

# Haematological disease

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## INTRODUCTION AND GENERAL ASPECTS

Blood consists of

- red cells
- white cells
- platelets
- plasma, in which the above elements are suspended.

Plasma is the liquid component of blood, which contains soluble fibrinogen. Serum is what remains after the formation of the fibrin clot.

### The formation of blood cells (haemopoiesis)

The haemopoietic system includes the bone marrow, liver, spleen, lymph nodes and thymus. The turnover of cells is enormous; red cells survive 120 days, platelets around 7 days but granulocytes only 7 hours. The production of as many as  $10^{13}$  new myeloid cells (all blood cells except for lymphocytes) per day in the normal healthy state obviously requires to be tightly regulated according to the needs of the body.

Blood islands are formed in the yolk sac in the third week of gestation and produce primitive blood cells which migrate to the liver and spleen. These organs are the chief sites of haemopoiesis from 6 weeks to 7 months, when the bone marrow becomes the main source of blood cells. The bone marrow is the only source of blood cells during normal childhood and adult life.

At birth, haemopoiesis is present in the marrow of nearly every bone. As the child grows the active red marrow is gradually replaced by fat (yellow marrow) so that haemopoiesis in the adult becomes confined to the central skeleton and the proximal ends (trabecular area) of the long bones. Only if the demand for blood cells increases and persists do the areas of red marrow extend. Pathological processes interfering with normal haemopoiesis may result in resumption of haemopoietic activity in the liver and spleen, which is referred to as *extra-medullary haemopoiesis*.

All blood cells are derived from pluripotent stem cells. These stem cells are supported by stromal cells (see below) which also influence haemopoiesis. The stem cell has two properties - the first is *self-renewal*, i.e. the production of more stem cells, and the second is its proliferation and differentiation into progenitor cells, committed to one specific cell line.

# 8

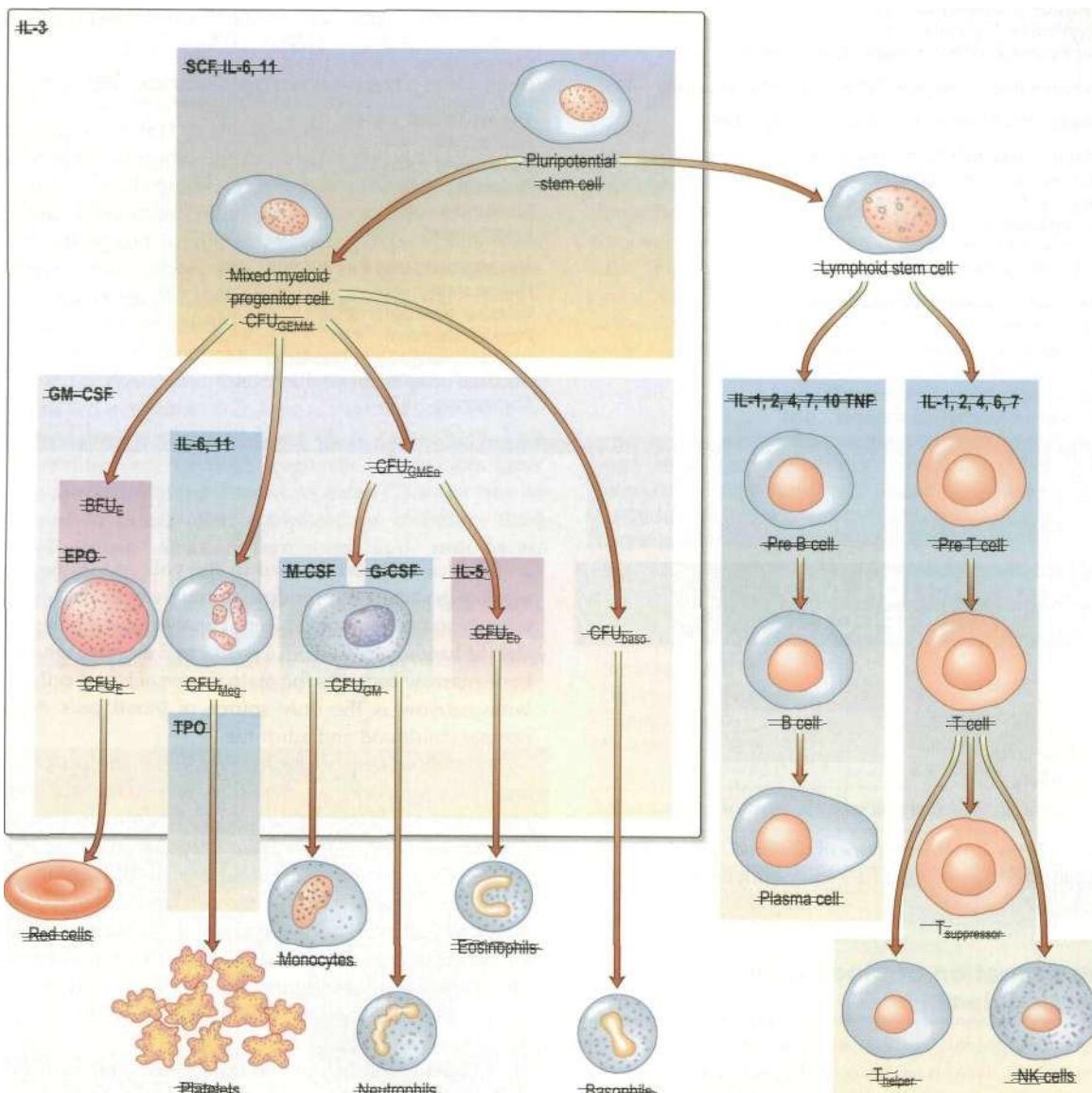
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There are two major ancestral cell lines derived from the pluripotential stem cell: lymphocytic and myeloid (non-lymphocytic) cells (Fig. 8.1). The former gives rise to T and B cells. The myeloid stem cell gives rise to the progenitor CFU-GEMM (colony-forming unit, granulocyte-erythrocyte-monocyte-megakaryocyte). The progenitor cells such as CFU-GEMM cannot be recognized in bone marrow biopsies but are recognized by their ability to form colonies when haemopoietic cells are immobilized in a soft gel matrix. The CFU-GEMM can go on to form CFU-GM, CFU-Eo, and CFU-Meg, each of which can produce a particular cell type (for example, neutrophils, eosinophils and platelets) under appropriate growth conditions. Haemopoiesis is under the control of

growth factors and inhibitors, and the microenvironment of the bone marrow also plays a role in its regulation.

### Haemopoietic growth factors

Haemopoietic growth factors are glycoproteins which regulate the differentiation and proliferation of haemopoietic progenitor cells and the function of mature blood cells. They act on receptors expressed on haemopoietic cells at various stages of development to maintain the haemopoietic progenitor cells and to stimulate increased production of one or more cell lines in response to stresses such as blood loss and infection (Fig. 8.1).



**Fig. 8.1 Role of growth factors in normal haemopoiesis.** Some of the multiple growth factors acting on stem cells and early progenitor cells are shown. BFU, burst-forming unit; CFU, colony-forming unit; CSF, colony-stimulating factor; E, erythroid; Eo, eosinophil; EPO, erythropoietin; G, granulocyte; GEMM, mixed granulocyte, erythroid, monocyte, megakaryocyte; GM, granulocyte, monocyte; IL, interleukin; M, monocyte; Meg, megakaryocyte; SCF, stem cell (Steel) factor or C-kit ligand; TNF, tumour necrosis factor; TPO, thrombopoietin.

The pluripotential stem cells are under the influence of a number of haemopoietic growth factors including interleukin-3 (IL-3), IL-6, -7, -11, fi-catenin and stem cell factor (SCF, Steel factor or C-kit ligand). Colony-stimulating factors (CSFs, the prefix indicating the cell type, see Fig. 8.1), as well as interleukins and erythropoietin (EPO) regulate the lineage committed progenitor cells. Thrombopoietin (TPO, which, like erythropoietin, is produced in the kidneys and the liver) along with IL-6 and IL-11 control platelet production. In addition to these factors stimulating haemopoiesis, other factors inhibit the process and include tumour necrosis factor (TNF) and transforming growth factor-p" (TGF-f3). Many of the growth factors are produced by activated T cells, monocytes and bone marrow stromal cells such as fibroblasts, endothelial cells and macrophages; these cells are also involved in inflammatory responses.

Many growth factors have been produced by recombinant DNA techniques and are being used clinically. Examples include G-CSF, which is used to accelerate haemopoietic recovery after chemotherapy and haemopoietic cell transplantation, and erythropoietin, which is used to treat anaemia in patients with chronic renal failure. Thrombopoietin is undergoing clinical trials in patients with idiopathic thrombocytopenic purpura.

### Peripheral blood

Automated cell counters are used to measure the level of haemoglobin (Hb) and the number and size of red cells, white cells and platelets (Table 8.1). Other indices can be derived from these values. The mean corpuscular volume (MCV) of red cells is the most useful of the indices and is used to classify anaemia (see p. 425).

The white cell count (WCC, or WBC, white blood count) gives the total number of circulating leucocytes, and many automated cell counters produce differential counts as well.

