of many pathological ring sideroblasts in the bone marrow (Fig. 3.14). These are erythroblasts containing numerous iron granules arranged in a ring or collar around the nucleus, instead of the one or two randomly distributed iron granules in normal erythroblasts (Fig. 3.10). There is also usually erythroid hyperplasia with ineffective erythropoiesis. Primary sideroblastic anaemia is diagnosed when 15% or more of marrow erythroblasts are ring sideroblasts but in the presence of the *SF3B1*mutation as few as 5% ring sideroblasts in

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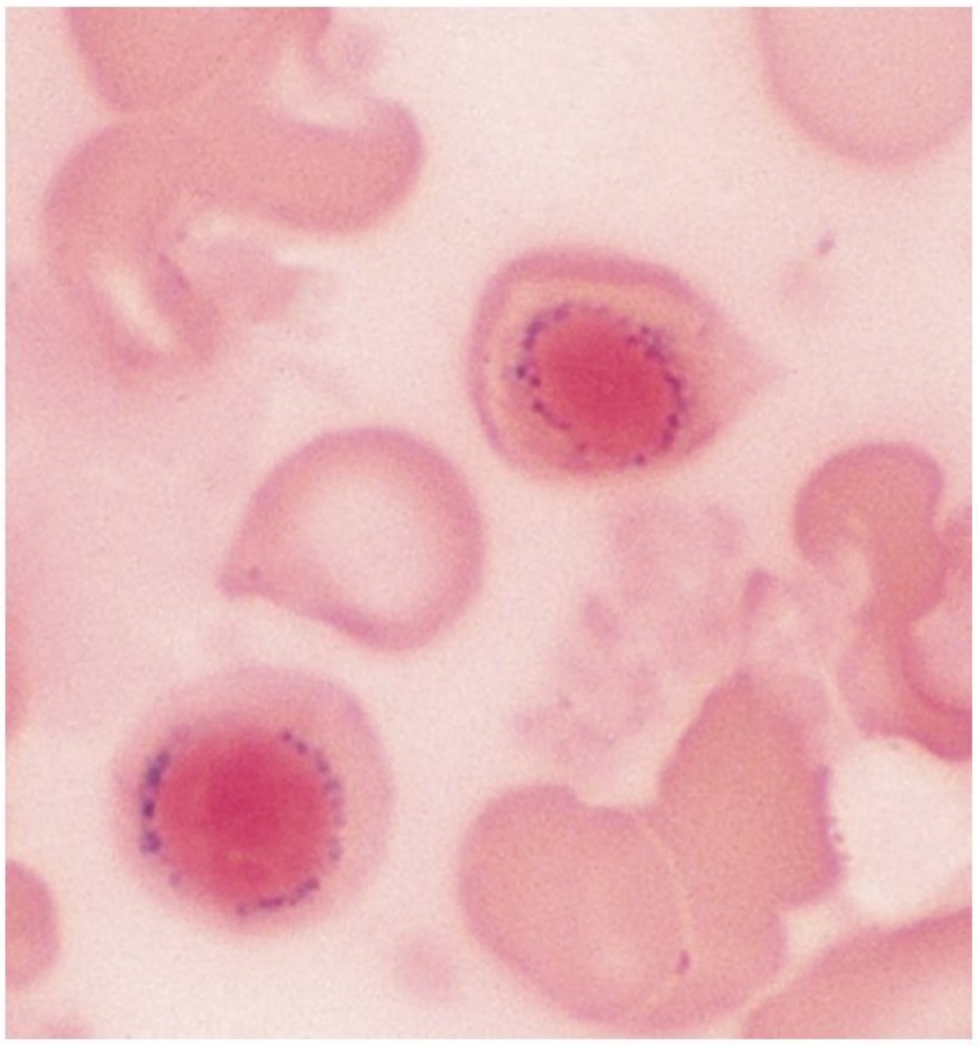


Figure 3.14 Ring sideroblasts with a perinuclear ring of iron granules in sideroblastic anaemia.

patients with myelodysplasia is still classified as a 'primary' sideroblastic anaemia (see below). Ring sideroblasts can also be found at lower numbers in a variety of other anaemias.

Sideroblastic anaemia is classified into different types (Table 3.9), the common link is a defect in haem synthesis. In the **hereditary forms,** the anaemia is usually markedly hypochromic and microcytic but some types show normocytic or macrocytic red cells. The most common mutations are in the *ALAS* gene, which is on the X chromosome. Pyridoxal-6-phosphate is a coenzyme for ALAS. Other rare types include an X-linked mitochondrial disease with spino-cerebellar degeneration and ataxia, mutations in other mitochondrial genes with the triad of anaemia, deafness and diabetes or Pearson syndrome (when there is also pancreatic insufficiency). Thiamine-responsive anaemia with both megaloblastic and sideroblastic erythropoiesis, accompanied by deafness and diabetes is due to mutations of the gene which codes for a thiamine transporter. Various congenital malformations of the limbs, face, nervous system, heart and other organs may be present in these rare syndromes.

The most frequent of the acquired sideroblastic anaemias is **refractory anaemia with ring sideroblasts** with the *SF3B1* mutation, a subtype of myelodysplasia (Chapter 16). Acquired reversible forms may be due to alcohol, lead and drugs, e.g. isoniazid.

In some patients, particularly with the hereditary *ALAS* mutated type, there is a response to pyridoxine therapy. High doses of thiamine (vitamin B1) improve those with the thiamine transporter gene mutation. Luspatercept (Chapters 7, 16) can be tried. Folate deficiency may occur and folic acid therapy may also be tried. Other treatments, e.g. erythropoietin and luspatercept, are effective in some patients with the myelodysplasia form (Chapter 16). In many severe cases, however, repeated blood transfusions are the only method of maintaining a satisfactory haemoglobin concentration, and transfusional iron overload requiring iron chelation therapy becomes a major problem.

Lead poisoning

Lead inhibits both haem and globin synthesis at a number of points. In addition, it interferes with the breakdown of RNA by inhibiting the enzyme pyrimidine 5′ nucleotidase, causing accumulation of denatured RNA in red cells, the RNA giving

Table 3.9 Classification of sideroblastic anaemia.

Hereditary
X chromosome-linked *ALAS* mutation
usually occurs in males, transmitted by females; also occurs rarely in females
X-linked mitochondrial gene mutation with spino-cerebellar ataxia.
Other rare autosomal types usually involving mitochondrial proteins or thiamine phosphorylation; deafness and diabetes often present
Acquired
Primary
Myelodysplasia (refractory anaemia with ring sideroblasts, as low as 5% with *SF3B1* mutation; see Chapter 16). N.B. Ring sideroblast formation (<15% of erythroblasts) may also occur in the bone marrow inother malignant diseases of the marrow, e.g. other types of myelodysplasia, myelofibrosis, myeloid leukaemia, myeloma, drugs, e.g. anti-tuberculosis (isoniazid, cycloserine), alcohol, lead other benign conditions, e.g. haemolytic anaemia, megaloblastic anaemia, rheumatoid arthritis

ALAS, δ-aminolaevulinic acid synthase.

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an appearance called basophilic stippling on the ordinary (Romanowsky) stain (Fig. 2.17). The anaemia may be hypochromic or predominantly haemolytic, and the bone marrow may show ring sideroblasts. Free erythrocyte protoporphyrin is raised.

Differential diagnosis of hypochromic anaemia

Table 3.8 lists the laboratory investigations that may be necessary. The clinical history is particularly important, as the source of the haemorrhage leading to iron deficiency or the presence of an inflammatory or other chronic disease may be revealed. The ethnic group and the family history may suggest a possible diagnosis of thalassaemia or other genetic defect of haemoglobin. Physical examination may also be helpful in determining a site of haemorrhage, features of a chronic inflammatory or malignant disease, koilonychia or, in some haemoglobinopathies, an enlarged spleen or bony deformities.

In thalassaemia trait the red cells tend to be very small, often with an MCV of 70 fL or less, even when anaemia is mild or absent; the red cell count is usually over 5.5×10^{12}/L. Conversely, in iron deficiency anaemia the indices fall progressively with the degree of anaemia and when anaemia is mild the indices may be normal or only just reduced, e.g. MCV 75–80 fL. In the anaemia of chronic disorders, the indices are also not markedly low, an MCV in the range 75–82 fL being usual.

It is usual to measure serum iron and TIBC, and/or serum ferritin to confirm iron deficiency. Haemoglobin high-performance liquid chromatography (HPLC) or electrophoresis with an estimation of Hb A_2 and Hb F is carried out in all patients suspected of thalassaemia or other genetic defect of haemoglobin, because of the family history, ethnic group, red cell indices and blood film. Iron deficiency or the anaemia of chronic disorders may also occur in these subjects. β-Thalassaemia trait is characterized by a raised Hb A_2 above 3.5%, but in α-thalassaemia trait there is no abnormality on simple haemoglobin studies, so the diagnosis is usually made by exclusion of all other causes of hypochromic red cells and by the presence of a red cell count $>5.5 \times 10^{12}$/L. DNA studies confirm the diagnosis.

Bone marrow examination is essential if a diagnosis of sideroblastic anaemia is suspected, but is not usually needed in diagnosis of the other hypochromic anaemias.

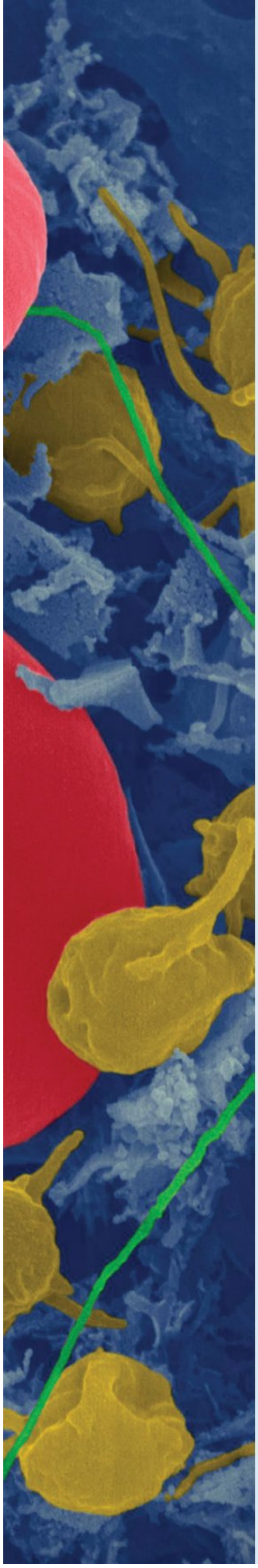
SUMMARY

- Iron is present in the body in haemoglobin, myoglobin, haemosiderin and ferritin, and in iron-containing enzymes. Transferrin is the main transport protein in blood.
- Hepcidin is the main regulator of iron absorption and iron release from macrophages and other cells.
- Iron metabolism is regulated according to iron status by intracellular iron regulatory proteins and by control of hepcidin synthesis. Hepcidin synthesis is also affected by erythroferrone secreted by erythroblasts and by inflammation.
- Iron deficiency is the most common cause of anaemia throughout the world. The red cells are hypochromic and microcytic. The serum ferritin, serum iron and saturation of the iron-binding capacity are reduced.
- In Western countries, it is usually caused by haemorrhage from the gastrointestinal or the female genital tract.
- Prolonged reduced dietary intake may cause the anaemia, particularly in developing countries, where hookworm and schistosomiasis may also be important causes of blood loss.
- It is treated by oral or less frequently parenteral iron to correct the anaemia and to restore body stores of iron, and by treating, as far as possible, the underlying cause.
- Other frequent causes of a hypochromic, microcytic anaemia are the anaemia of chronic disease, which occurs in patients with chronic inflammatory or malignant diseases, and α- or β-thalassaemia. Less common causes include sideroblastic anaemia (some cases), iron refractory iron deficiency anaemia and lead poisoning.
- Sideroblastic anaemias characterized by ring sideroblasts in the marrow are rare. They may be inherited or acquired; the most common subtype is myelodysplasia with the *SF3B1* mutation.



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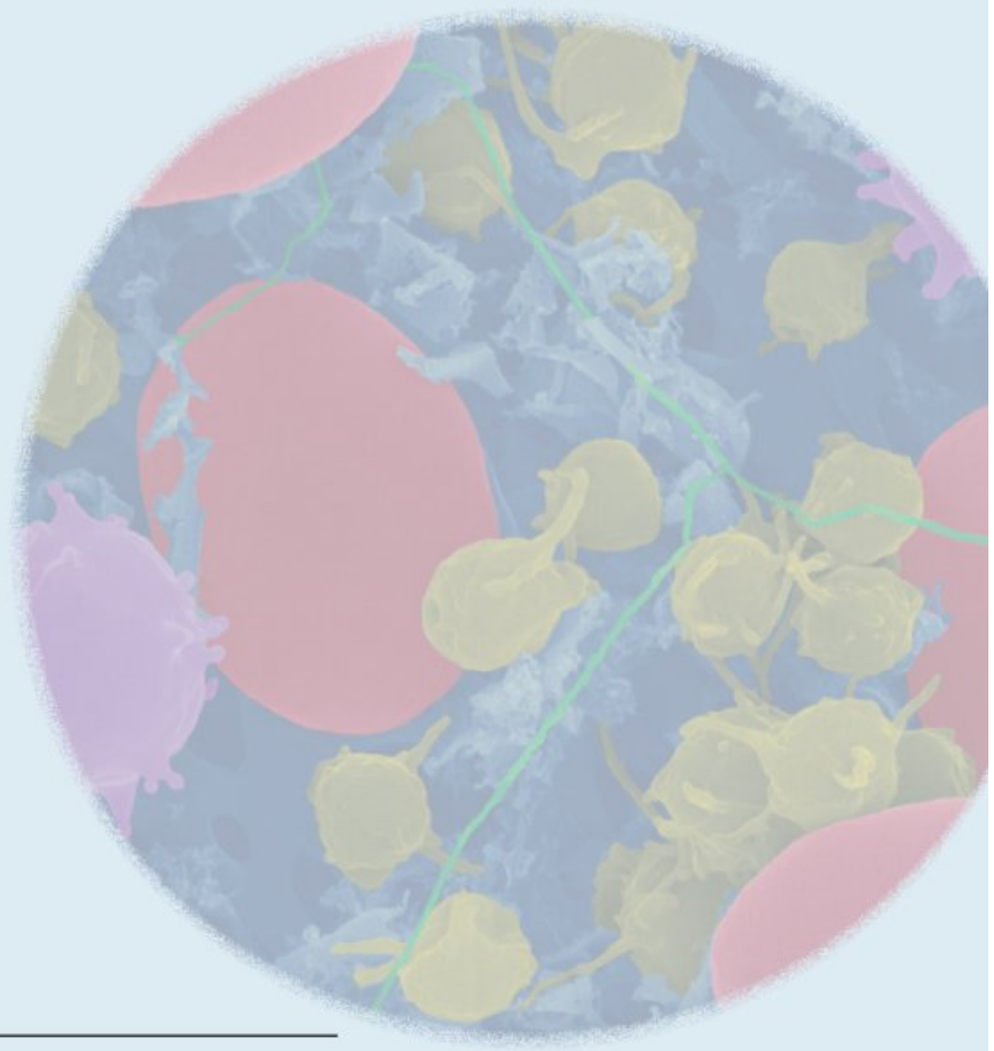


CHAPTER 4

Iron overload

Key topics

- Assessment of iron status and organ function 43
- Hereditary (genetic, primary) haemochromatosis 44
- Transfusional iron overload 45
- Iron chelation therapy 47



Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

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There is no physiological mechanism for eliminating excess iron from the body. Iron absorption is carefully regulated to avoid accumulation of excess iron. Iron overload (haemosiderosis) occurs in genetic disorders associated with inappropriately increased iron absorption (haemochromatosis), or in patients with severe chronic anaemias, especially those who receive regular blood transfusions. Excessive iron deposition in tissues may result in serious damage particularly to the heart, liver and endocrine organs. The causes of iron overload are listed in Table 4.1 and causes of hereditary haemochromatosis in Table 4.2.

Table 4.1 The causes of iron overload.

Increased iron absorption	Hereditary (genetic) haemochromatosis Ineffective erythropoiesis, e.g. non-transfusion-dependent thalassaemia or myelodysplastic syndromes Chronic liver disease
Increased iron intake	African siderosis (dietary and genetic components)
Repeated red cell transfusions	Transfusion siderosis

Table 4.2 Genetic causes of haemochromatosis and of hyperferritinaemia.

Type	Inheritance	Clinical condition	Gene defect
1	AR	Classical hereditary haemochromatosis	*HFE*
2	AR	Juvenile haemochromatosis	Hemojuvelin (*HJV*) Hepcidin (*HAMP*)
3	AR	Hereditary haemochromatosis	Transferrin receptor 2 (*TFR2*)
4a	AD	RE iron loading	Ferroportin (*SLC11A3*)
4b	AD	Parenchymal iron loading	Ferroportin *(SCL11A3*) (mutation at the hepcidin binding site)
5	AD	Hereditary hyperferritinaemia – cataract syndrome (no iron deposition)	Ferritin light chain (*FTL*)

AD, autosomal dominant; AR, autosomal recessive; RE, reticuloendothelial.

Assessment of iron status and organ function

The tests to assess iron overload and the degree of damage caused by iron are listed in Table 4.3. The serum ferritin is the most widely used test to assess iron overload and to monitor its treatment, although inflammatory and other diseases may increase serum ferritin levels even in the absence of iron overload. The percentage saturation of the iron-binding capacity (transferrin) is also valuable. Serum non-transferrin-bound (NTBI) iron, also known as labile plasma iron, is a toxic form of iron that occurs in severe transfusional iron overload but tests for NTBI are not widely available.

Aceruloplasminaemia is another rare genetic cause of iron loading.

Table 4.3 Assessment of iron overload.

Assessment of iron stores	
Serum ferritin	
Serum iron and percentage saturation of iron-binding capacity (transferrin)	
Serum non-transferrin-bound iron (NTBI)	
Bone marrow biopsy (Perls' stain) for iron within reticuloendothelial(RE) stores	
Liver biopsy (parenchymal and RE stores)	
Liver CT scan or MRI (T_2* or Ferriscan technique)	
Cardiac MRI (gated T_2* technique)	
Pancreas and pituitary MRI (not widely available) Annual transfusional calculated iron loading	
Assessment of tissue damage caused by iron overload	
Cardiac	Clinical; chest X-ray; ECG (24-h monitor); echocardiography (ECHO) to assess left and right ventricular ejection fraction at rest and with stress
Liver	Liver function tests, alpha-fetoprotein; liver ultrasound; MRI, fibroscan
Endocrine and bone	Clinical examination (including for growth and sexual development); oral glucose tolerance test; thyroid, parathyroid, gonadal, adrenal function and growth hormone assays; radiology for bone age; isotopic bone density study, vitamin D
Musculoskeletal	Hand X-rays with assessment of metacarpophalangeal joints

CT, computed tomography; ECG, electrocardiography; MRI, magnetic resonance imaging.

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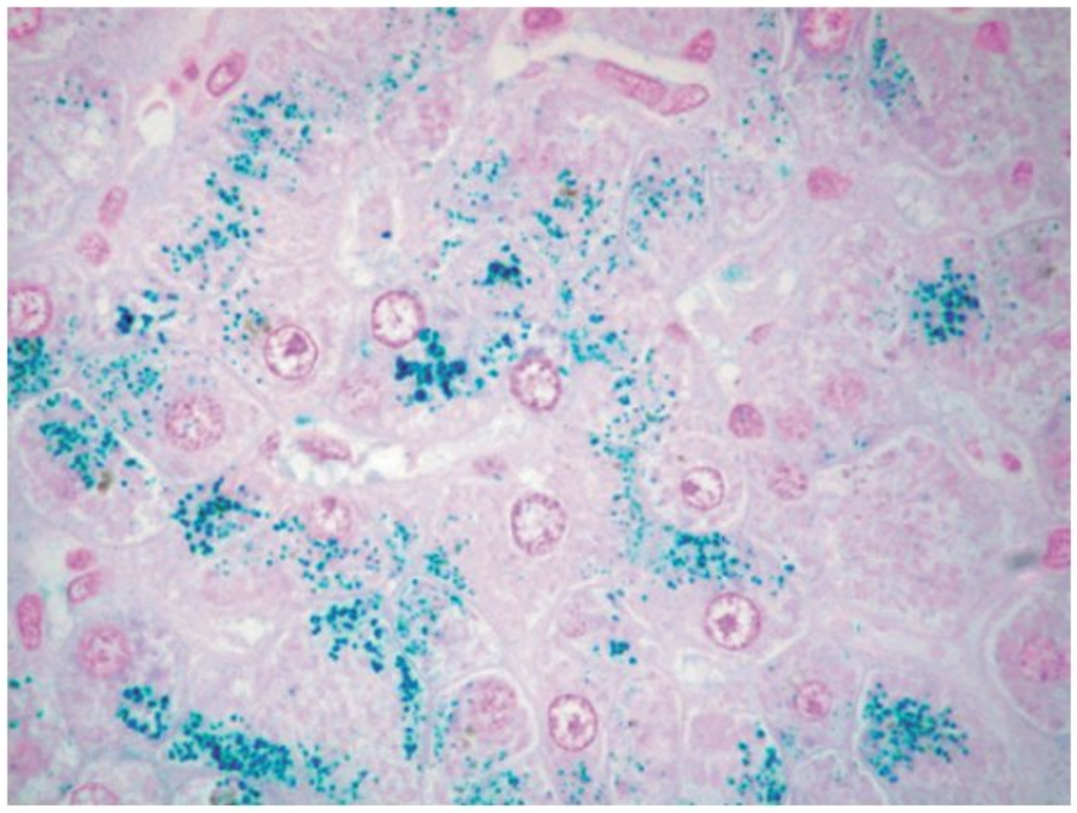


Figure 4.1 Liver biopsy in genetic haemochromatosis. Iron loading of hepatic parenchymal cells (Perls' stain). Source: Courtesy of Professor A.P. Dhillon.