Normally, less than 2% of the red cells are reticulocytes (p. 422). The reticulocyte count gives a guide to the erythroid activity in the bone marrow. An increased count

**Table 8.1 Normal values for peripheral blood**

	Male	Female
Hb (g/dL)	13.5-17.5	11.5-16
PCV (haematocrit; L7L)	0.4-0.54	0.37-0.47
RCC( $10^{12}/\text{L}$ )	4.5-6.0	3.9-5.0
MCV (fL)		80-96
MCH (pg)		27-32
MCHC (g/dL)		32-36
WBC( $10^9/\text{L}$ )		4.0-11.0
Platelets ( $10^7/\text{L}$ )		150-400
ESR (mm/h)		<20
Reticulocytes	0.5-2.5%	(50-100x10 <sup>7</sup> L)

ESR, erythrocyte sedimentation rate; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume of red cells; PCV, packed cell volume; RCC, red cell count; WBC, white blood count.

is seen with increased marrow maturity, e.g. following haemorrhage or haemolysis, and during the response to treatment with a specific haematinic. A low count in the presence of anaemia indicates an inappropriate response by the bone marrow and may be seen in bone marrow failure (from whatever cause) or where there is a deficiency of a haematinic.

A carefully evaluated blood film is still an essential adjunct to the above, as definitive abnormalities of cells can be seen.

### Erythrocyte sedimentation rate (ESR)

This is the rate of fall of red cells in a column of blood and is a measure of the acute-phase response. The pathological process may be immunological, infective, ischaemic, malignant or traumatic. A raised ESR reflects an increase in the plasma concentration of large proteins, such as fibrinogen and immunoglobulins. These proteins cause rouleaux formation, when cells clump together like a stack of coins, and therefore fall more rapidly. The ESR increases with age, and is higher in females than in males. It is low in polycythaemia vera, owing to the high red cell concentration, and increased in patients with severe anaemia.

### Plasma viscosity

Plasma viscosity measurement is used instead of the ESR in many laboratories. As with the ESR, the level is dependent on the concentration of large molecules such as fibrinogen and immunoglobulins. There is no difference between levels found in males and females, and viscosity increases only slightly in the elderly. It is not affected by the level of Hb and the result may be obtained within 15 minutes.

### C-reactive protein

C-reactive protein is a pentraxin, one of the proteins produced in the acute-phase response (see Table 4.4). It is synthesized exclusively in the liver and rises within 6 hours of an acute event. It rises with fever (possibly triggered by IL-1, IL-6 and TNF-a and other cytokines) and in inflammatory conditions and after trauma. It follows the clinical state of the patient much more rapidly than does the ESR and is unaffected by the level of Hb, but it is less helpful than the ESR or plasma viscosity in monitoring chronic inflammatory diseases. Its measurement is easy and quick to perform using an immunoassay that can be automated. High-sensitivity assays have shown that increased levels predict future cardiovascular disease (p. 802).

## THE RED CELL

### Erythropoiesis

Red cell precursors pass through several stages in the bone marrow. The earliest morphologically recognizable cells are pronormoblasts. Smaller normoblasts result from

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cell divisions, and precursors at each stage progressively contain less RNA and more Hb in the cytoplasm. The nucleus becomes more condensed and is eventually lost from the late normoblast in the bone marrow, when the cell becomes a reticulocyte.

Reticulocytes contain residual ribosomal RNA and are still able to synthesize Hb. They remain in the marrow for about 1-2 days and are released into the circulation, where they lose their RNA and become mature red cells (erythrocytes) after another 1-2 days. Mature red cells are non-nucleated biconcave discs.

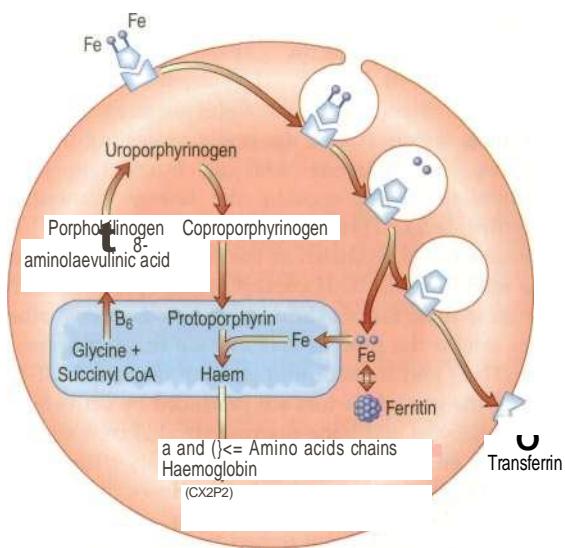
Nucleated red cells (*normoblasts*) are not normally present in peripheral blood, but are present if there is extramedullary haemopoiesis and in some marrow disorders (see leucoerythroblastic anaemia, p. 464).

About 10% of erythroblasts die in the bone marrow even during normal erythropoiesis. Such ineffective erythropoiesis is substantially increased in some anaemias such as thalassaemia major and megaloblastic anaemia.

*Erythropoietin* is a hormone which controls erythropoiesis. The gene for erythropoietin is on chromosome 7 and codes for a heavily glycosylated polypeptide of 165 amino acids. Erythropoietin has a molecular weight of 30 400 and is produced in the peritubular cells in the kidneys (90%) and in the liver (10%). Its production is regulated mainly by tissue oxygen tension. Production is increased if there is hypoxia from whatever cause - for example, anaemia or cardiac or pulmonary disease. The erythropoietin gene is one of a number of genes that is regulated by the hypoxic sensor pathway. The 3'-flanking region of the erythropoietin gene has a hypoxic response element which is necessary for the induction of transcription of the gene in hypoxic cells. Hypoxia-inducible factor 1 (HIF-1) is a transcription factor which binds to the hypoxia response element and acts as a master regulator of several genes that are responsive to hypoxia. Erythropoietin stimulates an increase in the proportion of bone marrow precursor cells committed to erythropoiesis, and CFU-E are stimulated to proliferate and differentiate. Increased 'inappropriate' production of erythropoietin is also seen in patients with renal disease and neoplasms in other sites which result in polycythaemia (see Table 8.16).

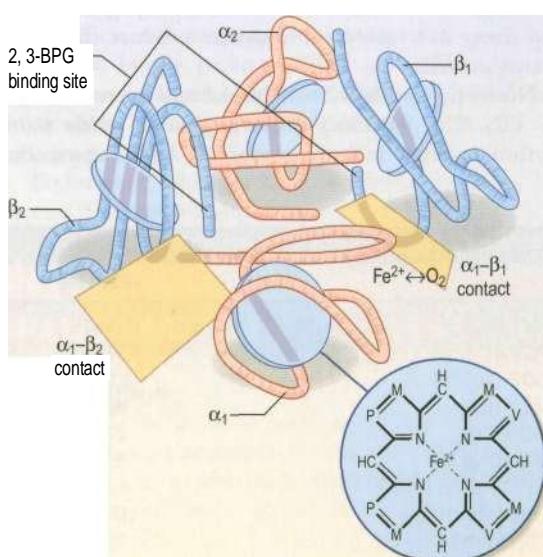
## Haemoglobin synthesis

Haemoglobin performs the main functions of red cells - carrying O<sub>2</sub> to the tissues and returning CO<sub>2</sub> from the tissues to the lungs. Each normal adult Hb molecule (Hb A) has a molecular weight of 68 000 and consists of two *a* and two *(3* globin polypeptide chains (0C2P2) which have 141 and 146 amino acids respectively. HbA comprises about 97% of the Hb in adults. Two other types, Hb A<sub>2</sub> (a<sub>2</sub>s<sub>2</sub>) and Hb F (a<sub>2</sub>y<sub>2</sub>), are found in adults in small amounts (1.5-3.2% and < 1%, respectively) (p. 440). Haemoglobin synthesis occurs in the mitochondria of the developing red cell (Fig. 8.2). The major rate-limiting step is the conversion of glycine and succinic acid to 8-aminolaevulinic acid (ALA) by ALA synthase (also see Fig. 19.22). Vitamin B<sub>6</sub> is a coenzyme for this reaction, which is inhibited by haem and stimulated by erythro-



**Fig. 8.2 Haemoglobin synthesis.** Transferrin attaches to a surface receptor on developing red cells. Iron is released and transported to the mitochondria, where it combines with protoporphyrin to form haem. Protoporphyrin itself is manufactured from glycine and succinyl-CoA. Haem combines with *a* and *p* chains (formed on ribosomes) to make haemoglobin.

poietin. Two molecules of 8-ALA condense to form a pyrrole ring (porphobilinogen). These rings are then grouped in fours to produce protoporphyrins. Finally, iron is inserted to form haem. Haem is then inserted into the globin chains to form Hb. The structure of Hb is shown in Figure 8.3.

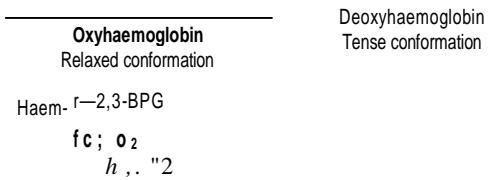


**Fig. 8.3 Model of the haemoglobin molecule showing *a* (pink) and *p* (blue) chains.** 2,3-BPG (bisphosphoglycerate) binds in the centre of the molecule and stabilizes the deoxygenated form by cross-linking the *p* chains (also see Fig. 8.4). M, methyl; P, propionic acid; V, vinyl.