Liver biopsy with staining for iron (Fig. 4.1) and chemical analysis of iron content is useful for assessment of both parenchymal iron (within hepatic cells) and reticuloendothelial iron (within Kupffer cells). Magnetic resonance imaging (MRI) using the T_2^* technique is the best non-invasive guide to liver and cardiac iron. A commercial Ferriscan MRI technique is widely used for measuring liver iron. Liver biopsy also allows estimation of the degree of fibrosis. Fibroscan (transient elastography) is a non-invasive ultrasound method of assessing liver fibrosis from any cause, including iron overload. Serum alpha-fetoprotein and liver ultrasound are used for serial screening for hepatocellular carcinoma in patients with known iron overload, previous hepatitis C or liver fibrosis typically on an annual basis. Monitoring for cardiac iron overload and of cardiac function are discussed on page xx under the heading of transfusional iron overload where cardiac disease is a major complication. Other complications of transfusional iron overload including liver, endocrine and bone disease are also discussed further under the heading thalassaemia major in Chapter 7.

Hereditary (genetic, primary) haemochromatosis

Hereditary haemochromatosis is a group of diseases in which there is from birth excessive absorption of iron from the gastrointestinal tract leading to iron overload of the parenchymal cells, dominantly of the liver (Fig. 4.1). At later stages endocrine and heart complications occur. In contrast to transfusional iron overload, the macrophages are not iron overloaded.

Most patients are homozygous for a missense mutation (C282Y) in the *HFE* gene, which leads to insertion of a tyrosine residue rather than cysteine in the mature protein. This allele has the highest prevalence (approximately 1 in 10) within populations of Northern European origin. However, gene penetrance is low; only a small proportion of this ethnic group who are homozygous for the mutation (about 1 in 300) present with clinical features of the disease. Affected individuals usually show a serum ferritin greater than 1000 µg/L. A second mutation H63D resulting in a histidine to aspartic acid substitution is found with the C282Y mutation in approximately 5% of patients with genetic haemochromatosis. Homozygotes for the H63D mutation usually do not have the disease. The H63D mutation has a broader global distribution than C282Y.

HFE is involved in regulation of hepcidin synthesis (Fig. 3.4). The C282Y mutation causes low plasma levels of hepcidin and so high levels of ferroportin in enterocytes, macrophages and other cells. Iron absorption and iron release from macrophages is therefore increased. Iron overload develops over decades and damages parenchymal cells, so that patients may present in adult life with hepatic disease (fibrosis, cirrhosis, hepatocellular carcinoma), endocrine disturbances (diabetes mellitus, hypothyroidism or impotence) or melanin skin pigmentation (Fig. 4.2). In some severe cases, there is cardiac failure or arrhythmia but these are more dominant features of transfusional iron overload. Homozygosity for the *HFE* mutation also underlies an arthropathy due to calcium pyrophosphate deposition and not related to the degree of iron overload. Most commonly affected are the second and third metacarpophalangeal joints.

Other non-*HFE* genetic factors as well as dietary iron intake, alcohol consumption, pregnancies, menstrual blood loss, other blood loss, e.g. gastrointestinal bleeding or blood donation affect the phenotype and time of presentation of the

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Figure 4.2 Melanin skin pigmentation. The right hand is of a teenager with iron overload caused by thalassaemia major. The left hand is of her mother, who has normal iron status.

disease. Regular blood donors on average present several years later than individuals who never donate blood.

The initial clinical presentation is often with non-specific symptoms such as fatigue, loss of libido or arthralgias. Diagnosis is suspected by the presence of increased levels of serum iron, serum transferrin saturation and ferritin. The diagnosis is confirmed by testing for the *HFE* mutation, but a negative result does not exclude the diagnosis, since mutations of other genes may cause a similar disease phenotype (Table 4.2 and below). Liver biopsy is useful to quantify the degree of iron overload and assess liver damage, but is associated with risks, including bleeding. MRI can be used instead to measure liver and cardiac iron.

Treatment is with regular venesection, initially at 1–2-week intervals, each unit of blood removing 200–250 mg of iron. Organ function may improve and liver fibrosis resolve, but the arthropathy does not respond to iron removal and response of the endocrine damage is variable. There are differences of opinion as to whether patients without evidence of organ dysfunction due to iron overload should be treated. Venesections are generally started when the serum ferritin is raised to over 1000 µg/L but some recommend starting if the serum ferritin is >300 µg/L in males or >200 µg/L in females. Venesection is monitored by serum ferritin and the aim is to restore this to normal, <100 µg/L, and then keep it there with further less frequent venesections, e.g. a few times per year. Some patients elect to become blood donors, but some blood banks and regulatory agencies do not allow blood donation from patients with haemochromatosis. Those with abnormal liver tests are excluded from donating due to the inability to exclude viral causes of hepatic enzyme elevation.

Rarer forms of genetic haemochromatosis are caused by mutations in genes for other iron regulatory proteins, including hemojuvelin, hepcidin and transferrin receptor 2. All (types II and III; Table 4.2) are associated, like homozygous *HFE* disease, with low plasma levels of hepcidin. They often present below the age of 30 years as severe (especially for Type II) iron overload with cardiomyopathy in children, adolescents or young adults. Hypogonadism is another particular feature, whereas arthropathy is absent. Genetic iron overload in Asian populations is usually due to these mutations rather than mutation of *HFE*.

On the other hand, ferroportin gene mutations (type IV disease) usually cause reticuloendothelial but not parenchymal cell iron overload. They do rarely cause parenchymal overload if the mutations in the ferroportin gene are at the hepcidin binding site. Aceruloplasminaemia is another rare genetic cause of iron loading. Mutations of the ferritin light chain gene (type V disease) cause a raised monoclonal serum ferritin with cataracts resulting from ferritin deposition in the eye, but no other tissue iron overload.

African iron overload

This occurs in sub-Saharan Africa through a combination of increased iron absorption due to a genetic defect, possibly in the ferroportin gene, and consumption of beverages, especially beer, with a high iron content due to the use of iron brewing or cooking pots.

Non-transfusion-dependent thalassaemia (thalassaemia intermedia)

Moderately severe forms of thalassaemia may lead to increased iron levels even in patients who do not need regular blood transfusions (Chapter 7). This is due to increased iron absorption due to raised plasma erythroferrone. This leads to increased levels of iron in the liver. The heart is spared. Blood transfusions at times of increased anaemia, e.g. with intercurrent infections, may increase the iron burden. Iron chelation is indicated if the liver iron concentration is above 5 mg/g dry weight or when the serum ferritin reaches 800 µg/L or when the iron leads to organ damage (see also Chapter 7). Other haemolytic anaemias including pyruvate kinase deficiency may also lead to iron loading due to low serum hepcidin levels caused by raised plasma erythroferrone due to ineffective erythropoiesis.

Transfusional iron overload

This develops in patients with chronic severe anaemia not due to haemorrhage who need regular blood transfusions. Each 500 mL of transfused blood contains 200–250 mg iron so iron overload is inevitable unless iron chelation therapy is given (Table 4.4). To make matters worse, iron absorption from food is increased in β-thalassaemia major and other anaemias secondary to ineffective erythropoiesis despite tissue iron overload. This is due to release from early erythroblasts of erythroferrone and of other proteins that inhibit hepcidin synthesis (Fig. 3.4). Non-transferrin-bound iron may appear in plasma when transferrin is >70% saturated. It causes widespread iron deposition in parenchymal tissues.

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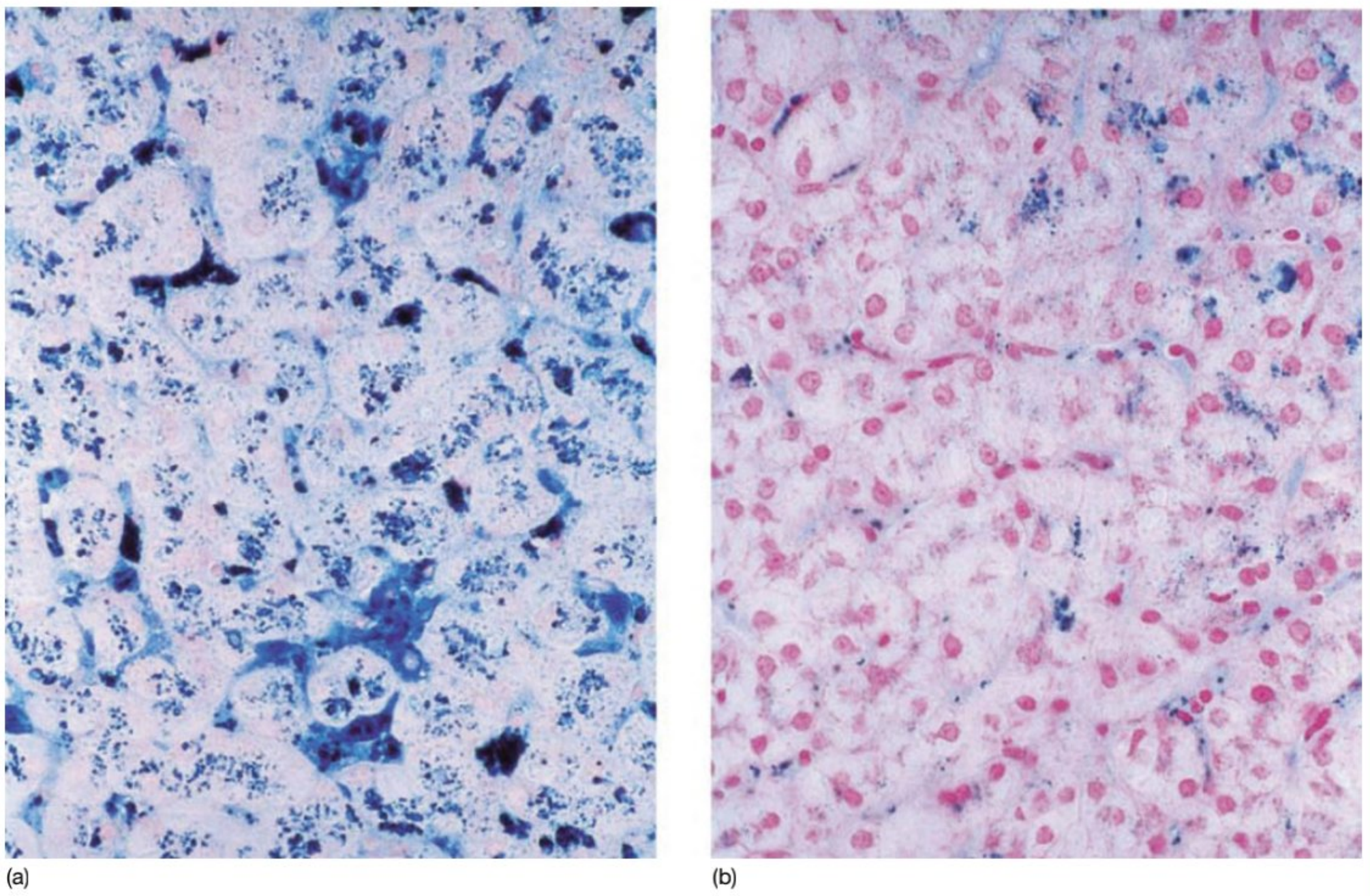
Table 4.4 Causes of anaemia that may lead to transfusional iron overload.

Congenital	Acquired
β-Thalassaemia major	Myelodysplastic neoplasias
β-Thalassaemia/Hb E disease	Red cell aplasia
Congenital dyserythropoietic anaemias	Acute leukaemias
Sickle cell anaemia (some cases)	Aplastic anaemia
Red cell aplasia (Diamond–Blackfan)	Primary myelofibrosis
Congenital sideroblastic anaemia	
Dyserythropoietic anaemia	

Hb, haemoglobin.

Cardiac damage due to iron is a dominant problem in transfusional iron overload. As for hereditary haemochromatosis, iron also damages the liver (Fig. 4.3) and the endocrine organs, including the hypothalamus and pituitary, with failure of growth, delayed or absent puberty, diabetes mellitus, hypothyroidism and hypoparathyroidism. Skin pigmentation as a result of excess melanin and haemosiderin gives a slate grey appearance even at an early stage of iron overload.

In the absence of intensive iron chelation, death occurs in the second or third decade of life in thalassaemia major, usually from congestive heart failure or cardiac arrhythmias. **T_2* MRI is a valuable measure of cardiac and liver iron loading (Fig. 4.4)**.



(a) (b)

Figure 4.3 β-Thalassaemia major: needle biopsy of liver. **(a)** Grade IV siderosis with iron deposition in the hepatic parenchymal cells, bile duct epithelium, macrophages and fibroblasts (Perls' stain). **(b)** Reduction of iron excess in liver after intensive chelation therapy.

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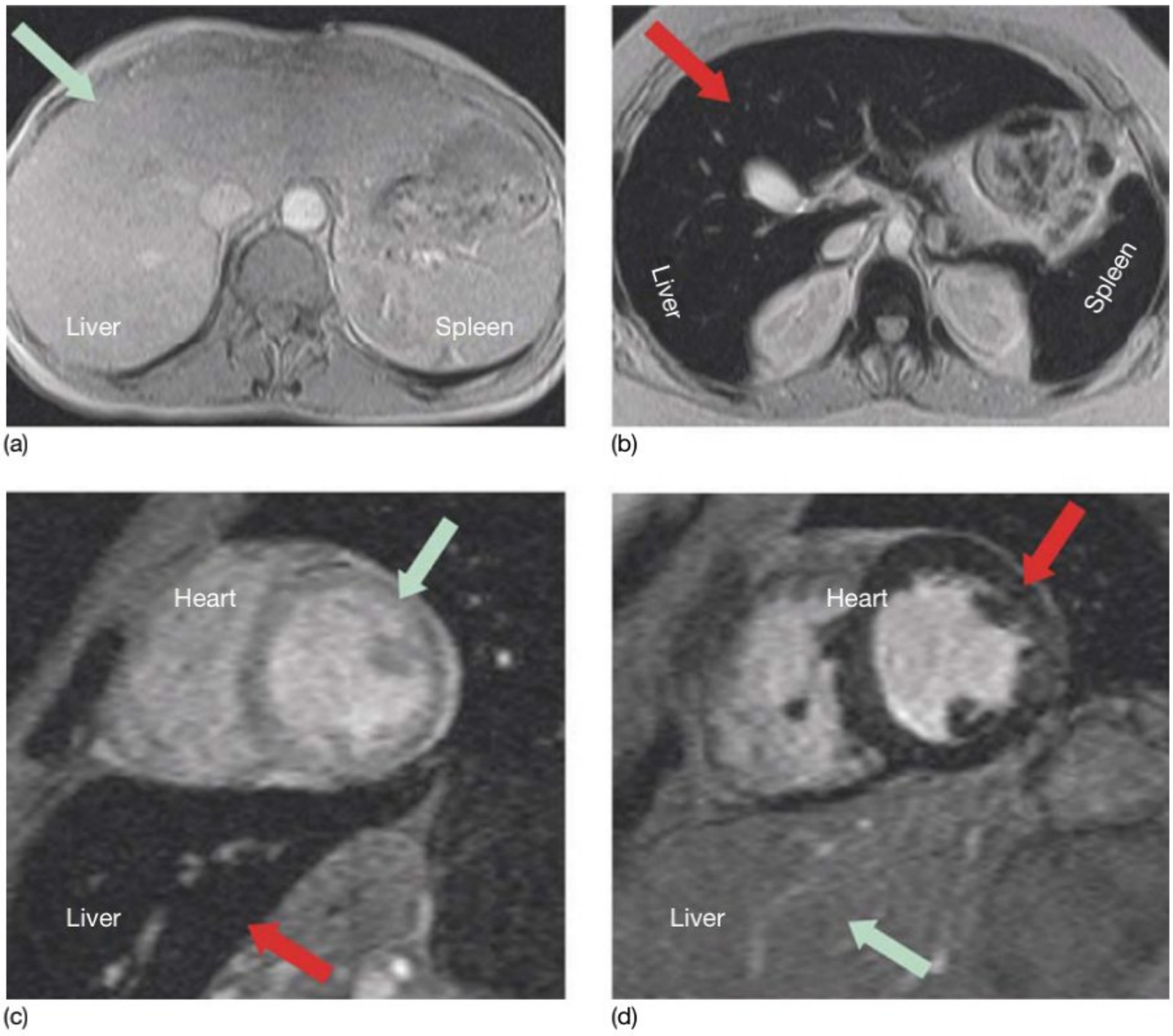


Figure 4.4 T_2* magnetic resonance images (MRIs) showing tissue appearance in iron overload: **(a)** normal volunteer, **(b)** severe iron overload. Green arrow, normal appearance; red arrow, iron overload. Lack of correlation: liver and cardiac iron in two cases of thalassaemia major, **(c)** and **(d)**.

It can detect increased cardiac iron and predict for cardiac failure or arrhythmia before sensitive tests detect impaired cardiac function. The shorter the relaxation time, the greater the cardiac iron burden and the greater risk of subsequent cardiac failure or arrhythmia (Fig. 4.5). Serum ferritin and liver iron correlate poorly with cardiac iron (Figs. 4.4 and 4.5). Moreover, serum ferritin is raised in viral hepatitis and other inflammatory disorders and should therefore be interpreted in conjunction with more accurate tests of iron status, such as T_2* MRI, Ferriscan R2 or liver biopsy. It is, however, useful for monitoring changes in iron burden when this is being treated by chelation therapy. It is important to monitor LVEF annually by echocardiography or MRI from the age of 8 in thalassaemia major. Monitoring for liver, endocrine and bone disease is also needed.

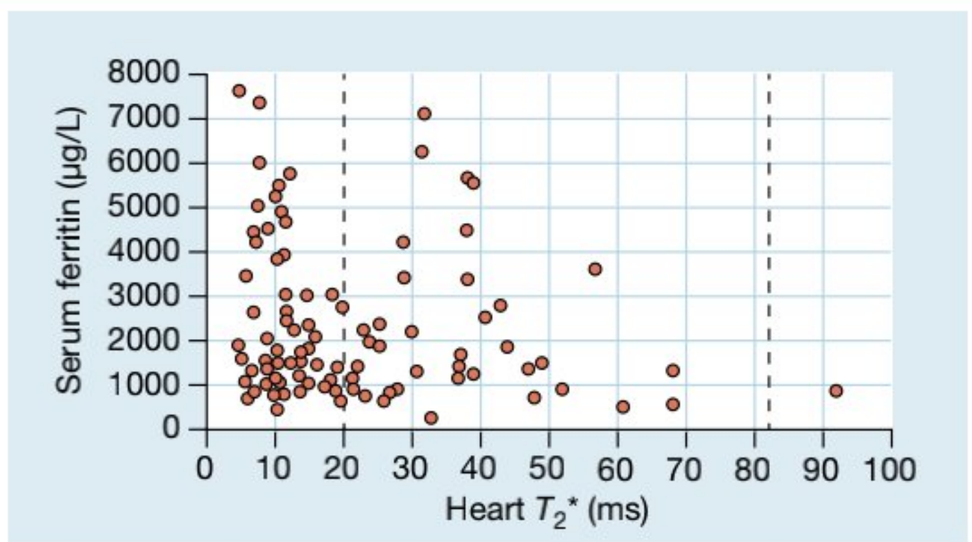


Figure 4.5 Comparison of T_2* magnetic resonance imaging (MRI) measurement of cardiac iron and serum ferritin in thalassaemia major patients. There are substantial numbers of patients with very high serum ferritin (>3000 µg/L) but normal heart iron (T_2* >20 msecs) and, conversely, many patients with serum ferritin levels <1000 µg/L with severe cardiac iron loading (T_2* <10 msecs). Source: L.J. Anderson *et al*. (2001) *Eur. Heart J.* 22: 2171–79.

Iron chelation therapy

Iron chelation therapy is used to treat transfusional iron overload. Three effective drugs are available: orally administered deferasirox and deferiprone, and parenterally administered

Table 4.5 Characteristics of desferrioxamine, deferiprone and deferasirox.

	Desferrioxamine (DFO)	Deferiprone (DFP)	Deferasirox (DFX)
Structure	Hexadentate	Bidentate	Tridentate
Molecular weight (Da)	560	139	373
Iron–chelator complex	1 : 1	1 : 3	1 : 2
Plasma clearance ($t_{1/2}$)	20 min	1–3 h	1–16 h
Absorption	Negligible	Peak 45 min	Peak 1–2.9 h
Iron excretion	Urine + faecal	Urine	Faecal
Therapeutic daily dose	40 mg/kg	75–100 mg/kg	20–40 mg/kg (dispersible tablet) 7–21 mg/kg (coated tablet)
Route	Parenteral	Oral	Oral
Clinical experience	>45 y	>35 y	>20 y
Side effects	Ototoxicity, retinal toxicity, growth defects, cartilage and bone abnormalities	Agranulocytosis, arthropathy, gastrointestinal disturbance, transient transaminitis, zinc deficiency	Skin rashes, gastrointestinal disturbance, rising serum creatinine

deferoxamine (Table 4.5). Thalassaemia major is the most frequent indication worldwide, but chelation is also used for iron overloaded, usually heavily transfused patients with the other anaemias (Table 4.4) and with other iron loading anaemias such as non-transfusion-dependent thalassaemia (Chapter 7) and pyruvate kinase deficiency (Chapter 6).

Deferasirox is given orally once daily and leads to iron loss in the faeces. Skin rashes and transient changes in liver enzymes and rise in serum creatinine are the main side effects. Licensing for young children and lack of major side effects have resulted in its widespread use, but high cost means that the other drugs may be preferred in some countries. Deferasirox removes iron primarily from the liver and is the least effective of the three drugs for eliminating cardiac iron.

Deferiprone is also an oral chelator and causes predominantly urinary iron excretion. It was usually given in three doses daily but a twice daily delayed release formulation has now been approved. It is licensed for first line treatment at varying starting ages in children in different countries. It may be used alone or, if this is inadequate, in combination with deferoxamine infused on one or more days a week, since the drugs have an additive or even synergetic effect on iron excretion. Alone it is the most effective of the three drugs at removing cardiac iron and improving left and right heart function. Side effects include an arthropathy, agranulocytosis (in about 1%), neutropenia, gastrointestinal disturbance and, rarely in patients with diabetes, zinc deficiency. Monitoring of the blood count weekly for the first 6 months, fortnightly for the next 6 months and then every 2–4 weeks or at time of blood transfusion is recommended for all patients receiving deferiprone. Combination therapy with the two orally active chelators, if either alone is not sufficiently effective, has been effective without unexpected toxicity in several trials, but is not yet licensed in any country.

The third drug, **deferoxamine**, was the first of the three drugs to be used in clinical practice. It is not active orally and is usually given by subcutaneous infusion over 8–12 hours for 5–7 days each week; vitamin C is given to increase iron excretion. Most iron is lost in the urine, but up to one-third is also excreted in the stools. Because of the difficult administration, lack of patient adherence is a major problem. It may be given on one or more each days each week in combination with daily deferiprone or deferasirox and can be used intravenously in combination with oral deferiprone in patients with severe iron overload at risk of dying from cardiac failure. Side effects are particularly frequent if high doses are used in children and in adults without heavy iron overload. These include high tone deafness, retinal damage, bone abnormalities and growth retardation. Patients receiving deferoxamine should have auditory and fundoscopic examinations annually.

All three chelators can be given in children. Deferasirox is most frequently used and a liquid formulation of deferiprone and a sprinkle form of deferasirox are available.

Chelation is typically started in thalassaemia major after 10–12 units have been transfused or the serum ferritin is >1000 µg/L. In other conditions such as myelodysplastic neoplasias, there is controversy about when to initiate chelation and hepatic and cardiac T_2^* MRI may help guide this decision.

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Chelation is given to keep the cardiac T_2^* at >20 msecs, liver at <7 mg/g dry weight and serum ferritin level at <1000–1500 µg/L, when the body iron stores are approximately 5–10 times normal. MRI assesses cardiac and liver iron accurately and should be repeated annually or more frequently if there is definite cardiac or liver damage (Fig. 4.5). Serum ferritin is useful in monitoring changes in iron stores, but as it is an acute phase reactant it may be elevated in the presence of recent infection trauma or surgery and this may falsely suggest inadequate chelation.

Serial tests of heart, liver and endocrine function are also needed to monitor therapy.

Life expectancy has improved dramatically for thalassaemia major patients since the introduction of iron chelation. Chelation may even reverse liver, endocrine and cardiac damage in cases where this has developed before effective chelation has been started.

SUMMARY

- Iron overload may be caused by excessive absorption of iron from food because of low plasma hepdidin levels due to hereditary (genetic) haemochromatosis or because of raised plasma erythroferrone levels due to ineffective erythropoiesis.
- Iron overload may also result from repeated blood transfusions in patients with refractory anaemias. Each unit of transfused blood contains 200–250 mg of iron.
- Excess iron absorbed from the gastrointestinal tract in hereditary haemochromatosis accumulates in the parenchymal cells of the liver, the endocrine organs and, in severe cases, the heart.
- Hereditary haemochromatosis is usually caused by homozygous mutation C282Y of the *HFE* gene resulting in the HFE protein change and a low serum hepcidin level. Rarer forms are caused by mutations of other genes coding for proteins hemojuvelin, hepcidin, transferrin receptor 2 and ferroportin involved in iron regulation.
- Repeated venesections are used to reduce the body iron burden in hereditary haemochromatosis.
- Transfusional iron overload most frequently occurs in thalassaemia major, but also in other transfusion-dependent refractory anaemias, e.g. some cases of myelodysplastic neoplasias, sickle cell anaemia, primary myelofibrosis, red cell aplasia and aplastic anaemia.
- Transfusional iron overload causes damage to the liver, endocrine organs and heart, with iron accumulation also in macrophages of the reticuloendothelial system.
- Cardiac failure or arrhythmia caused by cardiac siderosis, best detected by T_2^* MRI, is the most frequent cause of death from transfusional iron overload.
- Treatment is with iron chelating drugs: deferasirox and deferiprone which are active orally, or with deferoxamine, given subcutaneously or intravenously.
- Life expectancy has improved dramatically in thalassaemia major as a result of iron chelation therapy and the use of T_2^* MRI to accurately measure cardiac and liver iron.



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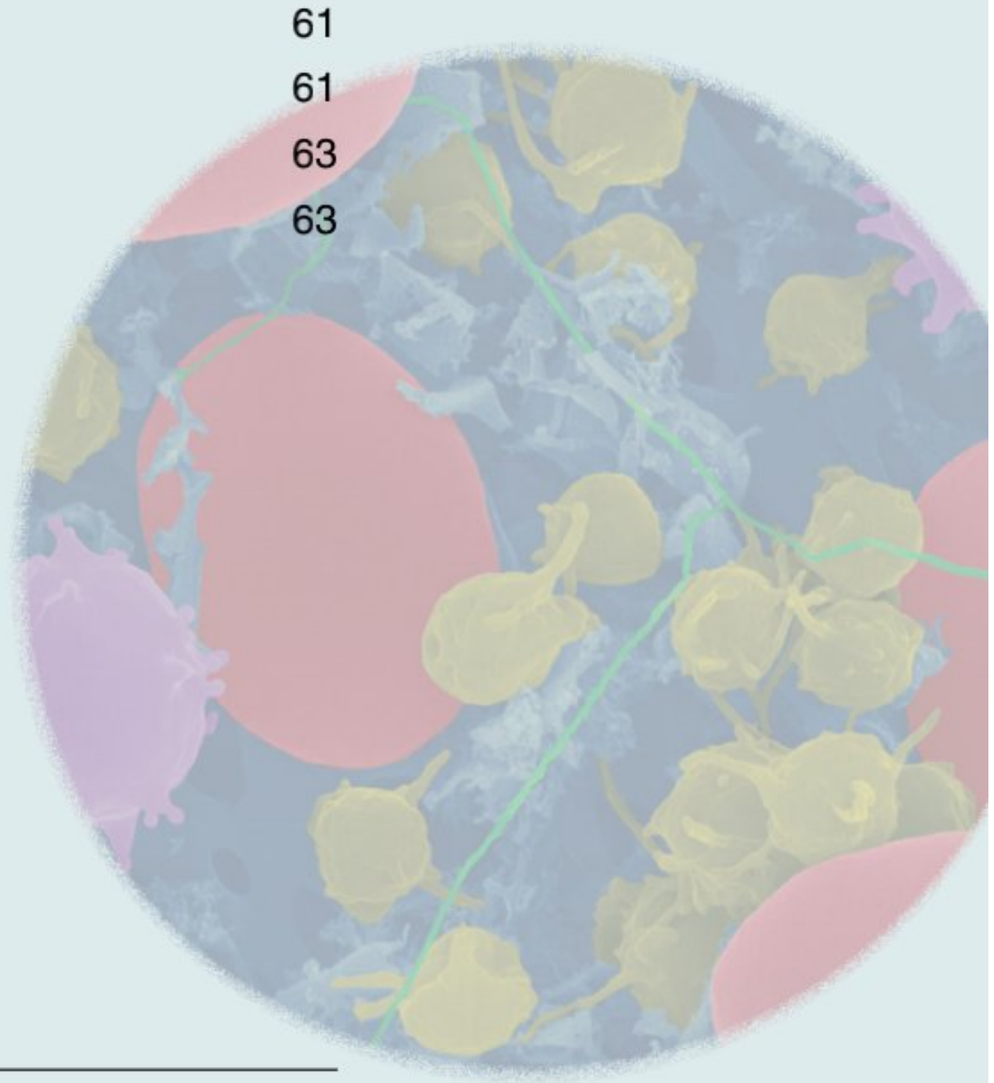


CHAPTER 5

Megaloblastic anaemias and other macrocytic anaemias

Key topics

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Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

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Introduction to macrocytic anaemia

In macrocytic anaemia, the red cells are abnormally large (mean corpuscular volume, MCV >98 fL). There are several causes (Table 2.5) broadly subdivided into megaloblastic and non-megaloblastic (Table 5.10), based on the appearance of developing erythroblasts in the bone marrow. An elevated MCV may also be an artefact reported by an automated cell counter if red cell agglutination or a paraprotein is present.

Megaloblastic anaemias

This is a group of anaemias in which the erythroblasts in the bone marrow show a characteristic abnormality: maturation of the nucleus is delayed relative to that of the cytoplasm. The underlying defect is defective DNA synthesis. This is usually caused by deficiency of vitamin B_{12} or folate. Less commonly, inherited or acquired, e.g. by drugs, abnormalities of the metabolism of these vitamins or inherited or acquired lesions in DNA synthesis may cause an identical haematological appearance (Table 5.1). Morphologically, this developmental asynchrony is manifest by a persistently open, loosely organized chromatin in the erythropoietic cell nucleus, while the cytoplasm exhibits staining changes of haemoglobinization typical of later stages of maturation (Fig 5.14).

Vitamin B_{12} (B_{12}, cobalamin)

Vitamin B_{12} is synthesized in nature by microorganisms. Animals acquire it by eating food of animal origin, by internal production from intestinal bacteria (not in humans) or by eating bacterially contaminated foods. Vitamin B_{12} consists of a small group of compounds, the cobalamins, which have the same basic structure, a cobalt atom at the centre of a corrin ring (Fig. 5.1). The reactive centre of the molecule is attached to either a cyano group (-CN, cyanocobalamin), a hydroxyl group (-OH, hydroxocobalamin), a methyl group (-CH_3, methylcobalamin) or 5-deoxyadenosyl (adenosylcobalamin). The vitamin is found in foods of animal origin such as liver, meat, fish and dairy produce, but does not occur in fruit, cereals or vegetables, except in small amounts due to contamination by insect parts in harvesting or by micro-organisms in a natural environment (Table 5.2).

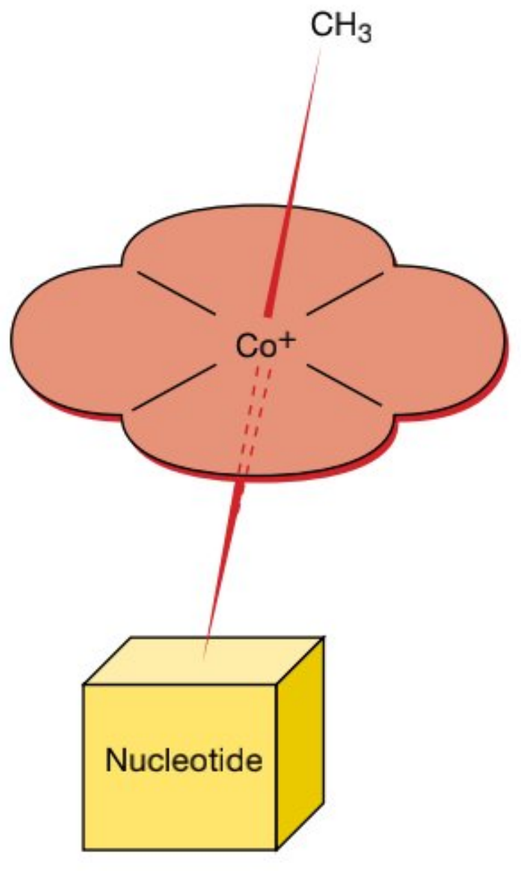


Figure 5.1 The structure of methylcobalamin, the main form of vitamin B_{12} in human plasma. Other forms include deoxyadenosylcobalamin, the main form in human tissues; hydroxocobalamin and cyanocobalamin, the main forms used in treatment of vitamin B_{12} deficiency and in multivitamin supplements.

Table 5.1 Causes of megaloblastic anaemia.

Vitamin B_{12} deficiency (causes are listed in Table 5.3)
Folate deficiency (causes are listed in Table 5.5)
Combined folate and B_{12} deficiency
Abnormalities of vitamin B_{12} or folate metabolism, e.g. transcobalamin deficiency, nitrous oxide, anti-folate drugs such as methotrexate, phenytoin or trimethoprim
Inherited defects of DNA synthesis
Congenital enzyme deficiencies, e.g. orotic aciduria, which impairs pyrimidine synthesis
Acquired enzyme deficiencies, e.g. due to hydroxyurea, purine synthesis antagonists such as 6-mercaptopurine, pyrimidine antagonists such as cytarabine

Absorption

A normal diet contains a large excess of B_{12} compared with daily needs (Table 5.2). B_{12} is released from protein binding in food by pepsin in the stomach. It is then mainly combined with the protein, **intrinsic factor (IF)**. IF is synthesized by the gastric parietal cells. The IF–B_{12} complex subsequently binds in the ileum to a specific surface receptor for IF, **cubam**, a complex of proteins cubilin and amnionless. Amnionless directs endocytosis of the cubilin IF–B_{12} complex into the ileal cell so that B_{12} is absorbed and IF destroyed (Fig. 5.2). The maximum amount of B_{12} that can be absorbed from a single oral dose (either in the form of food or a supplement) via the IF–cubam mechanism is about 1–2 µg.

Some dietary B_{12}, after release from food, binds to the glycoprotein haptocorrin (also known as R-factor, R-protein, or transcobalamin I) (Fig 5.2). This glycoprotein is present in plasma, milk, saliva and gastric juice. Release of dietary B_{12} from haptocorrin, making it available for binding to IF, depends largely on proteases from the pancreas.

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Table 5.2 Vitamin B_{12} and folate: nutritional aspects.

	Vitamin B_{12}	**Folate**
Typical daily dietary intake	7–30 μg	200–400 μg
Food sources	Animal products only	Many foods, especially liver, greens and yeast
Effect of cooking	Little effect	Easily destroyed
Minimal adult daily requirement	2 μg	100–200 μg
Body stores when replete	2–3 mg (sufficient for 2–4 years)	10–12 mg (sufficient for 4 months)
Absorption		
Site	Ileum	Duodenum and jejunum
Mechanism	Bound to intrinsic factor, absorbed by cubam	Conversion to methyltetrahydrofolate
Limit	2–3 μg/day	50–80% of dietary content
Enterohepatic circulation	5–10 μg/day	90 μg/day
Transport in plasma	Most bound to haptocorrin; TC essential for cell uptake	Weakly bound to albumin
Major intracellular physiological forms	Methyl- and deoxyadenosyl-cobalamin	Reduced polyglutamate derivatives
Usual therapeutic form	Hydroxocobalamin or cyanocobalamin	Folic (pteroylglutamic) acid

TC, transcobalamin (transcobalamin II); haptocorrin = transcobalamin 1.

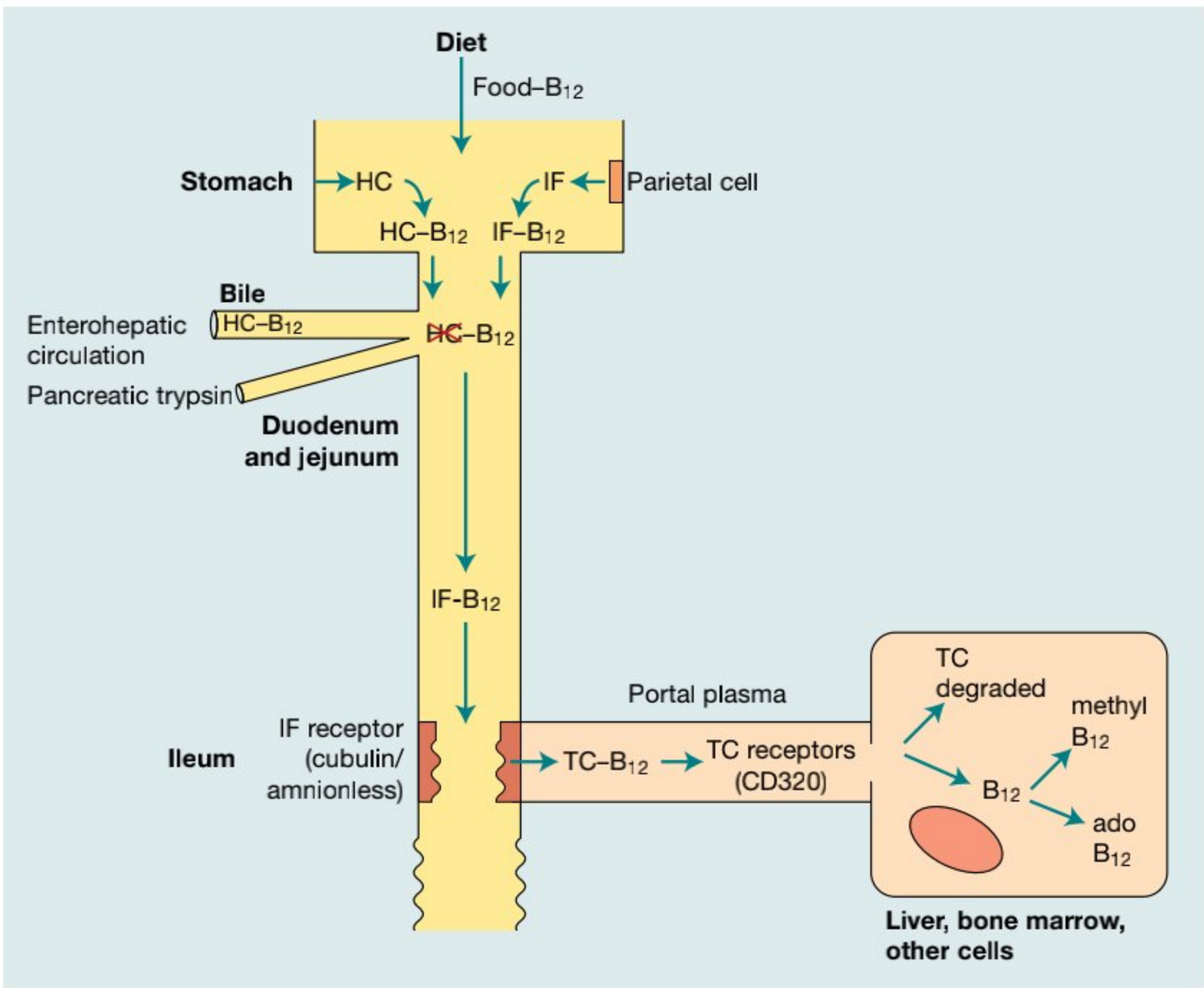


Figure 5.2 The absorption of dietary vitamin B_{12} after combination with intrinsic factor (IF), through the ileum. TC, transcobalamin; IF, intrinsic factor; HC, haptocorrin; ado B_{12}, see text.