## Haemoglobin function

The biconcave shape of red cells provides a large surface area for the uptake and release of oxygen and carbon dioxide. Haemoglobin becomes saturated with oxygen in the pulmonary capillaries where the partial pressure of oxygen is high and Hb has a high affinity for oxygen. Oxygen is released in the tissues where the partial pressure of oxygen is low and Hb has a low affinity for oxygen.

In adult haemoglobin (Hb A), a haem group is bound to each of the four globin chains; the haem group has a porphyrin ring with a ferrous atom which can reversibly bind one oxygen molecule. The haemoglobin molecule exists in two conformations, R and T. The T (taut) conformation of deoxyhaemoglobin is characterized by the globin units being held tightly together by electrostatic bonds (Fig. 8.4). These bonds are broken when oxygen binds to haemoglobin, resulting in the R (relaxed) conformation in which the remaining oxygen-binding sites are more exposed and have a much higher affinity for oxygen than in the T conformation. The binding of one oxygen molecule to deoxyhaemoglobin increases the oxygen affinity of the remaining binding sites - this property is known as 'cooperativity' and is the reason for the sigmoid shape of the oxygen dissociation curve. Haemoglobin is, therefore, an example of an allosteric protein. The binding of oxygen can be influenced by secondary effectors - hydrogen ions, carbon dioxide and red-cell 2,3-bisphosphoglycerate (2,3-BPG, formerly called 2,3-diphosphoglycerate (2,3-DPG)). Hydrogen ions and carbon dioxide added to blood cause a reduction in the oxygen-binding affinity of haemoglobin (the Bohr effect) (Fig. 15.5). Oxygenation of haemoglobin reduces its affinity for carbon dioxide (the Haldane effect). These effects help the exchange of carbon dioxide and oxygen in the tissues.



**Fig. 8.4 Oxygenated and deoxygenated haemoglobin molecule.** The haemoglobin molecule is predominantly stabilized by a-p chain bonds rather than a-a and p-p chain bonds. The structure of the molecule changes during O<sub>2</sub> uptake and release. When O<sub>2</sub> is released, the p chains rotate on the ctp<sub>2</sub> and o<sup>+</sup> contacts, allowing the entry of 2,3-BPG which causes a lower affinity of haemoglobin for O<sub>2</sub> and improved delivery of O<sub>2</sub> to the tissues. From Hoffbrand AV, Pettit JE (1993) *Essential Haematology*, 3rd edn. Oxford: Blackwell Scientific Publications, with permission.

Red cell metabolism produces 2,3-BPG from glycolysis. 2,3-BPG accumulates because it is sequestered by binding to deoxyhaemoglobin. The binding of 2,3-BPG stabilizes the T conformation and reduces its affinity for oxygen. The P<sub>50</sub> is the partial pressure of oxygen at which the haemoglobin is 50% saturated with oxygen. P<sub>50</sub> increases with 2,3-BPG concentrations (right-hand shift in Fig. 15.5), which increase when oxygen availability is reduced in conditions such as hypoxia or anaemia. P<sub>50</sub> also rises with increasing body temperature, which may be beneficial during prolonged exercise. Haemoglobin regulates oxygen transport as shown in the oxyhaemoglobin dissociation curve. When the primary limitation to oxygen transport is in the periphery, e.g. heavy exercise, anaemia, the P<sub>50</sub> is increased to enhance oxygen unloading. When the primary limitation is in the lungs, e.g. lung disease, high altitude exposure, the P<sub>50</sub> is reduced to enhance oxygen loading.

A summary of normal red cell production and destruction is given in Figure 8.5.

## FURTHER READING

- Pepys MB, Berger A (2001) The renaissance of C reactive protein. *British Medical Journal* **322**: 4—5.  
 Prychal JT (2003) Delivery on demand — a new era of gene therapy. *New England Journal of Medicine* **348**: 1282–1283.  
 Rosenthal N (2003) Prometheus's vulture and the stem-cell promise. *New England Journal of Medicine* **349**: 267–274.  
 Weatherall D, Provan D (2000) Red cells I: inherited anaemias. *Lancet* **355**: 1169–1175.

## ANAEMIA

Anaemia is present when there is a decrease in the level of haemoglobin in the blood below the reference level for the age and sex of the individual (Table 8.1). Alterations in the level of Hb may occur as a result of changes in the plasma volume, as shown in Figure 8.6. A reduction in the plasma volume will lead to a spuriously high Hb - this is seen with dehydration and in the clinical condition of apparent polycythaemia (see p. 455). A raised plasma volume produces a spurious anaemia, even when combined with a small increase in red cell volume as occurs in pregnancy. After a major bleed, anaemia may not be apparent for several days until the plasma volume returns to normal.

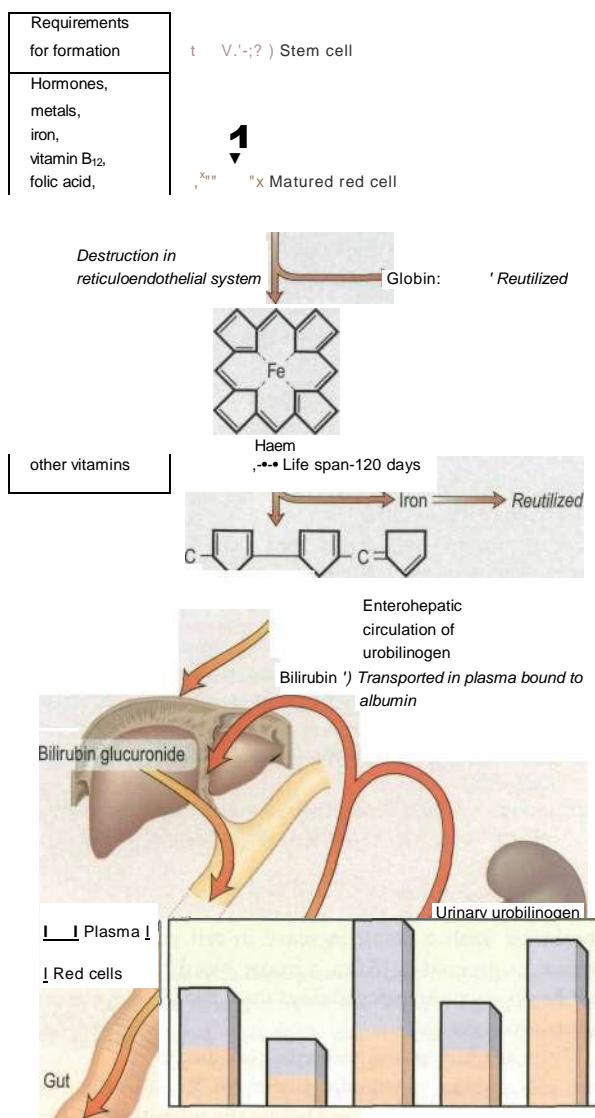
The various types of anaemia, classified in terms of the red cell indices, particularly the MCV, are shown in Figure 8.7, p. 425. There are three major types:

- hypochromic microcytic with a low MCV
- normochromic normocytic with a normal MCV
- macrocytic with a high MCV.

## Clinical features

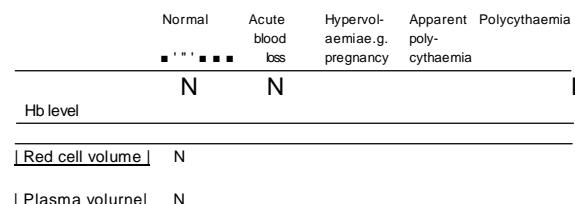
Patients with anaemia may be asymptomatic. A slowly falling level of Hb allows for haemodynamic compen-

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**Fig. 8.5 Red cell production and**

**breakdown** (see p. 350).



**Fig. 8.6 Alterations of haemoglobin in relation to plasma.**

sation and enhancement of the oxygen-carrying capacity of the blood. A rise in 2,3-BPG causes a shift of the oxygen dissociation curve to the right, so that oxygen is more readily given up to the tissues. Where blood loss is rapid, more severe symptoms will occur, particularly in elderly people.

### Symptoms (all non-specific)

- m* Fatigue
- Headaches
- Faintness

(The above three symptoms are all very common in the general population.)

- Breathlessness
- Angina
- Intermittent claudication
- Palpitations.

### Signs

- Pallor
- Tachycardia
- Systolic flow murmur
- Cardiac failure
- Rarely papilloedema and retinal haemorrhages after an acute bleed (can be accompanied by blindness).

Specific signs of the different types of anaemia will be discussed in the appropriate sections. Examples include:

- koilonychia - spoon-shaped nails seen in iron deficiency anaemia
- jaundice - found in haemolytic anaemia
- bone deformities - found in thalassaemia major
- leg ulcers - occur in association with sickle cell disease.

It must be emphasized that anaemia is not a diagnosis, and a cause must be found.