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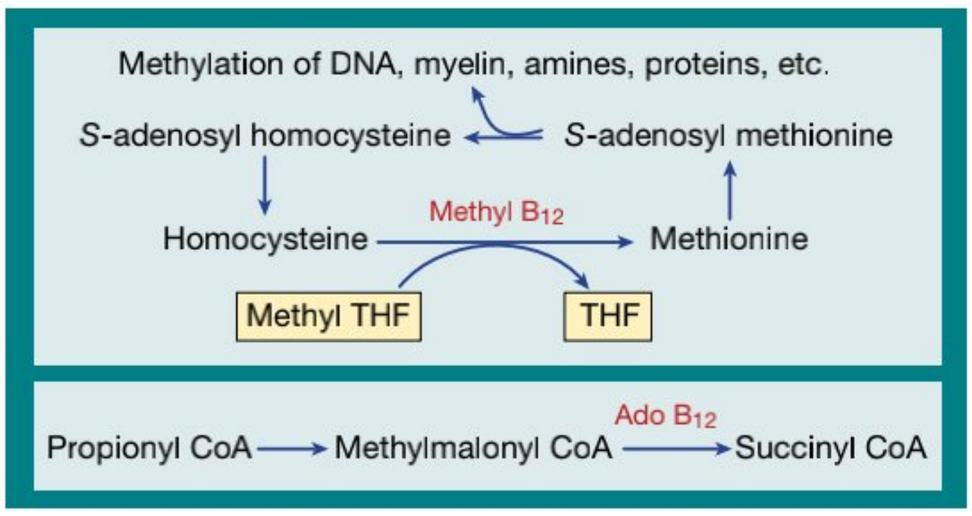


Figure 5.3 The biochemical reactions of vitamin B_{12} in humans. Ado B_{12}, deoxyadenosylcobalamin; CoA, coenzyme A; THF, tetrahydrofolate.

Transport of vitamin B_{12}: the transcobalamins

Vitamin B_{12} is absorbed from the ileal cell into portal blood, where it attaches to the plasma-binding protein transcobalamin (TC, also called transcobalamin II), which delivers B_{12} to the bone marrow and all other tissues. Although TC is the essential plasma protein for transferring B_{12} into the cells of the body, the amount of B_{12} on TC is normally very low (<50 ng/L).

Congenital TC deficiency due to germline mutations in the *TCN2* gene causes megaloblastic anaemia because of failure of B_{12} to enter marrow (and other cells) from plasma, but the serum B_{12} level in TC deficiency is normal. This is because most B_{12} in plasma is bound to haptocorrin. This glycoprotein is synthesized by granulocytes and macrophages. In myeloproliferative neoplasms where granulocyte production may be greatly increased, the haptocorrin and B_{12} levels in plasma both rise considerably. This also occurs in some liver diseases. B_{12} bound to haptocorrin in the blood does not transfer to marrow; the B12 is functionally 'dead'.

Biochemical functions

Vitamin B_{12} is a coenzyme for two biochemical reactions. First, as methyl B_{12} it is a cofactor for methionine synthase, the enzyme responsible for methylation of homocysteine to methionine which uses methyltetrahydrofolate (methylTHF) as methyl donor (Fig. 5.3). Second, as deoxyadenosyl B_{12} (ado B_{12}) it is coenzyme in the conversion of methylmalonyl coenzyme A (CoA) to succinyl CoA, a key intermediate in the citric acid cycle (Fig. 5.3).

Folate

Folic (pteroylglutamic) acid is the parent compound of a large group of compounds, the folates (Fig. 5.4). This family of compounds is also known as vitamin B9, a name which often appears on the packets of cereals that have been fortified with the vitamin.

Absorption, transport and function

Dietary folates are a complex mixture of variously reduced and polyglutamated folates. They are all converted to one compound, methylTHF, a reduced monoglutamate form which circulates in plasma (Fig. 5.5a). After entering cells, methylTHF is de-methylated to THF, and then converted to folate polyglutamate forms by addition of usually four, five or six glutamate moieties (Fig. 5.6). Folic (pteroylglutamic) acid itself is a poor substrate for reduction by dihydrofolate reductase. It is mainly converted during absorption to methylTHF at doses of 200–400 µg, larger oral doses of folic acid enter portal plasma unchanged and are then converted to physiological forms in the liver or excreted in the urine (Fig 5.5b).

Folates are needed in a variety of biochemical reactions in the body involving single carbon unit transfer, in amino acid interconversions, e.g. homocysteine conversion to methionine (Figs. 5.3 and 5.6) and serine to glycine, or in synthesis of pyrimidine and purine precursors of DNA.

Biochemical basis for megaloblastic anaemia

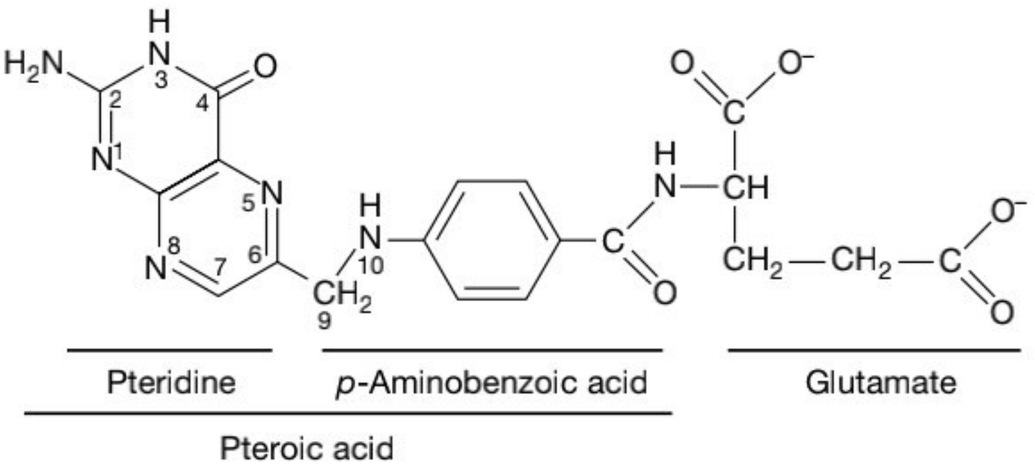
DNA is formed by polymerization of the four deoxyribonucleoside monophosphates derived from their triphosphates (Fig 5.6). Folate deficiency is thought to cause megaloblastic anaemia by limiting synthesis of thymidine monophosphate (dTMP) from deoxyuridine monophosphate, a rate-limiting step in DNA synthesis. This reaction needs 5,10-methylene THF polyglutamate as coenzyme. Consequent starvation of the precursor dTTP leads to prolongation of the S phase during mitosis, failure to form new double-stranded DNA and apoptotic cell death.

The role of B_{12} in DNA synthesis is indirect. B_{12} is needed in the conversion of methylTHF, which enters marrow and other cells from plasma, to THF and other active folates. In this reaction, homocysteine is converted to methionine. THF (but not methylTHF) is a substrate for folate polyglutamate synthesis. The folate polyglutamates are the intracellular folate coenzymes. B_{12} deficiency therefore reduces the supply of the critical folate coenzyme 5,10-methylene THF polyglutamate, needed for synthesis of thymidine monophosphate (dTMP) (Fig. 5.6). The block caused by B_{12} deficiency in the conversion of methylTHF either mono- or poly-glutamate, to other forms of folate has been termed the 'methylfolate trap'. Other congenital or acquired causes of megaloblastic anaemia, e.g. antimetabolite drug therapy, mainly inhibit purine or pyrimidine synthesis at one or another step. The result is a reduced supply of one or other of the four precursors needed for DNA synthesis.

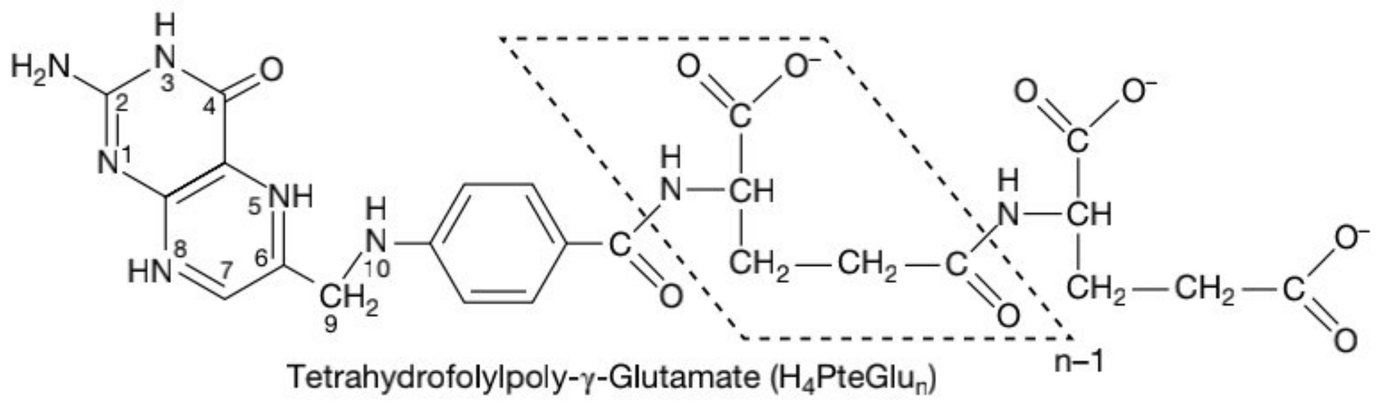
Folate reduction

During the synthesis of dTMP, the folate polyglutamate coenzyme becomes oxidized from the THF to the dihydrofolate (DHF) state (Fig. 5.6). The enzyme dihydrofolate reductase regenerates active THF from DHF. Inhibitors of this enzyme,

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Folic acid, Pteroylglutamate (PteGlu)



Tetrahydrofolylpoly-γ-Glutamate ($H_4PteGlu_n$)

One carbon substituent		Position	Oxidation state
Methyl	$-CH_3$	N-5	Methanol
Methylene	$-CH_2-$	N-5, N-10	Formaldehyde
Methenyl	$-CH=$	N-5, N-10	Formate
Formyl	$-CHO$	N-5 or N-10	Formate
Formimino	$HN=CH-$	N-5	Formate

Figure 5.4 The upper panel shows the chemical structure of folic acid (pteroylglutamic acid). The middle panel shows the structure of folic acid reduced to the tetrahydrofolate form and with one additional glutamic acid. The lower panel shows the five single carbon units that may be added to the folate molecule. Source: Courtesy of Professor Barry Shane.

e.g. methotrexate, inhibit folate-mediated biochemical reactions including in DNA synthesis (Fig. 5.6). Methotrexate is a useful drug, mainly in the treatment of malignant, e.g. acute lymphoblastic leukaemia (Chapter 17) or inflammatory disease, e.g. rheumatoid arthritis, psoriasis with excessive cell turnover. The weaker antagonist, pyrimethamine, is used primarily against toxoplasmosis. Trimethoprim, active against bacterial DHF reductase but only very weakly against the human enzyme, is used alone or in combination with a sulphonamide, as cotrimoxazole, especially to treat urinary tract infections. Toxicity caused by methotrexate or pyrimethamine may be reversed by the reduced folate, folinic acid (5-formyl THF). In the protocols used, this reversal does not eliminate the effectiveness of methotrexate when used in anti-cancer chemotherapy.

Causes of severe vitamin B_{12} deficiency

In developed countries, severe deficiency is usually caused by the auto-immune disease, pernicious anaemia (Table 5.3). Less commonly, severe deficiency may be caused by extreme lack of B_{12} in the diet (as in strict veganism without dietary supplements), total gastrectomy or small intestinal lesions. In vegetarians and people subsisting on a poor-quality diet low in B_{12}-rich foods, an intact entero-hepatic circulation usually but not invariably helps to protect them from severe B_{12} deficiency. The deficiency takes at least 2 years to develop, i.e. the time needed for body stores to deplete at the rate of 1–2 µg/day when there is severe malabsorption of B_{12}. There is no syndrome of B_{12} deficiency as a result of increased utilization or loss of the vitamin. Nitrous oxide, however, may rapidly inactivate body B_{12} (p. 63).

Pernicious anaemia

Pernicious anaemia (PA) is caused by autoimmune attack on the gastric mucosa, leading to atrophy of the stomach. The wall of the stomach becomes thin, with a plasma cell and lymphoid infiltrate of the lamina propria. Intestinal metaplasia may occur. Destruction of parietal cells results in achlorhydria and lack of secretion of IF. Serum gastrin levels are raised. *Helicobacter pylori* infection may initiate an autoimmune

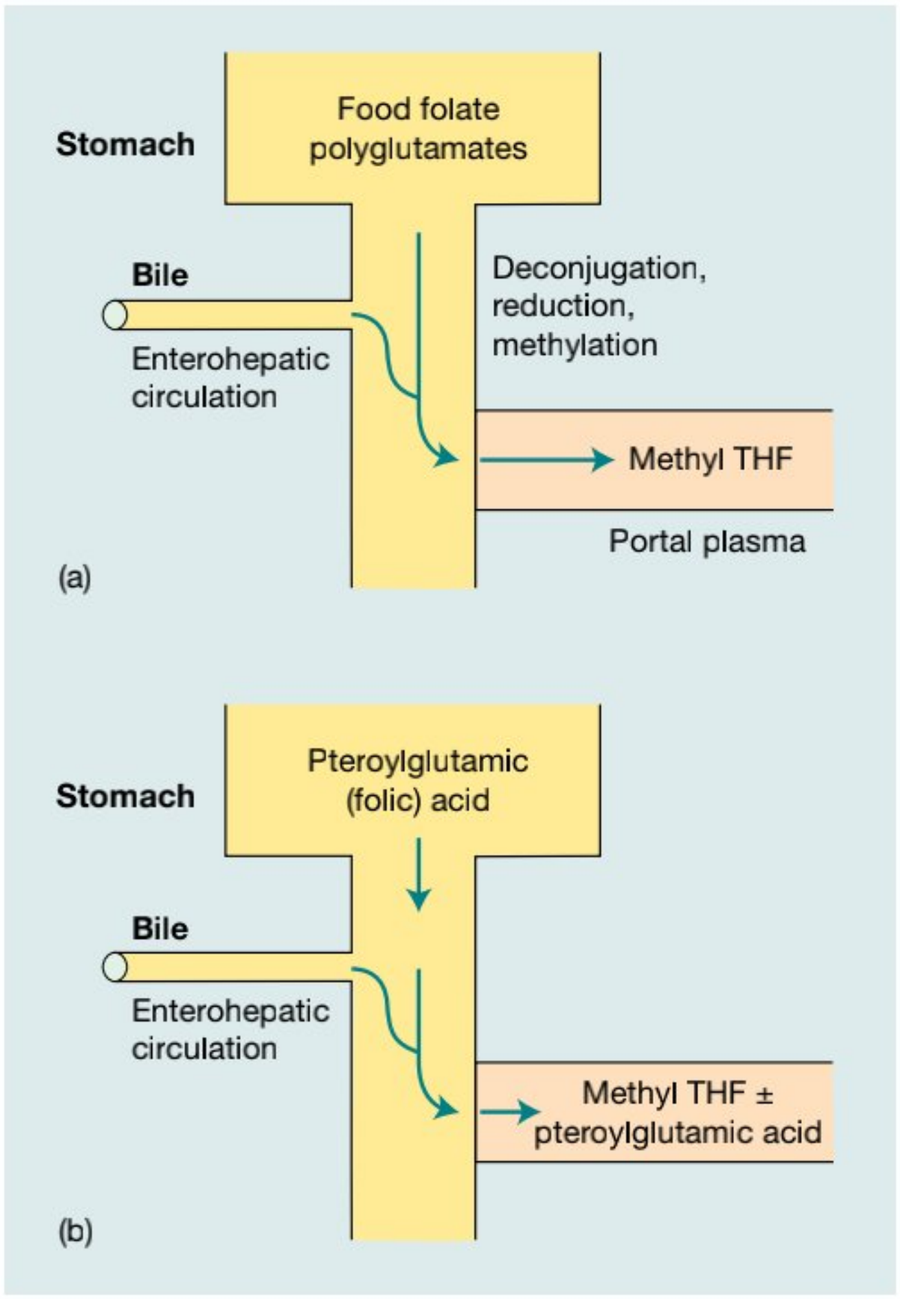


Figure 5.5 **(a)** The absorption of dietary folates; **(b)** The absorption of pteroylglutamic (folic) acid. At doses up to 400 μg, most pteroylglutamic (folic) acid is converted to methyltetrahydrofolate (methylTHF). At higher doses, unchanged pteroylglutamic acid enters portal plasma.

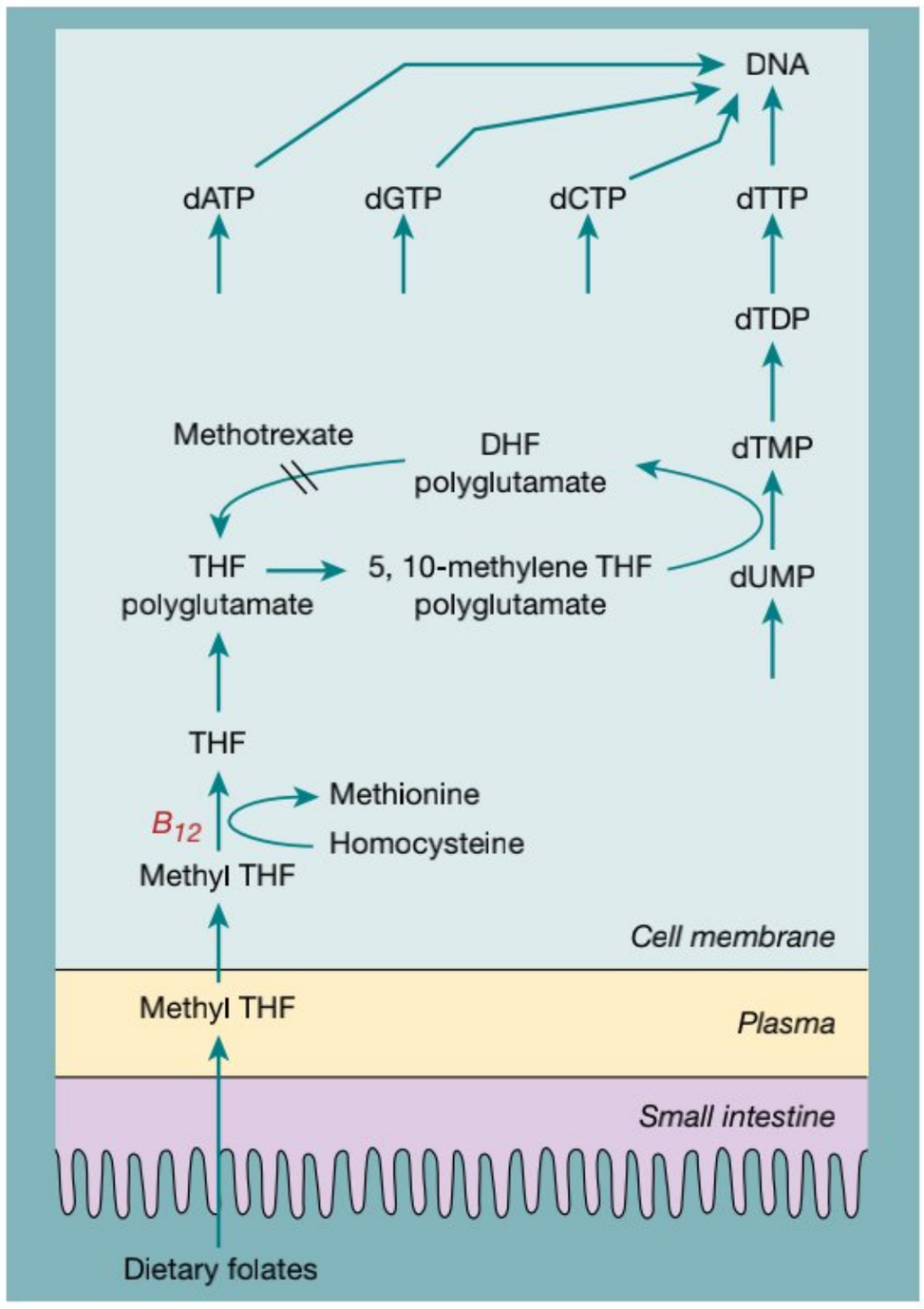


Figure 5.6 The biochemical basis of megaloblastic anaemia caused by vitamin B_{12} or folate deficiency. Folate is required in one of its coenzyme forms, 5,10-methylenetetrahydrofolate polyglutamate, in the synthesis of thymidine monophosphate from its precursor deoxyuridine monophosphate. Vitamin B_{12} is needed to convert methyltetrahydrofolate (methylTHF), which enters the cells from plasma, to THF, from which polyglutamate and other forms of folate are synthesized. Dietary folates are all converted to methylTHF (a monoglutamate) by the small intestine. The block in conversion of methylTHF to THF at the monoglutamate and polyglutamate levels and so to other forms of folate has been called the 'methylfolate trap'. A, adenine; C, cytosine; d, deoxyribose; DHF, dihydrofolate; DP, diphosphate; G, guanine; MP, monophosphate; T, thymine; TP, triphosphate; U, uracil.

gastritis, which presents in younger subjects as iron deficiency and in the elderly as PA.

More females than males are affected (1.6: 1), with a peak occurrence at 60 years, and there may be associated autoimmune disease, particularly thyroid diseases (Table 5.4). The disease is found in all races, but is most common in Northern Europeans. It tends to occur in families. There is also an increased incidence of carcinoma of the stomach (approximately 2–3% of all cases of pernicious anaemia).

Antibodies

Ninety per cent of patients with PA show in the serum parietal cell antibody directed against the gastric proton pump H^+/K^+-AT Pase. The antibody, however, is not specific for PA. PA is distinguished from simple atrophic (non-PA) autoimmune gastritis by the presence of antibodies to IF. **Fifty to seventy percent of PA patients show in serum an antibody to IF which inhibits IF binding to B_{12}**. An antibody blocking IF attachment to its ileal binding site is less frequent. IF antibodies also occur in PA in gastric juice, where they block any remaining IF binding B_{12}. IF antibodies are specific for PA, but as they occur in the serum of only half of patients, their absence from serum does not exclude the diagnosis. The more common parietal cell antibody is less specific, as it occurs quite commonly in older subjects, e.g. 16% of normal women over 60 years even without PA.

Other causes of severe vitamin B_{12} deficiency

Congenital lack or abnormality of IF due to mutations of its gene usually presents at approximately 2 years of age, when

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Table 5.3 Causes of severe vitamin B_{12} deficiency.

Nutritional
Especially strict vegans
Malabsorption
Gastric causes
Pernicious anaemia
Congenital lack or abnormality of intrinsic factor
Total or partial gastrectomy
Intestinal causes
Intestinal stagnant loop syndrome – jejunal diverticulosis, blind-loop, stricture, etc.
Chronic tropical sprue
Ileal resection and Crohn's disease
Congenital selective malabsorption with proteinuria (autosomal recessive megaloblastic anaemia)
Fish tapeworm

Table 5.4 Pernicious anaemia: associations.

Female	**Vitiligo**
Blue eyes	Myxoedema
Early greying	Hashimoto's disease
Northern European	Thyrotoxicosis
Familial	Addison's disease
Blood group A	Hypoparathyroidism Type I diabetes Hypogammaglobulinaemia Carcinoma of the stomach

stores of B_{12} derived from the mother *in utero* have been exhausted. Specific malabsorption of B_{12} is due to genetic mutation of the IF–B_{12} receptor proteins, cubilin or amnionless. It usually presents in infancy or childhood and is associated with proteinuria in 90% of cases. Infants born to and breast fed by B_{12}-deficient mothers may also develop symptomatic B_{12} deficiency.

Causes of mild vitamin B_{12} deficiency

The two most frequent causes worldwide of mild B_{12} deficiency, almost always insufficient to cause anaemia or a neuropathy, are an inadequate diet and malabsorption of food B_{12} due to atrophic gastritis (particularly in the elderly and with *Helicobacter* pylori infection). Less frequent causes are bariatric surgery, chronic pancreatitis, gluten-induced enteropathy, HIV infection, and prolonged treatment with proton pump inhibitors, metformin or cholestyramine. In the Zollinger–Ellison syndrome, the pH in the duodenum falls so low that pancreatic enzymes that normally release B_{12} from haptocorrin are inactivated. These malabsorption conditions do not usually lead to B_{12} deficiency sufficient to cause anaemia or neuropathy. In pregnancy, serum B_{12} levels may fall to below the normal range, but spontaneously return to normal after delivery (Chapter 34).

Table 5.5 Causes of folate deficiency.

Nutritional
Especially old age, institutions, poverty, famine, special diets, goat's milk anaemia etc.
Malabsorption
Tropical sprue, gluten-induced enteropathy (adult or child). Possible contributory factor to folate deficiency in some patients with partial gastrectomy, extensive jejunal resection or Crohn's disease
Excess utilization
Physiological
Pregnancy and lactation, prematurity
Pathological
Haematological diseases: haemolytic anaemias, myelofibrosis
Malignant disease: carcinoma, lymphoma, myeloma
Inflammatory diseases: Crohn's disease, tuberculosis, rheumatoid arthritis, psoriasis, exfoliative dermatitis, malaria
Excess urinary folate loss
Active liver disease, congestive heart failure
Drugs
Anticonvulsants, sulfasalazine
Mixed
Liver disease, alcoholism, intensive care

Folate deficiency

This is most often a result of a poor dietary intake of folate alone or in combination with a condition of increased folate utilization or malabsorption (Table 5.5). Excess cell turnover of any sort, including pregnancy, is a cause of an increased need for folate. Some of the folate coenzyme involved in synthesis of thymidine monophosphate is degraded at the C_9—N_{10} bond during the reaction. Consequently when DNA synthesis and so thymidine synthesis is increased, destruction of folate in increased. The mechanism by which anticonvulsants and barbiturates cause the deficiency is controversial.

Clinical features of megaloblastic anaemia

The onset is usually insidious, with gradually progressive symptoms and signs of anaemia (Chapter 2). The patient may be mildly jaundiced (lemon yellow; Fig. 5.7) because of the

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Figure 5.7 Megaloblastic anaemia: pallor and mild icterus in a patient with a haemoglobin count of 70.0 g/L and a mean corpuscular volume of 132 fL.



Figure 5.8 Megaloblastic anaemia: glossitis – the tongue is beefy red and painful.



Figure 5.9 Megaloblastic anaemia: angular cheilosis (stomatitis).

excess breakdown of haemoglobin resulting from increased ineffective erythropoiesis in the bone marrow. Glossitis (a beefy-red sore tongue; Fig. 5.8), angular cheilosis (Fig. 5.9) and mild symptoms of malabsorption with loss of weight because of the epithelial abnormality may be present. Purpura as a result of thrombocytopenia and widespread melanin pigmentation (the cause of which is unclear) are less frequent presenting features (Table 5.6). Many asymptomatic patients are diagnosed when a blood count performed for another reason reveals macrocytosis.

Vitamin B_{12} neuropathy (subacute combined degeneration of the cord)

Severe B_{12} deficiency can cause a progressive neuropathy affecting the peripheral sensory nerves and posterior and lateral spinal columns (Fig. 5.10). The neuropathy is symmetrical and affects the lower more than the upper limbs. The patient notices tingling in the feet, difficulty in walking, and may fall over in the dark due to loss of positional sense. Rarely, optic atrophy or severe psychiatric symptoms are present. Anaemia may be severe, mild or even absent. The

Table 5.6 Effects of vitamin B_{12} or folate deficiency.

Megaloblastic anaemia
Macrocytosis of epithelial cell surfaces
Neuropathy (for B_{12} deficiency only)
Sterility
Rarely, reversible melanin skin pigmentation
Decreased osteoblast activity
Neural tube defects in the foetus

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Figure 5.10 Cross-section of the spinal cord in a patient who died with subacute combined degeneration of the cord (Weigert–Pal stain). There is demyelination of the dorsal and dorsolateral columns.

duration and severity of the neurological defects predict the outcome. The peripheral neuropathy is usually reversible with B_{12} therapy, but spinal cord recovery is usually incomplete, especially if the neuropathy has been present for more than a few months. Prolonged deficiency in infants impairs motor function, which also may be irreversible.

The cause of the neuropathy is likely to be related to the accumulation of *S*-adenosyl homocysteine and reduced levels of *S*-adenosyl methionine in nervous tissue, resulting in defective methylation of myelin and other substrates.

Neural tube defect

Folate deficiency in the mother predisposes to the neural tube defects (NTDs) anencephaly, spina bifida or encephalocoele in the foetus. The neural tube is formed in the foetus from a plate of cells in the early embryo. It gives rise to the brain and spinal cord and their covering skull and spinal bones. The defect can affect the head when part or all the brain and skull are missing. More frequently it is the spine which is defective with failure of closure, often with protrusion of the spinal cord and its covering meninges in the lower back region (Fig. 5.11). The abnormality may be detected by ultrasound during the pregnancy when a therapeutic abortion can be performed. The foetus may also die *in utero* or soon after birth. An infant born with spina bifida often has paralysis of the lower limbs and may have urinary and faecal incontinence. Hydrocephalus due to obstruction of flow of cerebrospinal fluid is another serious complication.

The lower the maternal serum or red cell folate (or serum $B_{12)}$ levels, the higher the incidence of NTDs (Fig. 5.12). Moreover, supplementation of the diet with folic acid at the time of conception and in early pregnancy reduces the incidence of NTD by up to 83%. The exact mechanism is uncertain, but is thought to be related either to a fault in DNA synthesis or to the build-up of homocysteine and *S*-adenosyl homocysteine in the foetus, which may impair methylation of various proteins and lipids. A common polymorphism (677C→T) in the enzyme 5,10-methylene tetrahydrofolate reductase (5,10-MTHFR), which reduces conversion of 5,10-MTHF to methylTHF, results in higher serum homocysteine and lower serum and red cell folate levels compared with controls. The incidence of the mutation is higher in foetuses with NTD than in controls.



Figure 5.11 A baby with neural tube defect (spina bifida).
Source: Courtesy of Professor C.J. Schorah.

Other tissue abnormalities

Sterility is frequent in either sex with severe B_{12} or folate deficiency. Macrocytosis, excess apoptosis and other morphological abnormalities of cervical, buccal, bladder and other epithelia occur. Widespread reversible melanin pigmentation may also occur. B_{12} deficiency is associated with reduced osteoblastic activity.

Raised serum homocysteine levels and low serum or red cell folate and the polymorphism in the MTHFR enzyme (see

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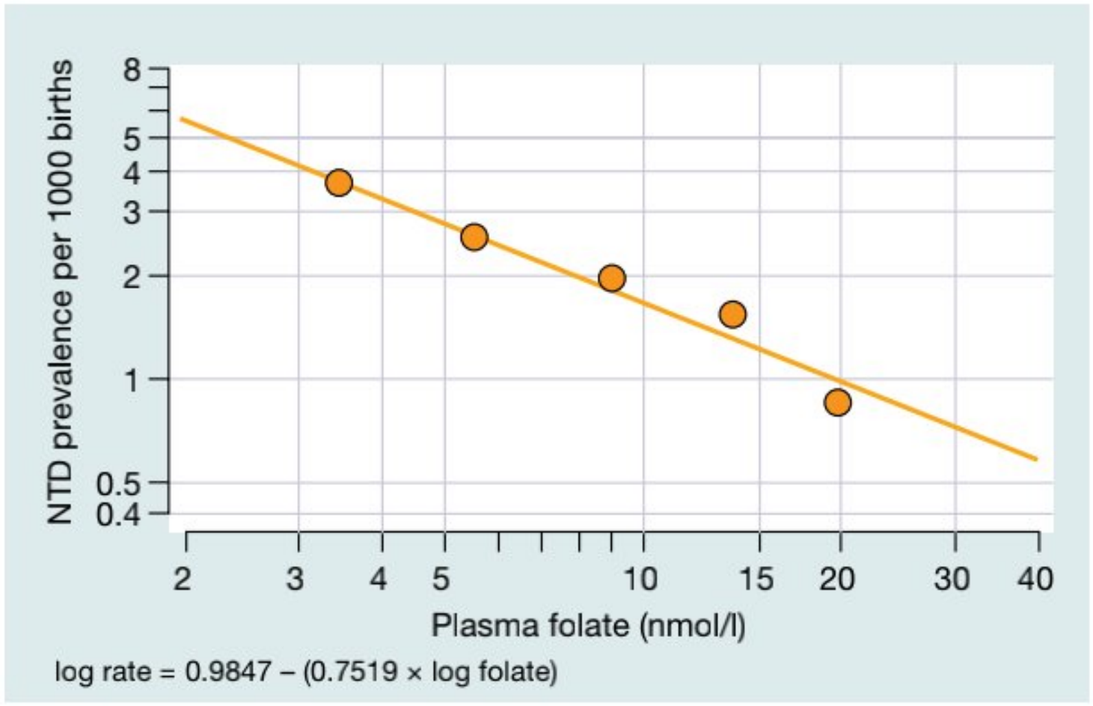


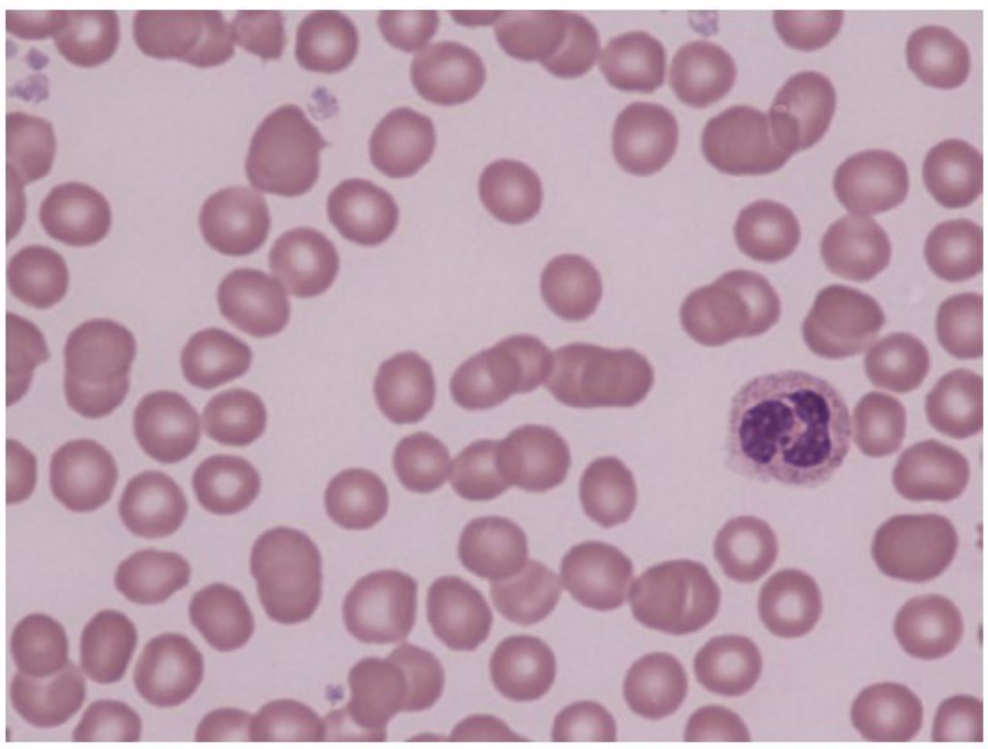
Figure 5.12 Linear relation between maternal plasma folate level and prevalence of a neural tube defect (NTD) birth. The lower the plasma folate, the higher the prevalence of an NTD pregnancy with no threshold. A similar linear relation is found between maternal red cell folate and prevalence of NTD births. 10 nmol/L = 3.8 μg/L. Source: N.J. Wald *et al*. (1998) Folic acid food fortification to prevent neural tube defects. *Lancet* 351: 834. N.J. Wald, J. Noble. In C.H. Rodeck, M.J. Whittle (eds) (1999) *Primary Prevention of Neural Tube Defects in Fetal Medicine: Basic Science and Clinical Practice*. London: Churchill Livingstone, pp. 283–90. L.E. Dale *et al*. (1995) Folate levels and neural tube defects: implications for prevention. *Journal of the American Medical Association* 274: 215–9.

above) have been associated with an increased incidence of cardiovascular diseases, including myocardial infarct, peripheral vascular diseases, stroke and venous thrombosis (Chapter 30). Folic acid prophylaxis, however, has not reduced the incidence of the arterial diseases or cardiovascular events, except for stroke in hypertensive subjects, where a reduction of 15% has been shown in large-scale controlled studies in China.

Various associations have been found between folate status and malignant diseases, but meta-analysis of subjects randomized to take folic acid or placebo in trials lasting two years or more does not show any difference in cancer incidence between those taking folic acid and the controls.

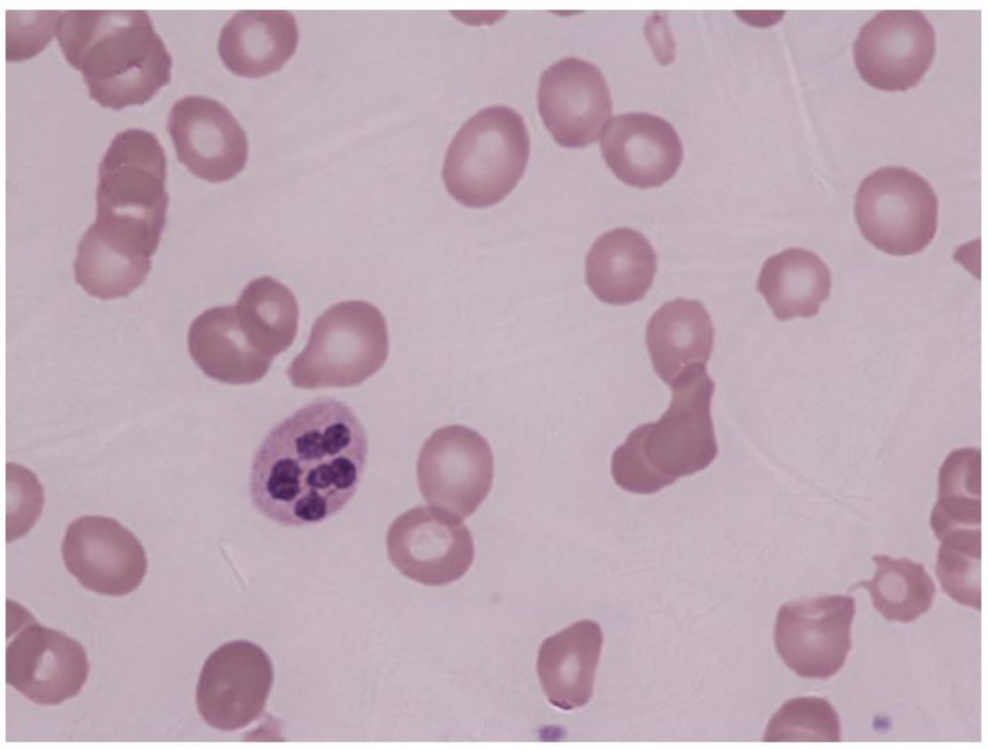
Laboratory findings

The anaemia is macrocytic (MCV >98 fL by definition and often as high as 120–140 fL in severe cases). The macrocytes are typically oval (Fig. 5.13a,b). In severe cases, megaloblasts



(a)

Figure 5.13(a) Normal blood film. The red cells are similar in shape (circular) and size. The white cell (neutrophil) to the right of the film has a nucleus with two major lobes.



(b)

Figure 5.13(b) Blood film from a patient with severe megaloblastic anaemia. Compared to a normal blood film at the same magnification, the red cells are larger and fewer in number. Many of the red cells are oval and misshapen. The neutrophil to the left of the centre has a nucleus with six lobes. Source: Courtesy (a) and (b) *Professor Barbara Bain*.

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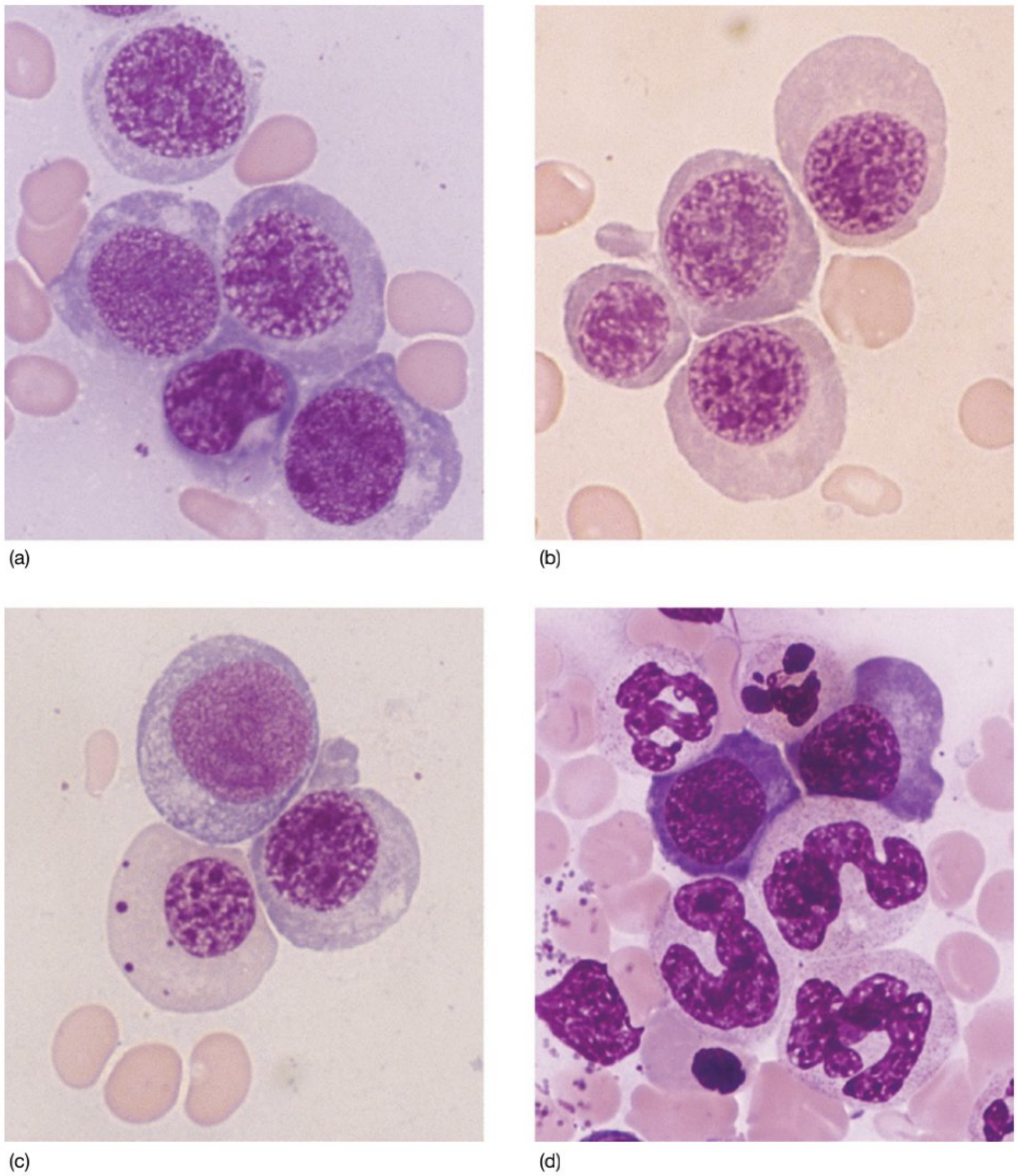


Figure 5.14 Megaloblastic changes in the bone marrow in a patient with severe megaloblastic anaemia. **(a–c)** Erythroblasts showing fine, open stippled (primitive) appearance of the nuclear chromatin even in late cells (pale cytoplasm with some haemoglobin formation). **(d)** Abnormal giant metamyelocytes and band forms.

may appear in the peripheral blood due to extramedullary haemopoiesis. If iron deficiency is also present the MCV may be normal, but the red cell distribution width (RDW) in such cases will be very wide and the blood film dimorphic, with distinct populations of large and small cells. The reticulocyte count is low and the total white cell and platelet counts may be reduced, especially in severely anaemic patients. **A proportion of the neutrophils show hypersegmented nuclei (six or more nuclear lobes)**. The bone marrow is usually hypercellular and the erythroblasts are large and show an open, fine, lacy primitive chromatin pattern despite normal cytoplasmic haemoglobinization (Fig. 5.14). Giant and abnormally shaped metamyelocytes are characteristic.