### Investigations

#### Peripheral blood

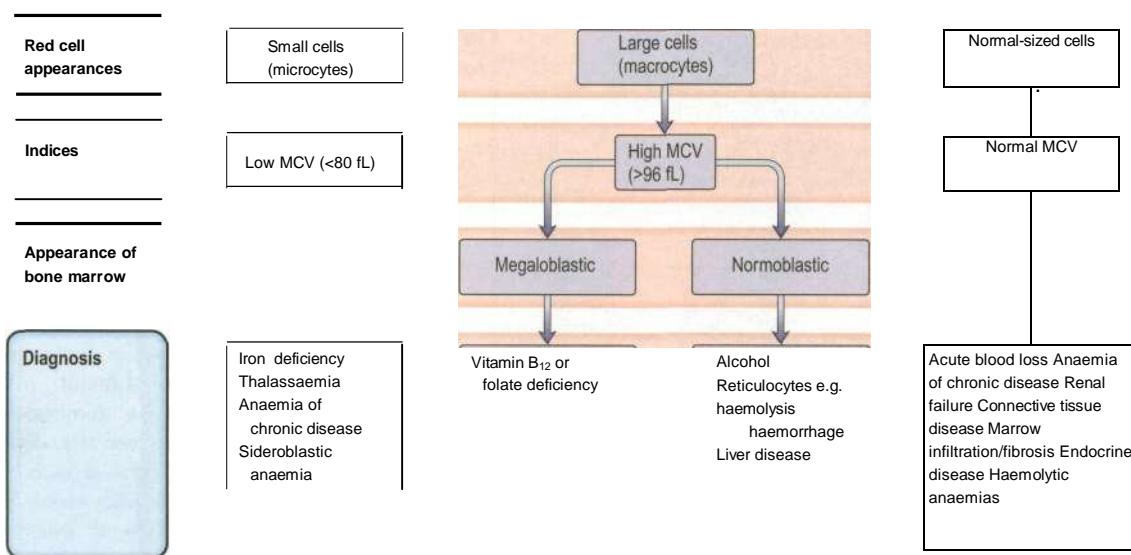
A low haemoglobin should always be considered in relation to:

- the white blood cell (WBC) count
- the platelet count
- the reticulocyte count (as this indicates marrow activity)
- the blood film, as abnormal red cell morphology (see Fig. 8.8) may indicate the diagnosis.

Where two populations of red cells are seen, the blood film is said to be dimorphic. This may, for example, be seen in patients with 'double deficiencies' (e.g. combined iron and folate deficiency in coeliac disease, or following treatment of anaemic patients with the appropriate haematinic).

#### Bone marrow

Examination of the bone marrow is performed to further investigate abnormalities found in the peripheral blood



**Fig. 8.7 Classification of anaemia.** MCV, mean corpuscular volume.

(Practical box 8.1). Aspiration provides a film which can be examined by microscopy for the morphology of the developing haemopoietic cells. The trephine provides a core of bone which is processed as a histological specimen and allows an overall view of the bone marrow architecture, cellularity and presence/absence of abnormal infiltrates.

The following are assessed:

- cellularity of the marrow
- type of erythropoiesis (e.g. normoblastic or megaloblastic)
- cellularity of the various cell lines
- infiltration of the marrow
- iron stores.

Special tests may be performed: cytogenetic, immunological, cytochemical markers, biochemical analyses (e.g. deoxyuridine suppression test), microbiological culture.

## MICROCYTIC ANAEMIA

Iron deficiency is the most common cause of anaemia in the world, affecting 30% of the world's population equivalent to 500 million people. This is because of the body's limited ability to absorb iron and the frequent loss of iron owing to haemorrhage. Although iron is abundant, most is in the insoluble ferric ( $Fe^{3+}$ ) form, which has poor bioavailability. Ferrous ( $Fe^{2+}$ ) is more readily absorbed. Free iron is toxic, and it is bound to various proteins for transport and storage.

The other causes of a microcytic hypochromic anaemia are anaemia of chronic disease, sideroblastic anaemia, and thalassaemia. In thalassaemia (p. 440) there is a defect in globin synthesis, in contrast to the other three causes of

### Practical Box 8.1

#### Techniques for obtaining "bone marrow"

The technique should be explained to the patient and consent obtained

##### Aspiration

Site - usually iliac crest

Give local anaesthetic injection

Use special bone marrow needle (e.g. Salah)

Aspirate marrow

Make smear with glass slide

Stain with:

Romanowsky technique

Perls' reaction (acid ferrocyanide) for iron.

##### Trephine

Indications include:

'Dry tap' obtained with aspiration Better assessment of cellularity, e.g. aplastic anaemia Better assessment of presence of infiltration or fibrosis.

##### Technique

Site - usually posterior iliac crest

Give local anaesthetic injection

Use special needle (e.g. Jamshidi - longer and wider than for aspiration)

Obtain core of bone

Fix in formalin; decalcify - this takes a few days

Stain with:

Haematoxylin and eosin

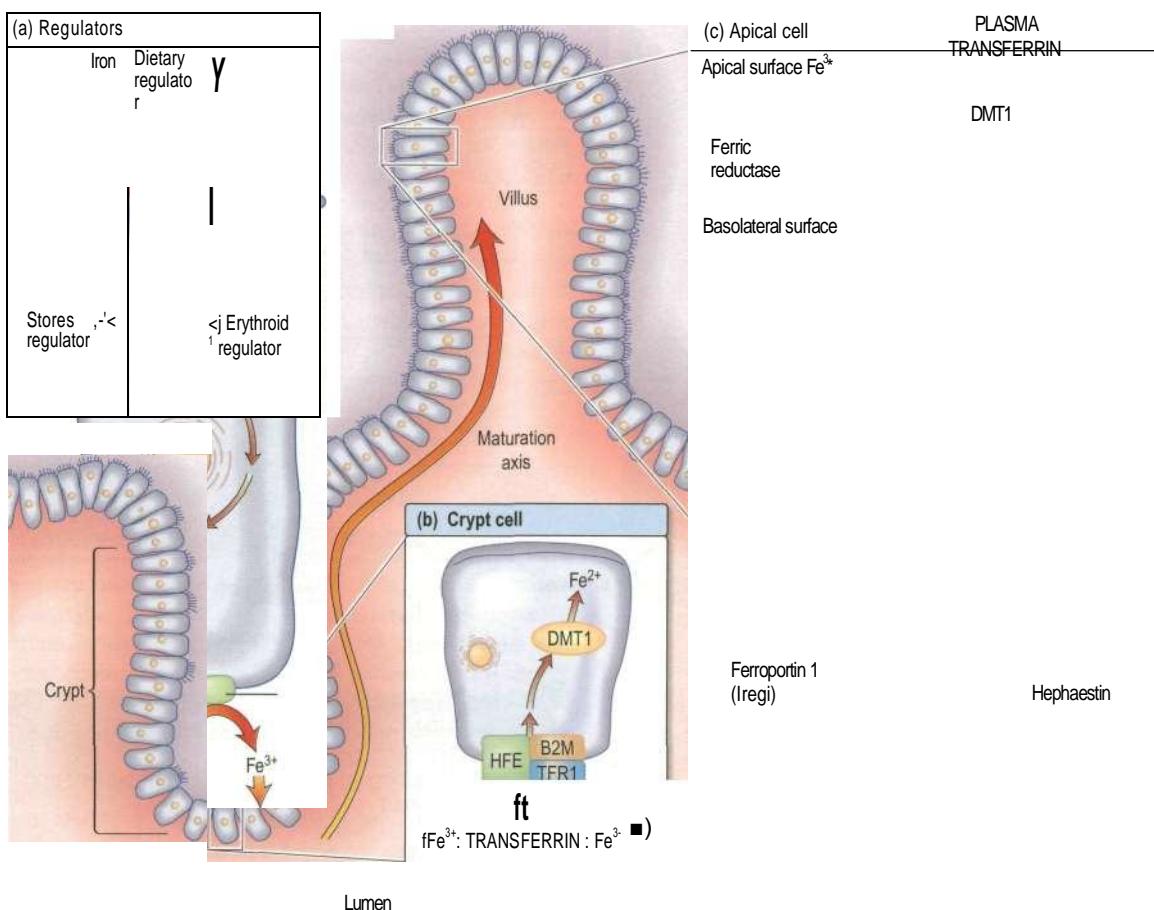
Reticulin stain.

microcytic anaemia where the defect is in the synthesis of haem.

## Iron

### Dietary intake

The average daily diet in the UK contains 15–20 mg of



**Fig. 8.8 (a)** Regulation of the absorption of intestinal iron. The iron-absorbing cells of the duodenal epithelium originate in the intestinal crypts and migrate toward the tip of the villus as they differentiate (maturation axis). Absorption of intestinal iron is regulated by at least three independent mechanisms. First, iron absorption is influenced by recent dietary iron intake (dietary regulator). After a large dietary bolus, absorptive cells are resistant to iron uptake for several days. Second, iron absorption can be modulated considerably in response to body iron stores (stores regulator). Third, a signal communicates the state of bone marrow erythropoiesis to the intestine (erythroid regulator). **(b)** Duodenal crypt cells sense body iron status through the binding of transferrin to the HFE/B2 microglobulinATR1 complex. Cytosolic enzymes change the oxidative state of iron from ferric ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ). A decrease in crypt cell iron concentration upregulates the divalent metal transporter (DMT1). This increases as crypt cells migrate up the villus and become mature absorptive cells. **(c)** Apical cell. Dietary iron is reduced from the ferrous to the ferric state by the brush border ferrireductase. DMT1 facilitates iron absorption from the intestinal lumen. The export proteins, e.g. ferroportin 1 (Iregi) and hephaestin, transfer iron from the enterocyte into the circulation. HFE, hereditary haemochromatosis gene; B2M, p<sub>2</sub>-microglobulin.

iron, although normally only 10% of this is absorbed. Absorption may be increased to 20–30% in iron deficiency and pregnancy.