The serum unconjugated bilirubin and lactate dehydrogenase are raised as a result of marrow cell breakdown (ineffective haemopoiesis).

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Table 5.7 Laboratory tests for vitamin B_{12} and folate deficiency.

			Result in	
Test	**Normal values***		**Vitamin B_{12} deficiency**	**Folate deficiency**
Serum vitamin B_{12}	160–925 ng/L	120–680 pmol/L	Low	Normal or borderline low
Serum folate	3.0–15.0 μg/L	4–30 nmol/L	Normal or raised	Low
Red cell folate	160–640 μg/L	360–1460 nmol/L	Normal or low	Low

*Normal values differ with different commercial kits.

Diagnosis of vitamin B_{12} or folate deficiency

It is usual to assay serum B_{12} and folate and in many laboratories also red blood cell folate (Table 5.7). The serum B_{12} is low in megaloblastic anaemia or neuropathy caused by B_{12} deficiency. In B_{12} deficiency, the serum folate tends to rise but the red cell folate falls. In the absence of B_{12} deficiency, however, the red cell folate is a more accurate guide to tissue folate status than the serum folate. Serum and red cell folate are both low in megaloblastic anaemia caused by folate deficiency. Serum folate in contrast to red cell folate is more labile, and even one or two nutritious meals in a hospitalized patient admitted with severe deficiency may normalize serum folate. However, red cell folate will remain low for some time.

Measurement of serum or urine methylmalonic acid is a test for B_{12} deficiency and measurement of homocysteine is a test for either folate or B_{12} deficiency. These are not specific, however, and it is difficult to establish normal levels in different age groups. These biochemical tests are also not widely available.

Tests for cause of vitamin B_{12} or folate deficiency

Useful tests are listed in Table 5.8. For B12 deficiency these are mainly concerned with assessing gastric function and testing for antibodies to gastric antigens. In all cases of pernicious anaemia, endoscopy studies should be performed at diagnosis to confirm the presence of gastric atrophy and exclude carcinoma of the stomach. For folate deficiency, the dietary history is most important, although it is difficult to estimate folate intake accurately. Unsuspected gluten-induced enteropathy or other underlying conditions should also be considered (Table 5.5).

Table 5.8 Tests for cause of vitamin B_{12} or folate deficiency.

Vitamin B_{12}	**Folate**
Diet history	Diet history
Serum gastrin	Tests for intestinal malabsorption
IF, parietal cell antibodies	Anti-transglutaminase and endomysial antibodies
Endoscopy	Duodenal biopsy Underlying disease

IF, intrinsic factor.

Treatment

Most cases only need therapy with the appropriate vitamin (Table 5.9). Patients with megaloblastic anaemia or neuropathy due to B_{12} deficiency should be treated initially with injections of B_{12}. If large doses of folic acid, e.g., 5 mg/day are given in B_{12} deficiency they cause a haematological response, but will allow a neuropathy to appear or progress as the B12 deficiency progresses. Folic acid in 5mg doses should therefore not be given alone for any prolonged period unless B_{12} deficiency has been excluded. In severely anaemic patients who need treatment urgently, it may be safer to initiate treatment with both vitamins after blood has been taken for B_{12} and folate assays. In the elderly, the presence of heart failure should be corrected with diuretics. Blood transfusion should be avoided if possible, as it may cause circulatory overload.

Parenteral B_{12} therapy is usually used for subsequent lifelong maintenance (Table 5.9). Large oral doses are needed daily to achieve sufficient B_{12} absorption for maintenance in PA and compliance with long-term therapy may be a problem. Oral therapy is more appropriate for those with mild degrees of B_{12} deficiency, e.g. dietary deficiency or with malabsorption of food B_{12}.Also for those with such disorders as haemophilia which make injections unsafe.

For folate deficiency, an oral dose of 5 mg daily is indicated initially, with parenteral therapy reserved for those receiving parenteral nutrition. It is usual to continue for 4 months and then to decide whether or not to continue this long term (Table 5.9).

Response to therapy

The patient usually begins to feel better after 24–48 hours of correct vitamin therapy, with increased appetite and well-being. The haemoglobin should rise by 20–30 g/L each fortnight. The white cell and platelet counts become normal in 7–10 days (Fig. 5.15) and the marrow is normoblastic in about 48 hours, although giant metamyelocytes persist for up to 12 days.

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Table 5.9 Treatment of megaloblastic anaemia.

	Vitamin B_{12} deficiency	Folate deficiency
Compound	Hydroxocobalamin*	Folic acid
Route	Intramuscular**	Oral
Dose	1000 µg	5 mg
Initial dose	6 × 1000 µg over 2–3 weeks	Daily for 4 months
Maintenance	1000 µg every 3 months*; usually life-long or daily large (500–1000 µg) oral doses of B_{12}	Depends on underlying disease; life-long therapy e.g. 5 mg once weekly may be needed in chronic inherited haemolytic anaemias, myelofibrosis, renal dialysis
Prophylactic	Following total gastrectomy or ileal resection Daily oral B_{12} for vegans and in developing countries during pregnancy and lactation	Pregnancy, severe haemolytic anaemias, dialysis, prematurity In over 80 countries the diet (grain, flour or rice) is fortified with folic acid to reduce the incidence of neural tube defect affected pregnancies and births

* In the USA cyanocobalamin is commonly used and maintenance B_{12} injections are then given monthly.
** Some clinicians recommend daily oral or sublingual therapy of vitamin B_{12} deficiency (see text).

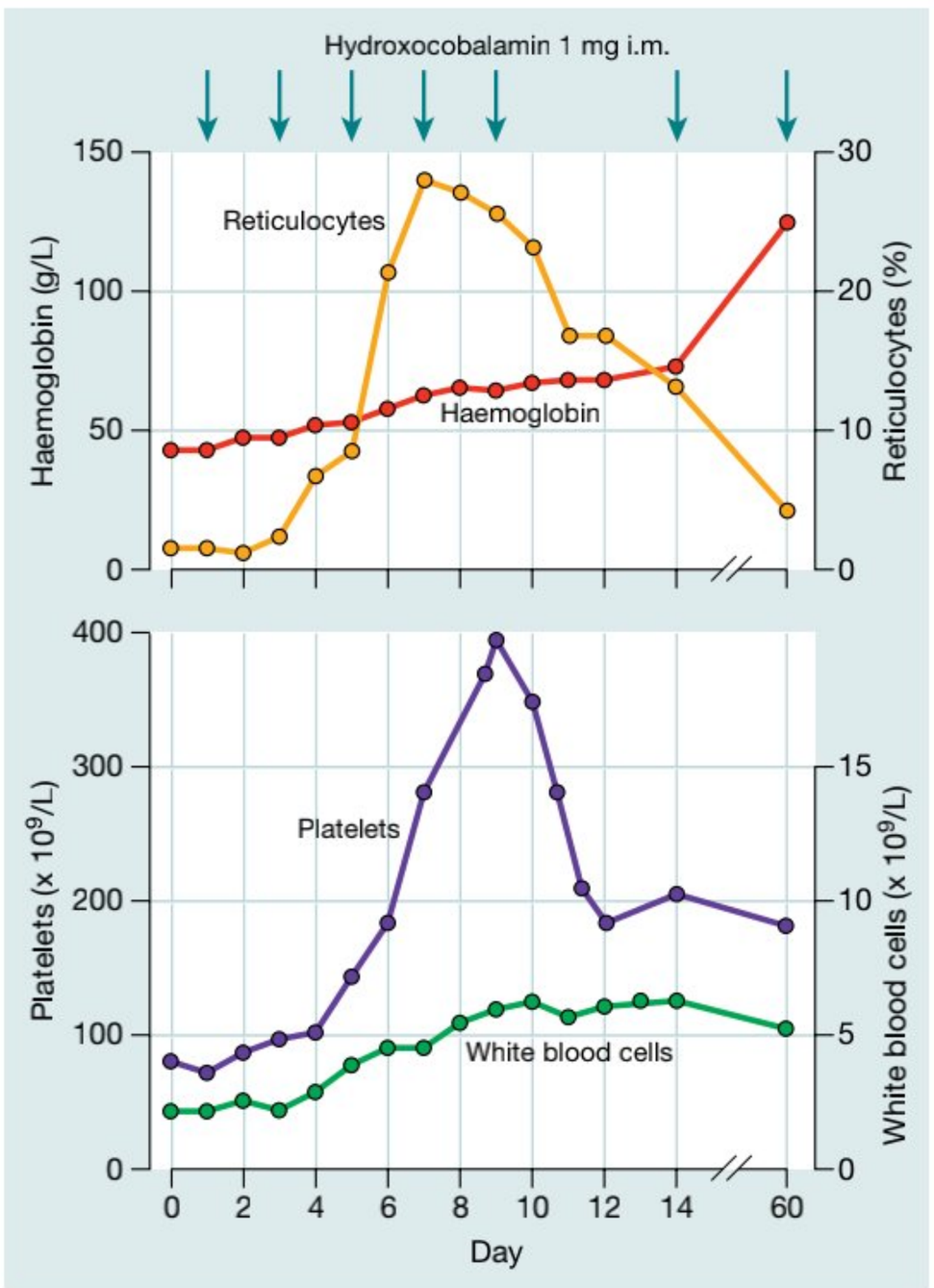


Figure 5.15 Typical haematological response to vitamin B_{12} (hydroxocobalamin) therapy in pernicious anaemia.

Prophylactic therapy

Vitamin B_{12} is given 3-monthly to those who have had a total gastrectomy or ileal resection. Oral supplements and consumption of dairy products or of foods fortified with B_{12} are recommended for vegetarians and those living because of poverty on a diet low in B_{12}-rich foods. In developing countries oral B_{12} supplements are recommended for all pregnant and lactating women.

Folic acid is given before and in pregnancy for the first 3 months at a typical dosage of 400 µg/day to reduce the risk of an NTD. The neural tube is formed within the first 4–6 weeks of gestation. In the UK a larger dose 5 mg daily is recommended throughout pregnancy for those considered at high risk e.g. having had a previous NTD pregnancy, with diabetes or sickle cell disease. This two dose recommendation seems unnecessary; all women should take the larger dose if they may become pregnant and in the first 3 months of pregnancy to get the greater benefit. All women of child-bearing age are also recommended to have an intake of at least 400 µg/day (by increased intake of folate-rich or folate-supplemented foods or as folic acid) to prevent a first occurrence of an NTD foetus. There have been no trials aimed at showing whether vitamin B12 fortification of the diet would also be beneficial in reducing the incidence of NTD pregnancies

Food fortification with folic acid, e.g., 150–220 ug/100 g of flour or grain is mandated in about 80 countries (including North America since 1998) and has reduced the prevalence of NTDs with no untoward effects reported. Folic acid supplementation of foods has not been approved in Europe though in 2021, the UK announced that it intended to introduce this. Many breakfast cereals are voluntarily fortified with folic acid by the manufacturers.

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Folic acid is also given to patients undergoing chronic dialysis, with severe chronic haemolytic anaemias, e.g. sickle cell anaemia, and to premature babies.

Other megaloblastic anaemias

The most frequent (Table 5.1) causes are drugs inhibiting DNA synthesis, e.g. hyroxycarbamide (hydroxyurea), cytarabine, or drugs inhibiting dihydrofolate reductase, e.g. methotrexate or pyrimethamine. Myelodysplastic neoplasias are also a relatively frequent cause in the elderly. Congenital deficiencies of enzymes involved in DNA synthesis, e.g. orotic aciduria, are extremely rare.

Abnormalities of vitamin B_{12} or folate metabolism

These include rare congenital deficiencies of enzymes concerned in B_{12} or folate intracellular trafficking or metabolism, or of the serum transport protein for B_{12}, TC. Nitrous oxide (N_2O) anaesthesia causes rapid inactivation of body B_{12} by oxidizing the reduced cobalt atom of methyl B_{12}. Megaloblastic marrow changes occur with several days of N_2O administration and can cause pancytopenia. Chronic exposure (as in dentists and anaesthetists) has been associated with neurological damage resembling B_{12} deficiency neuropathy. Antifolate drugs, particularly those which inhibit DHF reductase, e.g. methotrexate and pyrimethamine, may also cause megaloblastic change.

Other macrocytic anaemias

There are many non-megaloblastic causes of macrocytic anaemia (Table 5.10). The exact mechanism creating large red cells in each of these conditions is not clear, although increased lipid deposition on the red cell membrane or alterations of erythroblast maturation time in the marrow may be implicated. Alcohol is the most frequent cause of a raised MCV in the absence of anaemia. Reticulocytes are bigger than mature red cells, so haemolytic anaemia is a cause of macrocytic anaemia. Antimetabolite drugs such as hydroxycarbamide (Table 12.1) cause macrocytosis and the marrow may show megaloblastic changes. The other underlying conditions listed in Table 5.10 are usually easily diagnosed provided that they are considered and the appropriate investigations to exclude B_{12} or folate deficiency are carried out.

Table 5.10 Causes of macrocytosis other than megaloblastic anaemia.

Alcohol
Liver disease
Myxoedema
Myelodysplastic neoplasias; VEXAS (see page xxx)
Antimetabolite drugs, e.g. hydroxycarbamide
Aplastic anaemia
Pregnancy
Smoking
Reticulocytosis
Myeloma and paraproteinaemia
RBC agglutination (artefactual)

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 symmetrical 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, leucopenia and thrombocytopenia in megaloblastic anaemia, and the bone marrow appearance. Assays of serum B_{12} and folate are 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). The bone marrow erythropoiesis may be megaloblastic or normoblastic.
- Vitamin B_{12} or folate deficiency causes megaloblastic anaemia, in which the bone marrow erythroblasts have a typical abnormal appearance with delayed maturation of the nucleus compared to the degree of cytoplasmic haemoglobinization.
- Folates take part in biochemical reactions in DNA synthesis. B_{12} has an indirect role in DNA synthesis by its involvement in folate metabolism.
- B_{12} deficiency may also cause a neuropathy due to damage to the spinal cord and peripheral nerves.
- Severe B_{12} deficiency is usually caused by B_{12} malabsorption due to pernicious anaemia in which there is autoimmune gastritis, resulting in failure of synthesis of intrinsic factor, a protein 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

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by 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.
- Folate deficiency predisposes to neural tube defect (NTD) affected pregnancies. Supplements of folic acid before and during the early weeks of pregnancy and fortification of the diet with folic acid in about 80 countries that have mandated for this have successfully reduced the incidence of NTD affected pregnancies and births.
- 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.
- Causes of macrocytic red cells with or without anaemia and usually with normoblastic erythropoiesis include alcohol, liver disease, hypothyroidism, myelodysplasia, paraproteinaemia, cytotoxic drugs, aplastic anaemia, pregnancy and the neonatal period. In the non-anaemic patient, alcohol is the most frequent cause.



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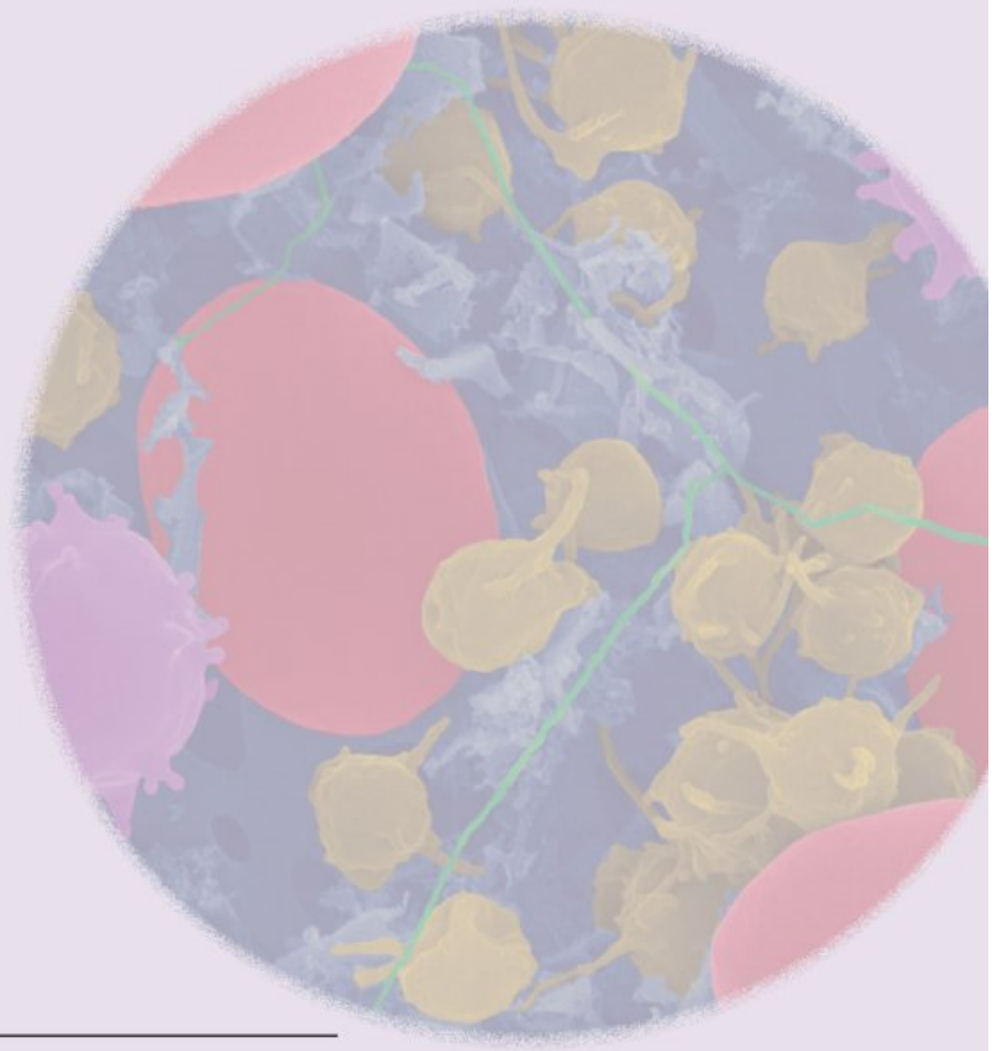


CHAPTER 6

Haemolytic anaemias

Key topics

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- Introduction to haemolytic anaemias 67
- Intravascular and extravascular haemolysis 68
- Hereditary haemolytic anaemias 69
- Acquired haemolytic anaemias 74



Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

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Normal red cell destruction

Normal (physiological) red cell destruction occurs after a mean life span of 120 days. Aged cells are removed extravascularly by the macrophages of the reticuloendothelial (RE) system, especially in the marrow but also in the liver and spleen. As red cells have no nucleus and therefore cannot synthesize new RNA and proteins, cell metabolism gradually deteriorates as enzymes are degraded, and the cells also become stiffer and less deformable in the microcirculation when structural proteins are degraded. Eventually senescent red cells become non-viable.

Breakdown of haem from haemoglobin liberates iron which macrophages export via ferroportin bound to plasma transferrin into plasma for recirculation. Protoporphyrin, the organic ring into which ferrous iron is embedded to form haem, is broken down in the macrophages to biliverdin and then to bilirubin. Bilirubin, which is lipid soluble, circulates bound to albumin to the liver where it is conjugated to glucuronides to make it water soluble and facilitate its excretion. Bilirubin glucuronides are excreted via bile into the gut where they are converted to stercobilinogen and stercobilin, which are brown in colour and excreted in faeces (Fig. 6.1). Stercobilinogen and stercobilin are partly reabsorbed after reduction by bacterial action in the intestines to urobilinogen and urobilin (also known as urochrome) which are excreted in the urine, urobilin being responsible for urine's yellow colour. Globin chains of haemoglobin are broken down to individual amino acids, which are reutilized for general protein synthesis in the body.