Non-haem iron is mainly derived from cereals, which are commonly fortified with iron; it forms the main part of dietary iron. Haem iron is derived from haemoglobin and myoglobin in red or organ meats. Haem iron is better absorbed than non-haem iron, whose availability is more affected by other dietary constituents.

#### Absorption (Fig. 8.8(a),(b))

Factors influencing iron absorption are shown in Table 8.2. Haem iron is partly broken down to non-haem iron, but some haem iron is absorbed intact into mucosal cells. Iron absorption occurs primarily in the duodenum. Non-haem iron is dissolved in the low pH of the stomach and reduced from the ferric to the ferrous form by a brush

border ferrireductase. Cells in duodenal crypts are able to sense the body's iron requirements and retain this information as they mature into cells capable of absorbing iron at the tips of the villi. A protein, divalent metal transporter 1 (DMT1), (formerly called divalent cation transporter [DCT1] or natural resistance-associated macrophage protein [NRAMP2]) transports iron (and other metals) across the apical (luminal) surface of the mucosal cells in the small intestine. Haem iron is absorbed in a separate less-well-characterized process.

Once inside the mucosal cell, iron may be transferred across the cell to reach the plasma, or be stored as ferritin; the body's iron status at the time the absorptive cell developed from the crypt cell is probably the crucial deciding factor. Iron stored as ferritin will be lost into the gut lumen when the mucosal cells are shed; this regulates iron balance. The mechanism of transport of iron across

**Table 8.2 Factors influencing iron absorption**

Haem iron is absorbed better than non-haem iron
Ferrous iron is absorbed better than ferric iron
Gastric acidity helps to keep iron in the ferrous state and soluble in the upper gut Formation of insoluble complexes with phytate or phosphate decreases iron absorption Iron absorption is increased with low iron stores and increased erythropoietic activity, e.g. bleeding, haemolysis, high altitude There is a decreased absorption in iron overload, except in hereditary haemochromatosis, where it is increased

the basolateral surface of mucosal cells involves a transporter protein, ferroportin 1 (Ireg1). This transporter protein requires an accessory, multicopper protein, hephaestin (see Fig. 8.9c).

The iron content of the body is kept within narrow limits and its loss and intake are normally finely balanced. Its absorption is closely related to the total iron stores of the body; in iron deficiency, absorption of iron may increase to 3–4 mg daily. The body is unable to excrete iron once it has been absorbed so the regulation of iron absorption is critical. Iron absorption is regulated in several ways (see Fig. 8.9) by dietary, stores and erythroid regulators. The liver peptide hepcidin may be the store and/or erythroid regulator. The hereditary haemochromatosis gene (*HFE*) plays a role in iron absorption, although the mechanism is not fully understood. It is not the prime regulator since iron absorption also responds to the stores of iron and erythroid regulators when *HFE* is either mutated or absent.

Anaemias with increased rates of erythropoiesis do not cause equal increases in iron absorption; for example, conditions with 'ineffective erythropoiesis' such as thalassaemia stimulate greater iron absorption than haemolytic anaemias such as hereditary spherocytosis and autoimmune haemolytic anaemia where red cell destruction occurs in the periphery.

### Transport in the blood

The normal serum iron level is about 13–32 µmol/L; there is a diurnal rhythm with higher levels in the morning. Iron is transported in the plasma bound to transferrin, a P-globulin that is synthesized in the liver. Each transferrin molecule binds two atoms of ferric iron and is normally one-third saturated. Most of the iron bound to transferrin comes from macrophages in the reticuloendothelial system and not from iron absorbed by the intestine. Transferrin-bound iron becomes attached by specific receptors to erythroblasts and reticulocytes in the marrow and the iron is removed (see Fig. 8.2).

In an average adult male, 20 mg of iron, chiefly obtained from red cell breakdown in the macrophages of the reticuloendothelial system, is incorporated into Hb every day.

### Iron stores

About two-thirds of the total body iron is in the circulation as haemoglobin (2.5–3 g in a normal adult man).

Iron is stored in reticuloendothelial cells, hepatocytes and skeletal muscle cells (500–1500 mg). About two-thirds of this is stored as ferritin and one-third as haemosiderin in normal individuals. Small amounts of iron are also found in plasma (about 4 mg bound to transferrin), with some in myoglobin and enzymes.

**Ferritin** is a water-soluble complex of iron and protein. It is more easily mobilized than haemosiderin for Hb formation. It is present in small amounts in plasma.

**Haemosiderin** is an insoluble iron-protein complex found in macrophages in the bone marrow, liver and spleen. Unlike ferritin, it is visible by light microscopy in tissue sections and bone marrow films after staining by Perls' reaction.

### Requirements

Each day 0.5–1.0 mg of iron is lost in the faeces, urine and sweat. Menstruating women lose 30–40 mL of blood per month, an average of about 0.5–0.7 mg of iron per day. Blood loss through menstruation in excess of 100 mL will usually result in iron deficiency as increased iron absorption from the gut cannot compensate for such losses of iron. The demand for iron also increases during growth (about 0.6 mg per day) and pregnancy (1–2 mg per day). In the normal adult the iron content of the body remains relatively fixed. Increases in the body iron content (haemochromatosis) are classified into:

- hereditary haemochromatosis (p. 386), where a mutation in the *HFE* gene causes upregulation of DMT1 and increased iron absorption
- secondary haemochromatosis (transfusion siderosis; see p. 441). This is due to iron overload in conditions where repeated transfusion is the only therapy.

### Iron deficiency

Iron deficiency anaemia develops when there is inadequate iron for haemoglobin synthesis. A normal level of Hb is maintained for as long as possible after the iron stores are depleted; latent iron deficiency is said to be present during this period.

### Causes

- Blood loss
- Increased demands such as growth and pregnancy
- Decreased absorption (e.g. postgastrectomy)
- Poor intake.

Most iron deficiency is due to blood loss, usually from the uterus or gastrointestinal tract. Premenopausal women are in a state of precarious iron balance owing to menstruation. Iron deficiency affects more than a quarter of the world's population, but isolated nutritional iron deficiency is rare in developed countries. The most common cause of iron deficiency world-wide is blood loss from the gastrointestinal tract resulting from hookworm infestation. The poor quality of the diet, predominantly containing vegetables, also contributes to the high prevalence of iron deficiency in developing countries. Even in developed countries, iron deficiency is not uncommon in infancy

## Haematological disease

where iron intake is insufficient for the demands of growth. It is more prevalent in infants born prematurely or where the introduction of mixed feeding is delayed.

### Clinical features

The symptoms of anaemia are described on page 424. Other clinical features occur as a result of tissue iron deficiency. These are mainly epithelial changes induced by the effect of inadequate iron in the cells:

- brittle nails
- spoon-shaped nails (koilonychia)
- atrophy of the papillae of the tongue
- angular stomatitis ..
- brittle hair
- a syndrome of dysphagia and glossitis (Plummer-Vinson or Paterson-Brown-Kelly syndrome; see p. 279).

The diagnosis of iron deficiency anaemia relies on a good clinical history with questions about dietary intake, regular self-medication with non-steroidal anti-inflammatory drugs (which may give rise to gastrointestinal bleeding), and the presence of blood in the faeces (which may be a sign of haemorrhoids or carcinoma of the lower bowel). In women, a careful enquiry about the duration of periods, the occurrence of clots and the number of sanitary towels or tampons (normal 3-5/day) used should be made.

### Investigations

#### Blood count and film

A characteristic blood film is shown in Figure 8.9. The red cells are microcytic ( $MCV < 80 \text{ fL}$ ) and hypochromic ( $MCH < 27 \text{ pg}$ ). There is poikilocytosis (variation in shape) and anisocytosis (variation in size). Target cells are seen.

#### Serum iron and iron-binding capacity

The serum iron falls and the total iron-binding capacity (TIBC) rises in iron deficiency compared with normal. Iron deficiency is regularly present when the transferrin saturation (i.e. serum iron divided by TIBC) falls below 19% (Table 8.3).

**Fig. 8.9 Hypochromic microcytic cells (arrow) on a blood film.** Poikilocytosis and anisocytosis are seen.

#### Serum ferritin

The level of serum ferritin reflects the amount of stored iron. The normal values for serum ferritin are 30-300  $\mu\text{g/L}$  (11.6-144 nmol/L) in males and 15-200 ng/L (5.8-96 nmol/L) in females. In simple iron deficiency, a low serum ferritin confirms the diagnosis. However, ferritin is an acute-phase reactant, and levels increase in the presence of inflammatory or malignant diseases. In these cases, measurement of serum iron/TIBC, serum ferritin and soluble transferrin receptors is used.

#### Serum soluble transferrin receptors

The number of transferrin receptors increases in iron deficiency. The results of this immunoassay compares well with results from bone marrow aspiration at estimating iron stores.

This assay can help to distinguish between iron deficiency and anaemia of chronic disease (see Table 8.3), and may avoid the need for bone marrow examination even in complex cases.

#### Bone marrow

Erythroid hyperplasia with ragged normoblasts is seen in the marrow in iron deficiency. Staining using Perls' reaction (acid ferrocyanide) does not show the characteristic Prussian-blue granules of stainable iron in the bone marrow fragments or in the erythroblasts.

Examination of the bone marrow is not essential for the diagnosis of iron deficiency but it may be helpful in

**Table 8.3 Microcytic anaemia: the differential diagnosis**

	Iron deficiency	Anaemia of chronic disease	Thalassaemia trait (a or p)	Sideroblastic anaemia
MCV	Reduced	Low normal or normal	Very low for degree of anaemia	Low in inherited type but often raised in acquired type
Serum iron	Reduced	Reduced	Normal	Raised
Serum TIBC	Raised	Reduced	Normal	Normal
Serum ferritin	Reduced	Normal or raised	Normal	Raised
Serum soluble transfer receptors	Increased	Normal	Normal or raised	Normal or raised
Iron in marrow	Absent	Present	Present	Present
Iron in erythroblasts	Absent	Absent or reduced	Present	Ring forms

TIBC, total iron binding capacity

the investigation of complicated cases of anaemia, e.g. to determine if iron deficiency is present in a patient with anaemia of chronic disease.

#### *Other investigations*

These will be indicated by the clinical history and examination. Investigations of the gastrointestinal tract are often required to determine the cause of the iron deficiency (see p. 293).

#### *Differential diagnosis*

The presence of anaemia with microcytosis and hypochromia does not necessarily indicate iron deficiency. The most common other causes are thalassaemia, sideroblastic anaemia and anaemia of chronic disease, and in these disorders the iron stores are normal or increased. The differential diagnosis of microcytic anaemia is shown in Table 8.3.

■ ..

#### *Treatment*

Iron deficiency is not a diagnosis per se. The correct management of iron deficiency is to find and treat the underlying cause, and to give iron to correct the anaemia and replace iron stores. The response to iron therapy can be monitored using the reticulocyte count and Hb level, with an expected rise in haemoglobin of 1 g/dL per week.

Oral iron is all that is required in most cases. The best preparation is ferrous sulphate (200 mg three times daily, a total of 180 mg ferrous iron) which is absorbed best when the patient is fasting. If the patient has side-effects such as nausea, diarrhoea or constipation, taking the tablets with food or reducing the dose using a preparation with less iron such as ferrous gluconate (300 mg twice daily, only 70 mg ferrous iron) is all that is usually required to reduce the symptoms. The use of expensive iron compounds, particularly the slow-release ones which release iron beyond its main sites of absorption, is unnecessary.

In developing countries, distribution of iron tablets is the main approach for the alleviation of iron deficiency. However, iron supplementation programmes have been ineffective, probably mainly because of poor compliance.

Oral iron should be given for long enough to correct the Hb level and to replenish the iron stores. This can take 6 months. The commonest causes of failure of response to oral iron are:

Failure to respond to oral iron

- lack of compliance
- continuing haemorrhage
- incorrect diagnosis, e.g. thalassaemia trait.

These possibilities should be considered before parenteral iron is used. However, parenteral iron is required by occasional patients, including those who have general intolerance of oral preparations even at low dose, those with severe malabsorption, and those who have chronic gastrointestinal diseases such as ulcerative colitis or Crohn's disease. Iron stores are replaced much faster with parenteral iron than with oral iron, but the haematological response is no quicker. Parenteral iron can be given as repeated deep intramuscular injections of

iron-sorbitol (1.5 mg of iron per kg body weight) or by slow intravenous infusion of iron-sucrose.

#### **Anaemia of chronic disease**

One of the most common types of anaemia, particularly in hospital patients, is the anaemia of chronic disease, occurring in patients with chronic infections such as infective endocarditis or tuberculosis and osteomyelitis in developing countries. Other causes include chronic inflammatory diseases such as Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus (SLE), polymyalgia rheumatica, and malignant disease. There is decreased release of iron from the bone marrow to developing erythroblasts, an inadequate erythropoietin response to the anaemia, and decreased red cell survival. The exact mechanisms responsible for these effects are not clear, but they seem to be mediated by inflammatory cytokines such as IL-1, tumour necrosis factor and interferons.

The serum iron and the TIBC are low, and the serum ferritin is normal or raised because of the inflammatory process. The serum soluble transferrin receptor level is normal (Table 8.3). Stainable iron is present in the bone marrow, but iron is not seen in the developing erythroblasts. Patients do not respond to iron therapy, and treatment is, in general, that of the underlying disorder. Recombinant erythropoietin therapy is used in the anaemia of renal disease (p. 673), inflammatory disease (rheumatoid arthritis, inflammatory bowel disease) and is being trialled in, for example, myelodysplasia).

#### **Sideroblastic anaemia**

Sideroblastic anaemias are inherited or acquired disorders characterized by a refractory anaemia, a variable number of hypochromic cells in the peripheral blood, and excess iron and ring sideroblasts in the bone marrow. The presence of ring sideroblasts is the diagnostic feature of sideroblastic anaemia. There is accumulation of iron in the mitochondria of erythroblasts owing to disordered haem synthesis forming a ring of iron granules around the nucleus that can be seen with Perls' reaction. The blood film is often dimorphic; ineffective haem synthesis is responsible for the microcytic hypochromic cells. Sideroblastic anaemias are classified as shown in Table 8.4. A structural defect in 8-aminolaevulinic acid (ALA) synthase, the pyridoxine-dependent enzyme responsible for the first step in haem synthesis (see Fig. 8.2), has been identified in one form of inherited sideroblastic anaemia. Primary acquired sideroblastic anaemia is one of the myelodysplastic syndromes (see p. 455). Lead toxicity is described on p. 1014.

#### *Treatment*

Some patients respond when drugs or alcohol are withdrawn, if these are the causative agents. In occasional cases, there is a response to pyridoxine. Treatment with folic acid may be required to treat accompanying folate deficiency.

**Table 8.4 Classification of sideroblastic anaemia**

**Inherited**

X-linked disease - transmitted by females

**Acquired**

Myelodysplasia

Myeloproliferative disorders

Myeloid leukaemia Drugs, e.g.

isoniazid Alcohol abuse Lead

toxicity

Other disorders, e.g. rheumatoid arthritis, carcinoma, megaloblastic and haemolytic anaemias

### FURTHER READING

- Bomford A (2002) Genetics of haemochromatosis. *Lancet* 360:1673-1681. Fleming RE, Bacon BR (2005) Orchestration of iron homeostasis. *New England Journal of Medicine* 352: 1741-1744. Provan D, Weatherall D (2000) Red cells II: acquired anaemias. *Lancet* 355:1260-1268. Spivak JL (2000) The blood in systemic disorders. *Lancet* 355:1707-1712.

## NORMOCYTIC ANAEMIA

Normocytic, normochromic anaemia is seen in anaemia of chronic disease, in some endocrine disorders (e.g. hypopituitarism, hypothyroidism and hypoadrenalinism) and in some haematological disorders (e.g. aplastic anaemia and some haemolytic anaemias) (see Fig. 8.7). In addition, this type of anaemia is seen acutely following blood loss. ■

## MACROCYTIC ANAEMIAS

These can be divided into megaloblastic and non-megaloblastic types, depending on bone marrow findings.

### MEGALOBLASTIC ANAEMIA

Megaloblastic anaemia is characterized by the presence in the bone marrow of erythroblasts with delayed nuclear maturation because of defective DNA synthesis (megaloblasts). Megaloblasts are large and have large immature nuclei. The nuclear chromatin is more finely dispersed than normal and has an open stippled appearance (Fig. 8.10). A characteristic abnormality of white cells, giant metamyelocytes, is frequently seen in megaloblastic anaemia. These cells are about twice the size of normal cells and often have twisted nuclei. Megaloblastic changes occur in:

- vitamin B<sub>12</sub> deficiency or abnormal vitamin B<sub>12</sub> metabolism
- folic acid deficiency or abnormal folate metabolism

**Fig. 8.10 Megaloblasts (arrowed) in the bone marrow.**

- other defects of DNA synthesis, such as congenital enzyme deficiencies in DNA synthesis (e.g. orotic aciduria), or resulting from therapy with drugs interfering with DNA synthesis (e.g. hydroxycarbamide (hydroxyurea), azathioprine, zidovudine - AZT)
- myelodysplasia due to dyserythropoiesis.

### Haematological values

Anaemia may be present. The MCV is characteristically > 96 fL unless there is a coexisting cause of microcytosis when there may be a dimorphic picture with a normal/low average MCV. The peripheral blood film shows macrocytes with hypersegmented polymorphs with six or more lobes in the nucleus (see Fig. 8.11). If severe, there may be leucopenia and thrombocytopenia.

### Biochemical basis of megaloblastic anaemia

The key biochemical problem common to both vitamin B<sub>12</sub> and folate deficiency is a block in DNA synthesis owing to an inability to methylate deoxyuridine monophosphate to deoxythymidine monophosphate, which is then used to build DNA (Fig. 8.12). The methyl group is supplied by the folate coenzyme, methylene tetrahydrofolate.