Haptoglobins are proteins in normal plasma which bind haemoglobin if either intravascular or significant extravascular haemolysis is present. The haemoglobin–haptoglobin complex is removed from plasma by the RE system. Intravascular haemolysis, i.e. the breakdown of red cells within blood vessels, plays little or no part in normal red cell destruction, but is important in some pathological states, discussed later.

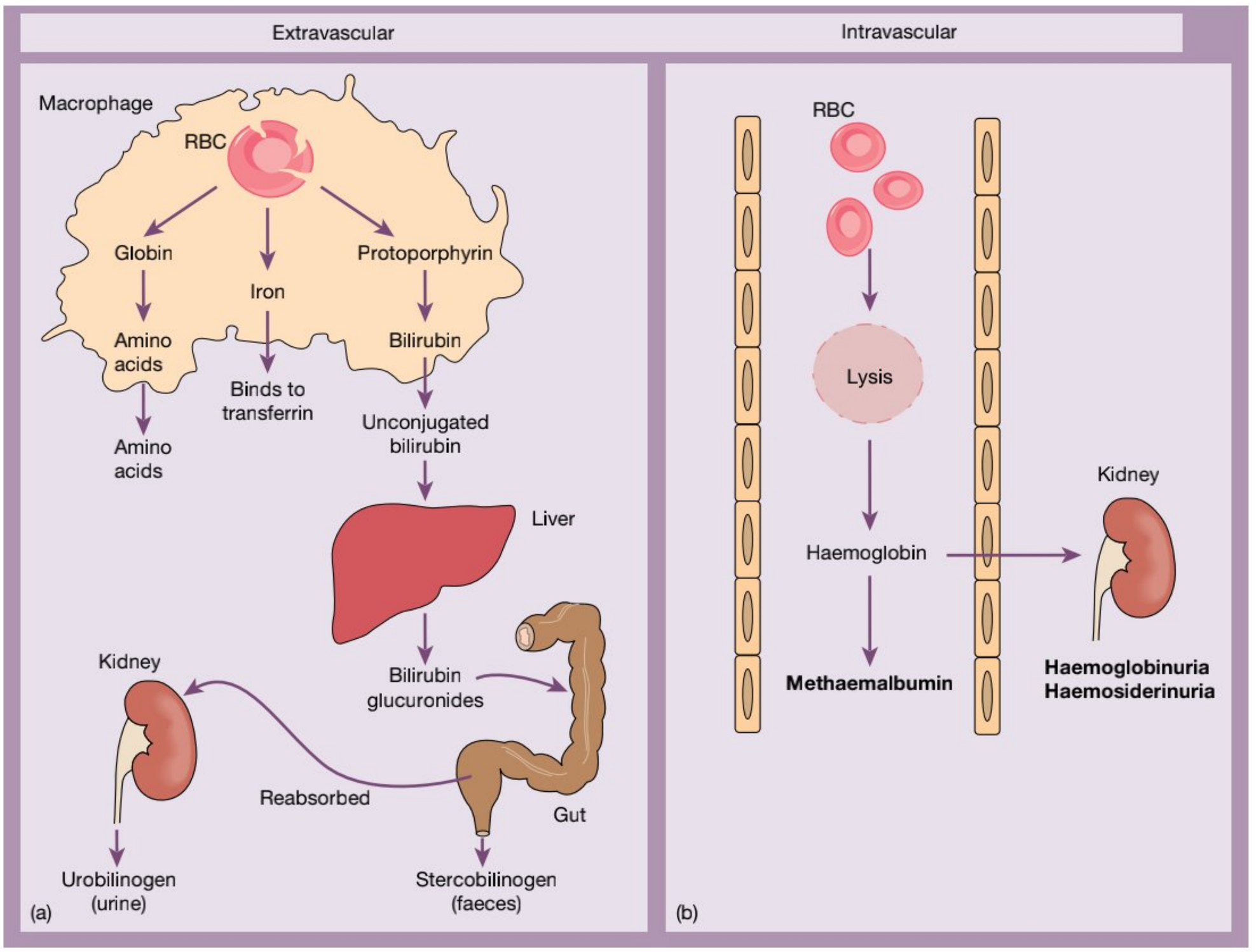


Figure 6.1 **(a)** Normal red blood cell (RBC) breakdown. This takes place extravascularly in the macrophages of the reticuloendothelial system. **(b)** Intravascular haemolysis within the blood vessel occurs in some pathological disorders, and is associated with haemoglobinaemia, haemoglobinuria and haemosiderinuria.

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Introduction to haemolytic anaemias

Haemolytic anaemias are defined as anaemias that result from an increase in the rate of red cell destruction. Because of erythropoietic hyperplasia and anatomical extension of bone marrow, red cell destruction may increase several-fold before anaemia develops – this is called compensated haemolytic disease. The normal adult marrow, after full expansion, is able to produce red cells at 6–8 times the normal rate. Therefore, anaemia due to haemolysis may not be seen until the red cell life span drops to less than 30 days. The expanded red cell production leads to a marked reticulocytosis.

Classification

Table 6.1 is a simplified classification of the haemolytic anaemias. **Hereditary haemolytic anaemias are typically the result of 'intrinsic' red cell defects, whereas acquired haemolytic anaemias are usually the result of an 'extracorpuscular' or 'environmental' change**. Paroxysmal nocturnal haemoglobinuria (PNH) is an exception because, although it is an acquired disorder, the PNH red cells have an intrinsic defect. PNH is associated with marrow hypoplasia and is discussed with aplastic anaemia in Chapter 24.

Clinical features

The patient may show pallor of the mucous membranes, mild fluctuating jaundice and splenomegaly. There is no bilirubin in urine, but the urine may turn dark on standing because of excess urobilinogen, which is colourless but oxidizes in light to highly coloured urobilin. Pigment (bilirubin) gallstones may complicate the condition (Fig. 6.2).

Some patients with haemolysis develop disease-specific complications. For instance, patients with sickle cell disease, hereditary spherocytosis, or rarely other haemolytic anaemias may develop ulcers around the ankle (Fig. 7.20), while those with thalassaemia major who do not receive regular transfusions develop bone deformities (Figs. 7.9 and 7.10). Patients with cold-reactive autoantibodies may exhibit discoloration in peripheral (acral) regions of the body such as the earlobes or fingertips, and patients with PNH may develop thrombosis.

Aplastic crises may occur, usually precipitated by infection with parvovirus, which 'switches off' erythropoiesis. These crises are characterized by a sudden fall in haemoglobin level and in reticulocyte count (Fig. 24.7). Rarely, folate deficiency may cause an aplastic crisis in which the bone marrow becomes megaloblastic.

Laboratory findings

The laboratory findings are conveniently divided into three groups.

1 **Features of increased red cell breakdown:**
   - **(a)** serum bilirubin raised, unconjugated and bound to albumin;
   - **(b)** urine urobilinogen increased;
   - **(c)** serum haptoglobins absent because the haptoglobins become saturated with haemoglobin and the complex is removed by RE cells.

Table 6.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane defects Hereditary spherocytosis, hereditary elliptocytosis, others *Metabolic disorders* G6PD deficiency, pyruvate kinase deficiency	*Autoimmune* Warm antibody type (see Table 6.5) Cold antibody type
Haemoglobinopathies and thalassaemias Hb SS, Hb SC, Hb CC, unstable haemoglobins, thalassaemia major and others; Chapter 7	*Alloimmune* Haemolytic transfusion reactions (acute or delayed) Haemolytic disease of the newborn Allografts, especially in stem cell transplantation Drug-associated *Red cell fragmentation syndromes* (Table 6.6) Microangiopathy, e.g. disseminated intravascular coagulation March haemoglobinuria *Infections:* malaria, clostridia, tick-borne illnesses such as babesiosis *Chemicals:* especially drugs, industrial/domestic substances, e.g. naphthalene in moth balls Severe burns *Secondary:* severe liver and renal disease *Paroxysmal nocturnal haemoglobinuria* (Chapter 24)

G6PD, glucose-6-phosphate dehydrogenase; Hb, haemoglobin.

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Figure 6.2 Ultrasound of multiple small pigment gallstones typical of those associated with hereditary spherocytosis. Source: Courtesy of Dr P. Wylie.

(d) Serum lactate dehydrogenase (LDH) may be raised in both severe extravascular and in intravascular haemolysis

2 Features of increased red cell production:

(a) reticulocytosis;

(b) bone marrow erythroid hyperplasia – the normal marrow myeloid: erythroid ratio of 2 : 1 to 12 : 1 is reduced to 1 : 1 or reversed.

3 Damaged red cells, visualized by:

(a) routine blood film morphology e.g. microspherocytes, elliptocytes, fragments;

(b) flow cytometry after eosin-5-maleimide (EMA) staining, see Fig 6.5;

(c) specific enzyme, protein or DNA tests.

Table 6.2 Causes of intravascular haemolysis.

Mismatched blood transfusion (usually ABO)
G6PD deficiency with oxidant stress
Red cell fragmentation syndromes
Some severe autoimmune haemolytic anaemias
Some drug- and infection-induced haemolytic anaemias
Paroxysmal nocturnal haemoglobinuria
March haemoglobinuria
Unstable haemoglobin

G6PD, glucose-6-phosphate dehydrogenase.

Intravascular and extravascular haemolysis

There are two mechanisms whereby red cells are destroyed in haemolytic anaemias. There may be excessive removal of red cells by cells of the RE system (**extravascular haemolysis**) or red cells may be broken down directly in the circulation (**intravascular haemolysis**) (Fig. 6.1; Table 6.2). Whichever mechanism dominates will depend on the pathology involved.

In intravascular haemolysis, free haemoglobin is released, which rapidly saturates plasma haptoglobins. The excess free haemoglobin is filtered by the glomerulus. If the rate of haemolysis saturates the renal tubular reabsorptive capacity, free haemoglobin enters urine (Fig. 6.3). Iron released from haemoglobin in the renal tubules is seen as haemosiderin in a urinary deposit. Oxidized haem bound to albumin (methaemalbumin) is also formed from the process of intravascular haemolysis.

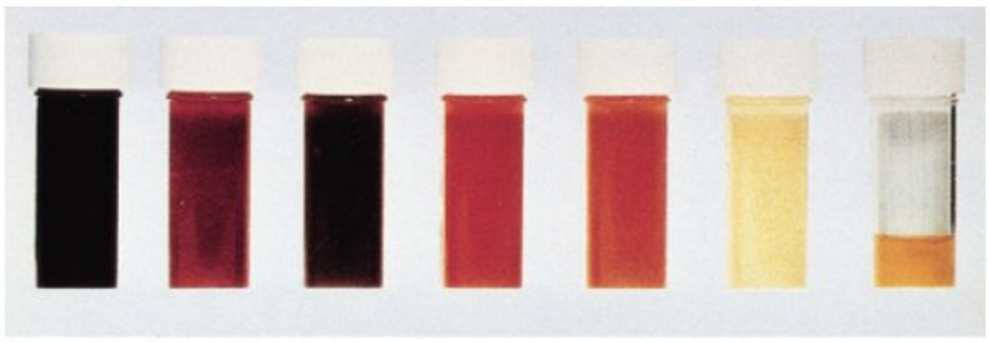
The main laboratory features of intravascular haemolysis therefore are (Fig. 6.3):

1 Haemoglobinaemia and haemoglobinuria.

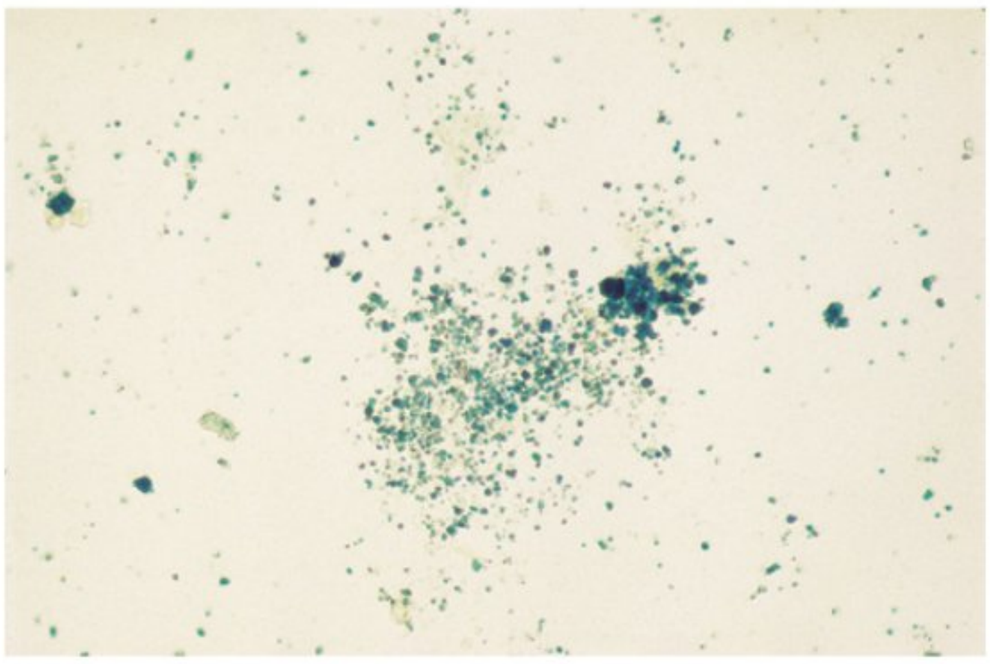
2 Haemosiderinuria.

3 Methaemalbuminaemia (detected spectrophotometrically).

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(a)



(b)

Figure 6.3 **(a)** Progressive urine samples in an acute episode of intravascular haemolysis showing haemoglobinuria of decreasing severity. **(b)** Prussian blue-positive deposits of haemosiderin in a urine spun deposit (Perls' stain).

Hereditary haemolytic anaemias

Membrane defects

Hereditary spherocytosis

Hereditary spherocytosis (HS) is the most common hereditary haemolytic anaemia in Northern Europeans.

Pathogenesis

HS is usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell (Table 6.3; Fig. 2.12). The marrow produces red cells of normal biconcave shape, but these lose membrane and become increasingly spherical (loss of surface area relative to volume) as they circulate through the spleen and the rest of the RE system. The loss of membrane may be caused by the release of parts of the lipid bilayer that are not supported by the skeleton. Ultimately, the spherocytes are unable to pass through the microcirculation, especially in the spleen, and die prematurely.

Clinical features

The inheritance is usually autosomal dominant with variable expression; rarely, it may be autosomal recessive. In about 25% of cases the mutation is new (spontaneous) with no family history of the disease. The anaemia may present at any age from infancy to old age. Jaundice is typically fluctuating and is particularly marked if the haemolytic anaemia is associated with Gilbert's disease (a defect of hepatic conjugation of bilirubin). Splenomegaly occurs in most patients. Pigment gallstones are

Table 6.3 Molecular basis of hereditary spherocytosis and elliptocytosis.

Hereditary spherocytosis
■ Ankyrin deficiency or abnormalities (most common cause – about 50% of patients) ■ α- or β-spectrin deficiency or abnormalities ■ Band 3 abnormalities ■ Pallidin (protein 4.2) abnormalities
Hereditary elliptocytosis
■ α- or β-spectrin mutants leading to defective spectrin dimer formation ■ α- or β-spectrin mutants leading to defective spectrin–ankyrin associations ■ Protein 4.1 deficiency or abnormality ■ Southeast Asian ovalocytosis due to a band 3 deletion

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frequent (Fig. 6.2); aplastic crises, usually precipitated by parvovirus infection, may cause a sudden increase in the severity of anaemia (Fig. 24.8).

Haematological findings

Anaemia is usual but not invariable; its severity tends to be similar in members of the same family. Reticulocytes are usually 5–20%. The blood film shows microspherocytes (Fig. 6.4a), which are densely staining with smaller diameters than normal red cells.

Investigation and treatment

A rapid flow cytometric analysis of eosin-5-maleimide (EMA) bound to erythrocytes is used as a test for HS and membrane protein deficiency (Fig. 6.5). EMA is a dye that binds specifically to band 3 of the red blood cell cytoskeleton and measures the content of erythrocyte structural proteins, which is altered in HS. The EMA test has replaced the osmotic fragility test, which showed the red cells to be excessively fragile compared to normal red cells in dilute saline solutions. Identification of the exact molecular defect is not needed for management, but electrophoresis of membrane proteins is carried out in difficult cases. The direct antiglobulin (Coombs') test is normal in HS, excluding an autoimmune cause of spherocytosis and haemolysis.

The principal form of treatment is splenectomy, preferably laparoscopic, although this should not be performed unless clinically indicated by symptomatic anaemia or gallstones, leg

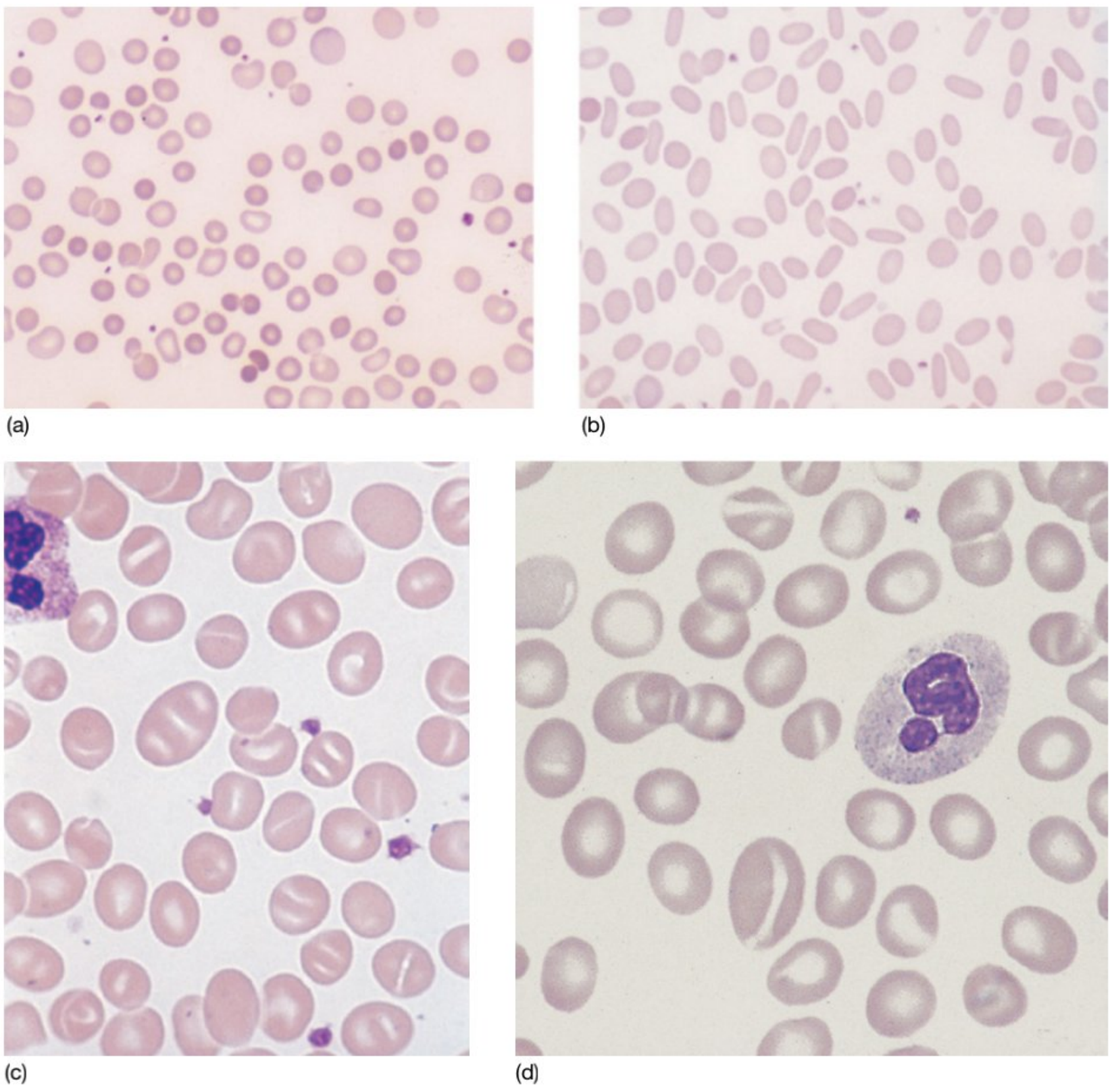


Figure 6.4 **(a)** Blood film in hereditary spherocytosis. The spherocytes are deeply staining and of small diameter. Larger polychromatic cells are reticulocytes (confirmed by supravital staining). **(b)** Blood film in hereditary elliptocytosis. **(c)** Hereditary stomatocytosis: peripheral blood film showing many cells with the characteristic loosely folded appearance of the membrane. **(d)** Southeast Asian ovalocytosis. Source: A.V. Hoffbrand *et al*. (2019) *Color Atlas of Hematology*, 5th edn. Reproduced with permission of John Wiley & Sons.