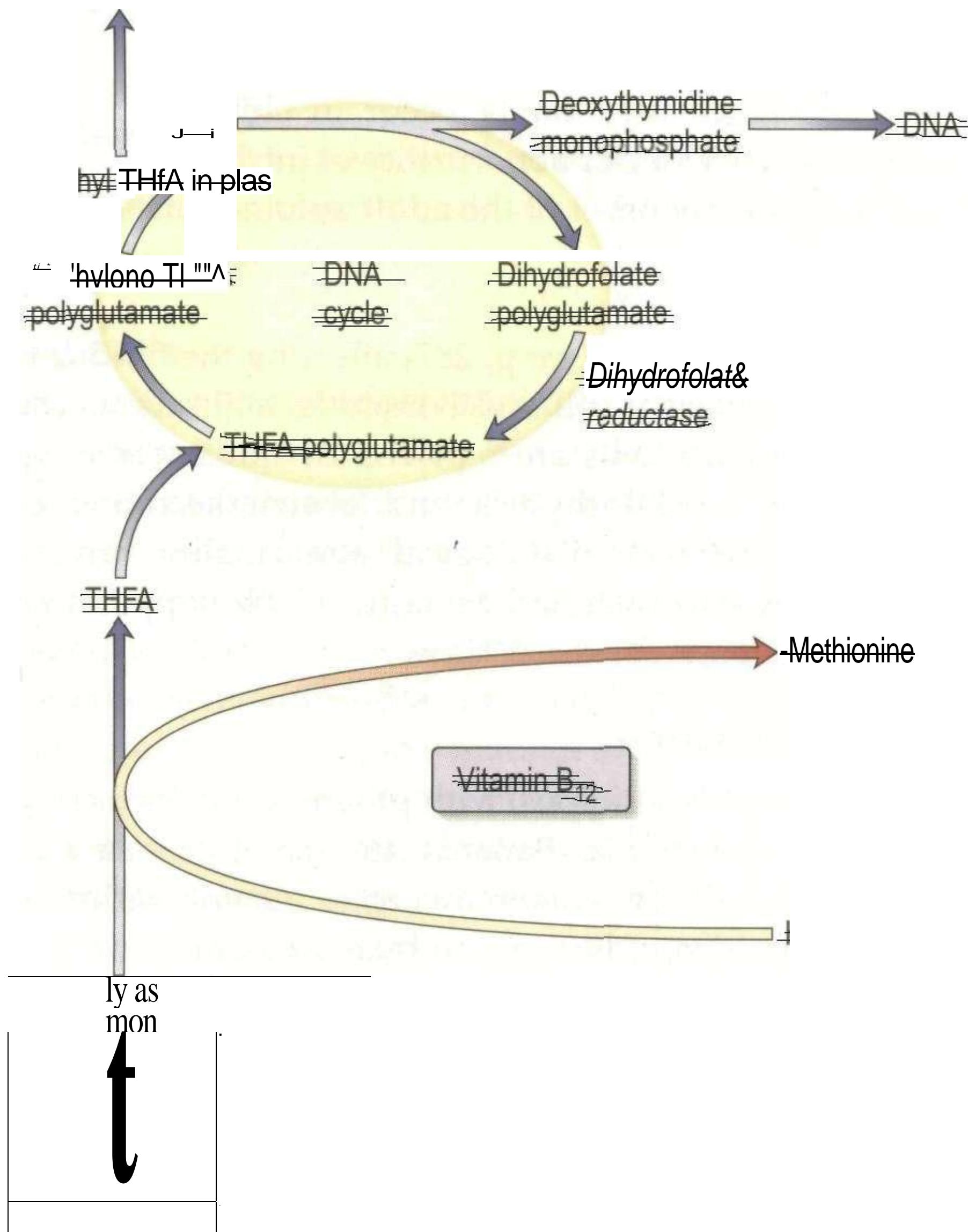
Deficiency of folate reduces the supply of this coenzyme; deficiency of vitamin B<sub>12</sub> also reduces its supply by slowing the demethylation of methyltetrahydrofolate (methyl THF) and preventing cells receiving tetrahydrofolate for synthesis of methylene tetrahydrofolate polyglutamate.

Other congenital and acquired forms of megaloblastic anaemia are due to interference with purine or pyrimidine synthesis causing an inhibition in DNA synthesis.

### Deoxyuridine suppression test

This is a useful method for rapidly determining the nature and severity of the vitamin B<sub>12</sub> or folate deficiency in severe or complex cases of megaloblastic anaemia.

**Fig. 8.11 Macrocytes and a hypersegmented neutrophil (arrowed) on a peripheral blood film.**



**Fig. 8.12 Biochemical basis of megaloblastic anaemia.**

The metabolic relationship between vitamin B<sub>12</sub> and folate and their role in DNA synthesis. THF, tetrahydrofolate.

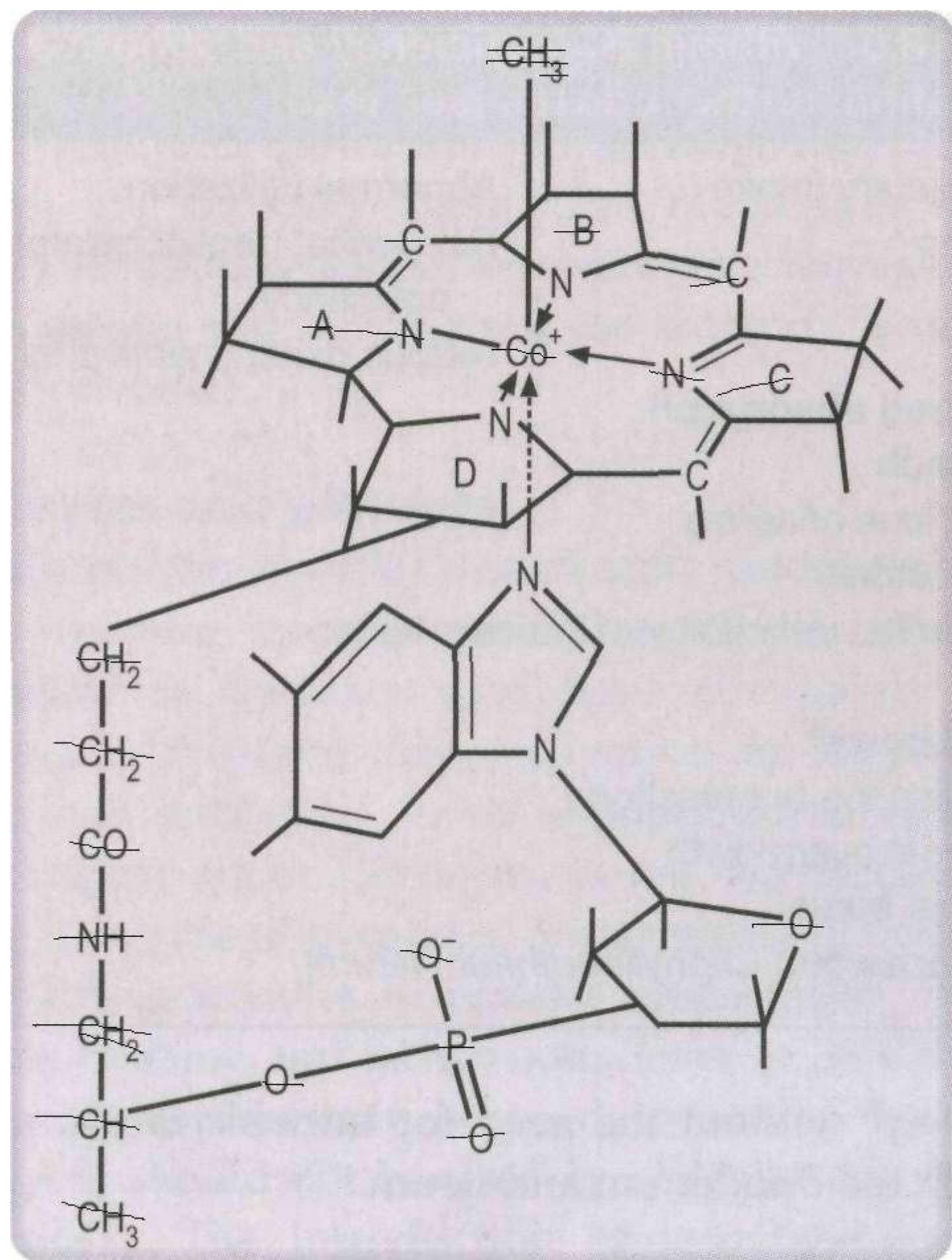
Tritiated thymidine is added to the patient's bone marrow in vitro. In a normoblastic marrow, the thymidine requirement is supplied by the methylation of deoxyuridine and this 'suppresses' the requirement for preformed tritiated thymidine to less than 5%. In a megaloblastic marrow, however, much more tritiated thymidine is used (5-50%). If the addition of B<sub>12</sub> corrects the abnormality, it suggests that B<sub>12</sub> is the cause of the deficiency. The addition of folate corrects the abnormality in both vitamin B<sub>12</sub> and folate deficiency.