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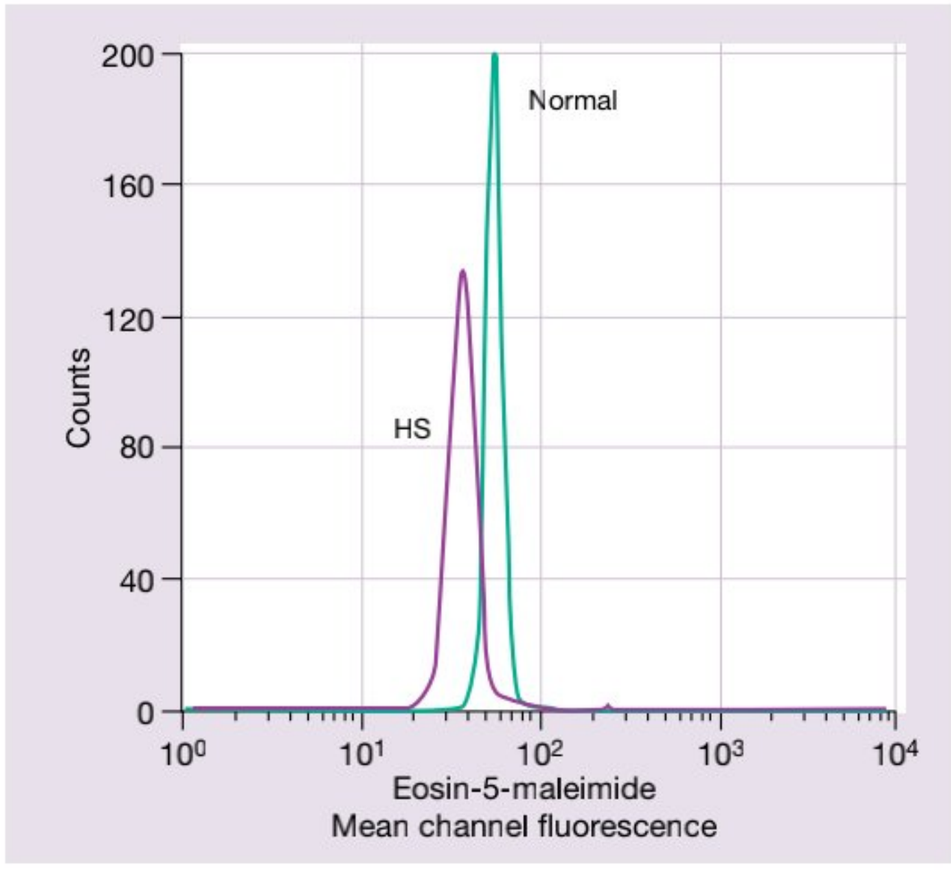


Figure 6.5 Eosin-5-maleimide (EMA) staining in hereditary spherocytosis (HS) showing reduced mean channel fluorescence due to membrane band 3 protein deficiency. Source: Courtesy of Mr G. Ellis.

ulcers or growth retardation. Because of the risk of post-splenectomy sepsis, particularly in early childhood (Chapter 10), the frequency of splenectomy has fallen over recent decades and the age at splenectomy has risen. Cholecystectomy should be performed with splenectomy if symptomatic gallstones are present. Unless there is additional pathology, splenectomy in HS should always produce a rise in the haemoglobin level to normal, even though microspherocytes formed in the rest of the RE system will remain. Folic acid, e.g. 5 mg weekly, is given in severe cases to prevent folate deficiency.

Hereditary elliptocytosis

Hereditary elliptocytosis (HE) has similar clinical and laboratory features to HS, except for the appearance of the blood film (Fig. 6.4b). HE is usually a clinically mild disorder and is predominantly discovered by chance on a blood film. There may be no evidence of haemolysis. Occasional patients require splenectomy. The basic defect is a failure to form normal spectrin heterodimers. Genetic mutations affecting horizontal interactions of red cell skeletal proteins have been detected (Table 6.3).

Patients with homozygous or doubly heterozygous elliptocytosis present with a severe haemolytic anaemia termed hereditary pyropoikilocytosis; this is most common in patients of African descent. In severe cases, splenectomy can be performed which results in clinical improvement. Folic acid supplementation is also appropriate.

Hereditary stomatocytosis

This is a group of rare red cell membrane defects including xerocytosis and overhydrated stomatocytosis, in which the red cells have mouth-like slits in a stained blood film (Fig 6.4c). The cells leak cations and anaemia is variable. Stomatocytosis may also occur as an artefact if blood films are badly prepared.

Southeast Asian ovalocytosis

This is common in Melanesia, Malaysia, Indonesia and the Philippines and is caused by a 9-amino acid deletion at the junction of the cytoplasmic and transmembrane domains of the band 3 protein. The cells are rigid and resist invasion by malarial parasites. The blood film shows ovalocytes and stomatocytes (Fig 6.4d). Most cases are not anaemic and asymptomatic. Treatment is rarely required.

Defective red cell metabolism

Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase (G6PD) functions to reduce nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. This reaction is the only source of NADPH needed for the production of reduced glutathione. The gene coding G6PD is on the X chromosome. G6PD deficiency renders the red cell susceptible to oxidant stress (Fig. 6.6).

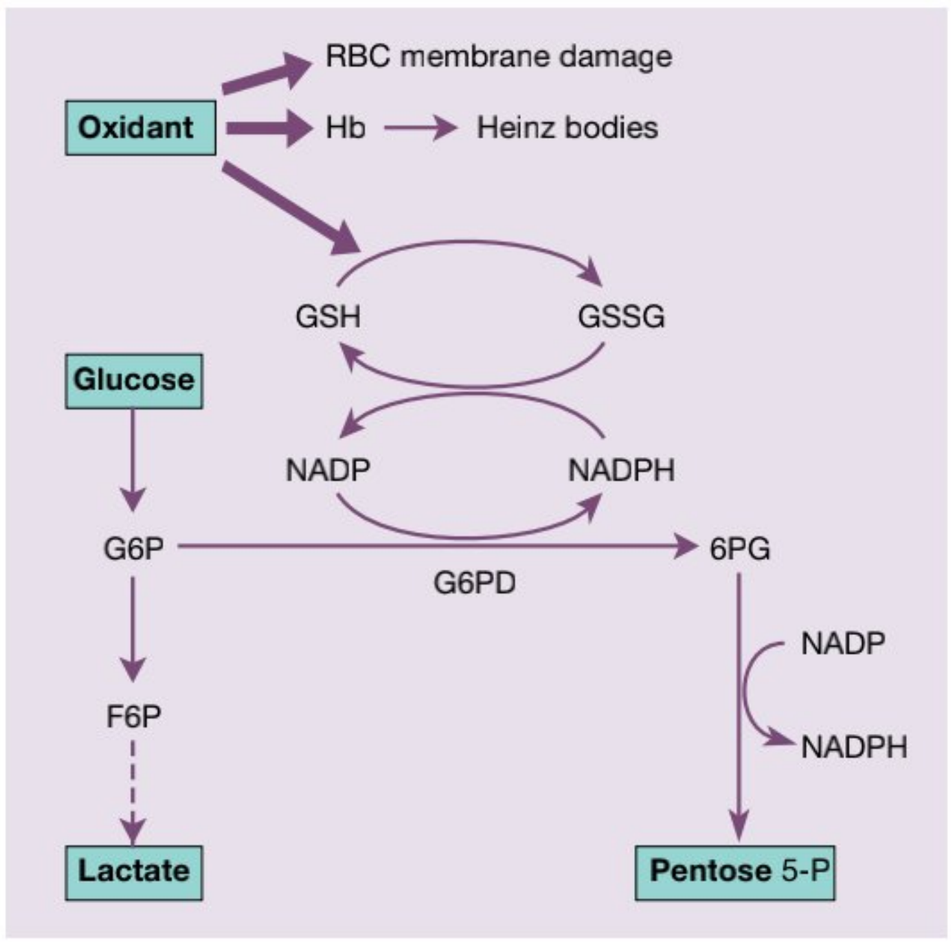


Figure 6.6 Haemoglobin and red blood cell (RBC) membranes are usually protected from oxidant stress by reduced glutathione (GSH). In G6PD deficiency, NADPH and GSH synthesis is impaired. F6P, fructose-6-phosphate;G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; 6-PG, 6-phosphogluconate; GSSG, glutathione (oxidized form); NADP, NADPH, nicotinamide adenine dinucleotide phosphate.

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Epidemiology

There is a wide variety of normal genetic variants of the enzyme G6PD. The most common isoforms are type B and type A, which is predominant in Africans. They have the same enzyme activity. In addition, more than 400 variants caused by point mutations or deletions that alter the activity of the G6PD enzyme have been characterized, and worldwide over 400 million people are G6PD deficient (Fig. 6.7).

The inheritance is sex-linked, dominantly affecting males, and carried by females who show approximately half the normal red cell G6PD values. Because of lyonization, heterozygote females may have a substantial proportion of G6PD deficient red cells and be susceptible to haemolysis. The female heterozygotes have an advantage of resistance to potentially lethal *Falciparum* malaria. The degree of deficiency varies with ethnic group and G6PD genotype, often being mild (10–60% of normal activity) in black Africans, more severe in Middle Eastern and South-East Asians, and most severe in Mediterranean populations, e.g. <10% of normal activity with G6PD Mediterranean. The degree of enzyme activity and the clinical severity of the syndrome do not always correlate. Severe deficiency occurs occasionally in Northern European people.

Clinical features

G6PD deficiency is usually asymptomatic, with a normal blood count between attacks of haemolysis. The main clinical syndromes that occur with red cell G6PD levels between 2% and 60% of normal are:

1 **Acute haemolytic anaemia in response to oxidant stress**, e.g. drugs, fava beans or infections (Table 6.4). Fava beans (*Vica fava*) contain an oxidant chemical, divicine. The degree of anaemia it causes is dose related. The acute haemolytic anaemia is caused by rapidly developing intravascular haemolysis with haemoglobinuria (Fig. 6.3a). Depending on the G6PD genotype, the anaemia may be self-limiting, as new young red cells are made with near normal enzyme levels, or may be life-threatening.
2 **Neonatal jaundice**, usually without haemolysis.
3 **Rarely, a congenital non-spherocytic haemolytic anaemia**. This chronic anaemia may result from different types of severe enzyme deficiency (levels <2% of normal).

Diagnosis

During a crisis the blood film may show contracted and fragmented cells, 'bite' cells and 'blister' cells (Fig. 6.8), which have had Heinz bodies removed by the spleen. Heinz bodies (oxidized, denatured and insoluble haemoglobin that precipitates in the red cell) may be seen in the reticulocyte preparation, particularly if the spleen is absent (Fig. 2.17). There are also features of intravascular haemolysis.

Between acute crises (except in the rare cases of congenital non-spherocytic haemolytic anaemia) the blood count is

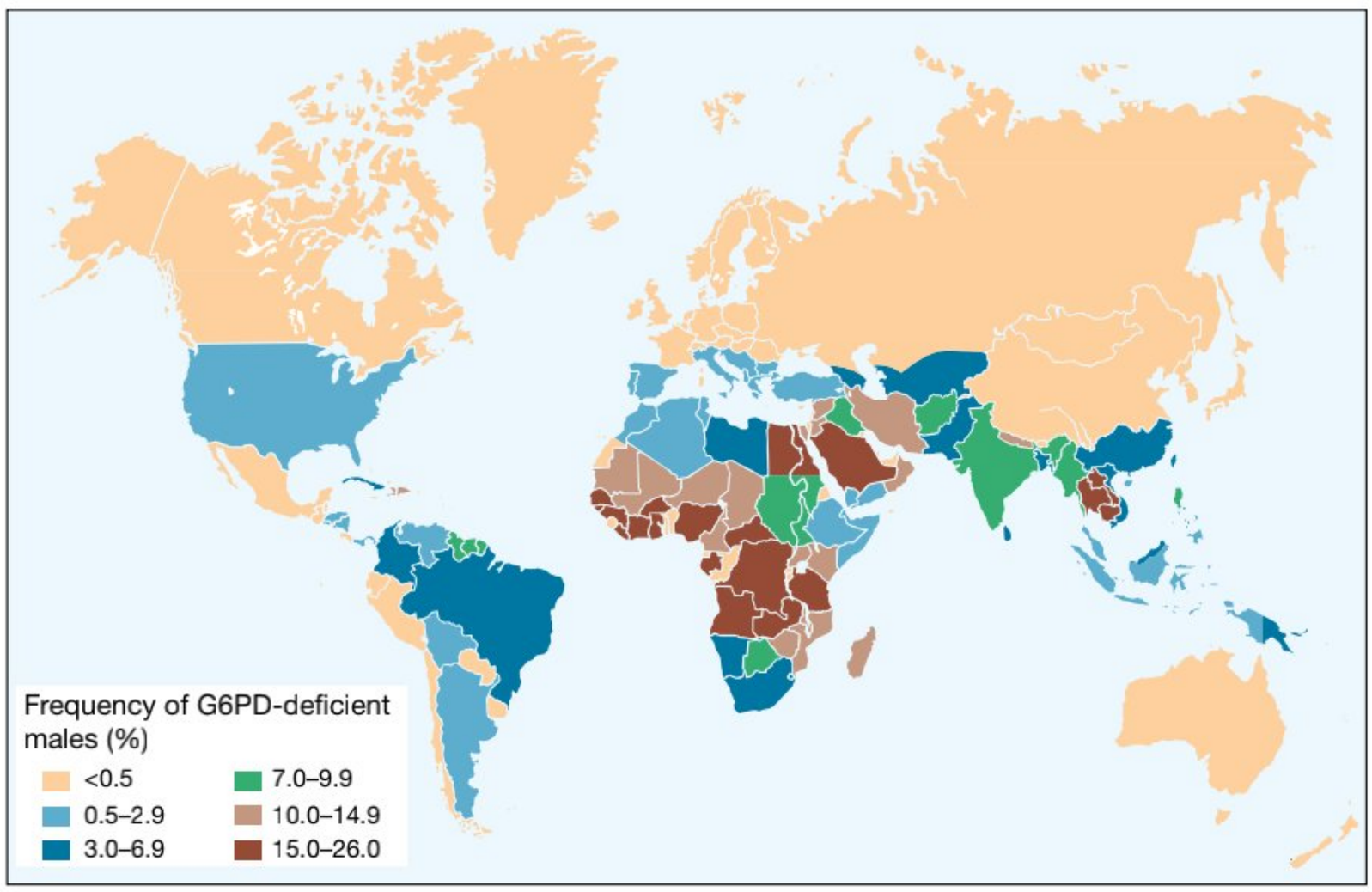


Figure 6.7 Global distribution of *G6PD* gene variants causing glucose-6-phosphate dehydrogenase (G6PD) deficiency. Shaded areas indicate the prevalence of G6PD deficiency. The distribution is similar to that of malaria; in countries where endogenous G6PD deficiency is not found, the distribution reflects immigration patterns. Source: Adapted from L. Luzzatto, R. Notaro (2001) *Science* 293: 442.

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Table 6.4 Agents that may cause haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Infections and other acute illnesses, e.g. diabetic ketoacidosis

Drugs

- Antimalarials, e.g. primaquine, pamaquine, chloroquine, Fansidar, Maloprim, quinine
- Sulphonamides and sulphones, e.g. co-trimoxazole, sulfanilamide, dapsone, sufasalazine
- Other antibacterial agents, e.g. quinolones, nitrofurans, nalidixic acid, chloramphenicol, ciprofoxcin,
- Analgesics, e.g. aspirin (moderate doses are safe)
- Anti-helminths, e.g. β-naphthol, stibophen
- Miscellaneous, e.g. vitamin K analogues, rasburicase, glibenclamide, isoniazid, naphthalene (mothballs), probenecid

Fava beans

Chemical oxidants

N.B. Many common drugs have been reported to precipitate haemolysis in G6PD deficiency in some patients, e.g. aspirin, quinine and penicillin, but not at conventional dosage.

normal. The enzyme deficiency is detected by one of a number of screening tests or by direct enzyme assay of red cells. Because of the higher enzyme level in young red cells in some G6PD genotypes, red cell enzyme assay may give a 'false' normal level in the phase of acute haemolysis with a reticulocyte response. Subsequent assay after the acute phase reveals the low G6PD level when the red cell population is of normal age distribution.

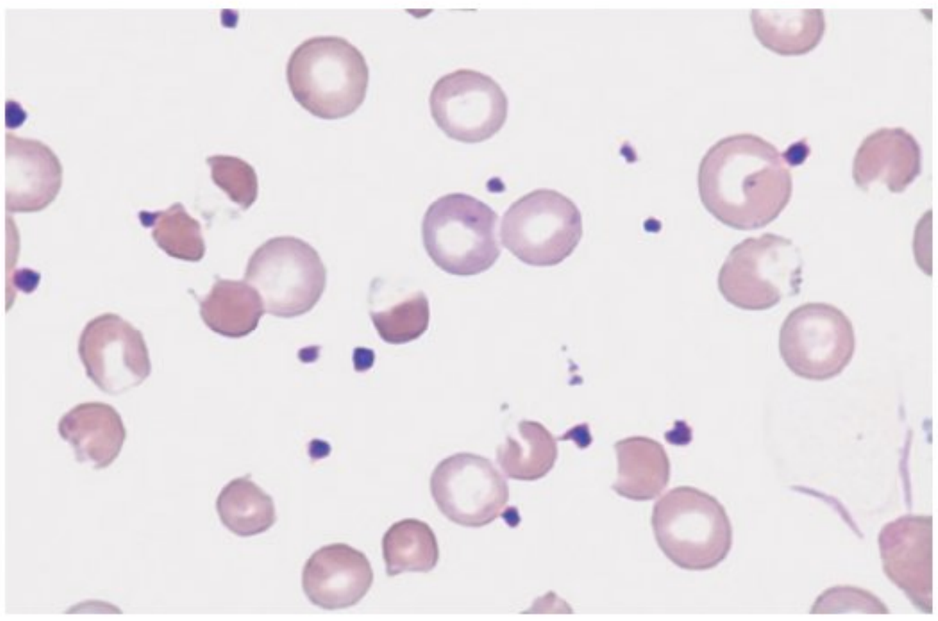


Figure 6.8 Blood film in glucose-6-phosphate dehydrogenase (G6PD) deficiency with acute haemolysis after an oxidant stress. Some of the cells show loss of cytoplasm with separation of remaining haemoglobin from the cell membrane ('blister' cells). There are also numerous contracted and deeply staining cells. Supravital staining (as for reticulocytes) showed the presence of Heinz bodies (Fig. 2.17).

Treatment

The offending drug is stopped, any underlying infection is treated, a high urine output is maintained with fluid supplementation. Blood transfusion is undertaken where necessary for severe anaemia. G6PD-deficient babies are prone to neonatal jaundice and in severe cases phototherapy and exchange transfusion is needed to avoid kernicterus (Chapter 34). The jaundice is usually not caused by excess haemolysis, but by deficiency of G6PD affecting neonatal liver function. Gilbert's syndrome (the genetic defect reducing the liver's ability to metabolise bilirubin) may also be present.

Glutathione deficiency and other syndromes

Other defects in the pentose phosphate pathway leading to similar syndromes to those of G6PD deficiency have been described – particularly defects causing glutathione deficiency.

Glycolytic (Embden–Meyerhof) pathway defects

These are all uncommon and lead to a congenital non-spherocytic haemolytic anaemia. In some there are defects of other systems e.g. a myopathy.

Pyruvate kinase deficiency

The most frequently encountered is the autosomal recessive condition pyruvate kinase deficiency (Fig. 2.11). The affected patients are homozygous or more frequently doubly heterozygous for mutations in the gene *PKLR*. Over 350 different mutations have been described. The red cells become rigid as a result of reduced adenosine triphosphate (ATP) formation. The severity of the anaemia varies widely (haemoglobin 40–100 g/L). It results in relatively mild symptoms because of a shift to the right in the oxygen-dissociation curve caused by a rise in intracellular 2,3-diphosphoglycerate (2,3-DPG). Clinically, jaundice is usual and gallstones are frequent. Frontal bossing and leg ulcers (Chapter 7) may be present. Iron loading occurs not only from transfusions but also due to raised plasma erythroferrone levels, consequent on the increased and ineffective erythropoiesis, which inhibit hepcidin synthesis. Perinatal complications including hydrops, prematurity, neonatal jaundice and anaemia are common.

The blood film shows poikilocytosis and distorted 'prickle' cells, particularly post-splenectomy. Direct enzyme assay and genomic sequencing are needed to make the diagnosis.

Treatment is with the drug Mitapivat, a small-molecule oral activator of wild and most mutant pyruvate kinases. It is associated with a rapid increase in haemoglobin in 40–50% of adults with PK deficiency. Features of haemolysis are reduced. It has only low grade and transient side effects. Responses have been durable for up to a year and longer follow up studies in adults and studies in children are ongoing. It is unclear whether the genotype predicts for response. Splenectomy may alleviate the anaemia but does not cure it. It increases the risk of infections and of venous thrombosis so is indicated only in those

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