## Vitamin B

Vitamin B<sub>12</sub> is synthesized by certain microorganisms, and humans are ultimately dependent on animal sources. It is found in meat, fish, eggs and milk, but not in plants. Vitamin B<sub>12</sub> is not usually destroyed by cooking. The average daily diet contains 5-30 µg of vitamin B<sub>12</sub>, of which 2-3 µg is absorbed. The average adult stores some 2-3 mg, mainly in the liver, and it may take 2 years or more after absorptive failure before B<sub>12</sub> deficiency develops, as the daily losses are small (1-2 µg).

### Structure and function

Cobalamins consist of a planar group with a central cobalt atom (corrin ring) and a nucleotide set at right-



**Fig. 8-13 Methylcobalamin structure.** This is the main form of vitamin B<sub>12</sub> in the plasma.

angles (Fig. 8.13). Vitamin B<sub>12</sub> was first crystallized as cyanocobalamin, but the main natural cobalamins have deoxyadenosyl-, methyl- and hydroxocobalamin groups attached to the cobalt atom.

The main function of B<sub>12</sub> is the methylation of homocysteine to methionine with the demethylation of methyl THF polyglutamate to THF. THF is a substrate for folate polyglutamate synthesis.

Deoxyadenosylcobalamin is a coenzyme for the conversion of methylmalonyl CoA to succinyl CoA. Measurement of methylmalonic acid in urine was used as a test for vitamin B<sub>12</sub> deficiency but it is no longer carried out routinely.

### Absorption and transport

Vitamin B<sub>12</sub> is liberated from protein complexes in food by gastric enzymes and then binds to a vitamin B<sub>12</sub>-binding protein ('R' binder) related to plasma *transcobalamin I* (TC I), derived from saliva. Vitamin B<sub>12</sub> bound to 'R' binder is released by pancreatic enzymes and becomes bound to intrinsic factor.

Intrinsic factor is a glycoprotein with a molecular weight of 45 000. It is secreted by gastric parietal cells along with H<sup>+</sup> ions. It combines with vitamin B<sub>12</sub> and carries it to specific receptors on the surface of the mucosa of the ileum. Vitamin B<sub>12</sub> enters the ileal cells and intrinsic factor remains in the lumen. Vitamin B<sub>12</sub> is transported from the enterocytes to the bone marrow and other tissues by the glycoprotein *transcobalamin II* (TCII). Although TC II is the essential carrier protein for vitamin B<sub>12</sub>, the amount of B<sub>12</sub> on TCII is low; it has a rapid clearance and is able to deliver cobalamin to all cells of the body. Vitamin B<sub>12</sub> in plasma is mainly bound to TC I (70-90%), but the functional role of this protein is unknown. About 1% of an oral dose of B<sub>12</sub> is absorbed

## Haematological disease

**Table 8.5 Vitamin B<sub>12</sub> deficiency and abnormal B<sub>12</sub> utilization: further causes (see text)**

Low dietary intake	Abnormal utilization	Congenital transcobalamin II deficiency
Vegans	Nitrous oxide (inactivates B- <sup>+</sup> )	
<b>Impaired absorption</b>		
<b>Stomach</b>		
Pernicious anaemia		
Gastrectomy		
Congenital deficiency of intrinsic factor		
<b>Small bowel</b>		
Heal disease or resection		
Bacterial overgrowth		
Tropical sprue		
Fish tapeworm ( <i>Diphyllobothrium latum</i> )		

'passively' without the need for intrinsic factor, mainly through the duodenum and ileum.

### Vitamin B<sub>12</sub> deficiency

There are a number of causes of B<sub>12</sub> deficiency and abnormal B<sub>12</sub> metabolism (Table 8.5). The most common cause of vitamin B<sub>12</sub> deficiency in adults is pernicious anaemia. Malabsorption of vitamin B<sub>12</sub> because of pancreatitis, coeliac disease or treatment with metformin is mild and does not usually result in significant vitamin B<sub>12</sub> deficiency.

### Pernicious anaemia

Pernicious anaemia (PA) is an autoimmune disorder in which there is atrophic gastritis with loss of parietal cells in the gastric mucosa with consequent failure of intrinsic factor production and vitamin B<sub>12</sub> malabsorption.

#### Pathogenesis of pernicious anaemia

This disease is common in the elderly, with 1 in 8000 of the population aged over 60 years being affected in the UK. It can be seen in all races, but occurs more frequently in fair-haired and blue-eyed individuals, and those who have the blood group A. It is more common in females than males.

There is an association with other autoimmune diseases, particularly thyroid disease, Addison's disease and vitiligo. Approximately one-half of all patients with PA have thyroid antibodies. There is a higher incidence of gastric carcinoma with PA than in the general population; the incidence in PA is 1-3%.

Parietal cell antibodies are present in the serum in 90% of patients with PA and also in many older patients with gastric atrophy. Conversely, intrinsic factor antibodies, although found in only 50% of patients with PA, are specific for this diagnosis. Two types of intrinsic factor antibodies are found: a blocking antibody, which inhibits binding of intrinsic factor to B<sub>12</sub>, and a precipitating antibody, which inhibits the binding of the B<sub>12</sub>-intrinsic factor complex to its receptor site in the ileum.

B<sub>12</sub> deficiency may rarely occur in children from a congenital deficiency or abnormality of intrinsic factor, or as a result of early onset of the adult autoimmune type.

#### Pathology

Autoimmune gastritis (see p. 287) affecting the fundus is present with plasma cell and lymphoid infiltration. The parietal and chief cells are replaced by mucin-secreting cells. There is achlorhydria and absent secretion of intrinsic factor. The histological abnormality can be improved by corticosteroid therapy, which supports an autoimmune basis for the disease.

#### Clinical features

The onset of PA is insidious, with progressively increasing symptoms of anaemia. Patients are sometimes said to have a lemon-yellow colour owing to a combination of pallor and mild jaundice caused by excess breakdown of haemoglobin. A red sore tongue (glossitis) and angular stomatitis are sometimes present.

The neurological changes, if left untreated for a long time, can be irreversible. These neurological abnormalities occur only with very low levels of serum B<sub>12</sub> (less than 60 ng/L) and occasionally occur in patients who are not clinically anaemic. The classical neurological features are those of a polyneuropathy progressively involving the peripheral nerves and the posterior and eventually the lateral columns of the spinal cord (subacute combined degeneration; p. 1263). Patients present with symmetrical paraesthesiae in the fingers and toes, early loss of vibration sense and proprioception, and progressive weakness and ataxia. Paraplegia may result. Dementia, psychiatric problems, e.g. depression, hallucinations, delusions, and optic atrophy also occur from vitamin B<sub>12</sub> deficiency.

#### Investigations

- **Haematological findings** show the features of a megaloblastic anaemia as described on page 430.
- **Bone marrow** shows the typical features of megloblastic erythropoiesis (Fig. 8.10), although it is frequently not performed in cases of straightforward macrocytic anaemia and a low serum vitamin B<sub>12</sub>.
- **Serum bilirubin** may be raised as a result of ineffective erythropoiesis. Normally a minor fraction of serum bilirubin results from premature breakdown of newly formed red cells in the bone marrow. In many megaloblastic anaemias, where the destruction of developing red cells is much increased, the serum bilirubin can be increased.
- **Serum vitamin B<sub>12</sub>** is usually well below 160 ng/L, which is the lower end of the normal range. Serum vitamin B<sub>12</sub> can be assayed using radioisotope dilution or immunological assays.
- **Serum folate level** is normal or high, and the red cell folate is normal or reduced owing to inhibition of normal folate synthesis.

#### Absorption tests

Vitamin B<sub>12</sub> absorption tests are performed only occasionally when the underlying cause of the B<sub>12</sub> deficiency is not

obvious. They cannot be performed in the UK as radioactive B<sub>12</sub> is not available. However, the principle of the absorption test is useful.

**Schilling test.** Radioactive B<sub>12</sub> is given orally followed by an i.m. injection of non-radioactive B<sub>12</sub> to saturate B<sub>12</sub> binding proteins and to flush out <sup>58</sup>Co-B<sub>12</sub>. The urine is collected for 24 hours and > 10% of the oral dose would be excreted in a normal person. If this is abnormal, the test is repeated with the addition of oral intrinsic factor capsules. If the excretion is now normal, the diagnosis is pernicious anaemia or gastrectomy. If the excretion is still abnormal, the lesion must be in the terminal ileum or there may be bacterial overgrowth. The latter could be confirmed by repeating the test after a course of antibiotics.

#### Gastrointestinal investigations

In PA there is achlorhydria. Intubation studies can be performed to confirm this but are rarely carried out in routine practice. Endoscopy or barium meal examination of the stomach is performed only if gastric symptoms are present.

#### Differential diagnosis

Vitamin B<sub>12</sub> deficiency must be differentiated from other causes of megaloblastic anaemia, principally folate deficiency, but usually this is quite clear from the blood level of these two vitamins.

Pernicious anaemia should be distinguished from other causes of vitamin B<sub>12</sub> deficiency (see Table 8.5). Any disease involving the terminal ileum or bacterial overgrowth in the small bowel can produce vitamin B<sub>12</sub> deficiency (see p. 304). Gastrectomy can lead, in the long term, to vitamin B<sub>12</sub> deficiency. Vegans are strict vegetarians and eat no meat or animal products. A careful dietary history should be obtained.

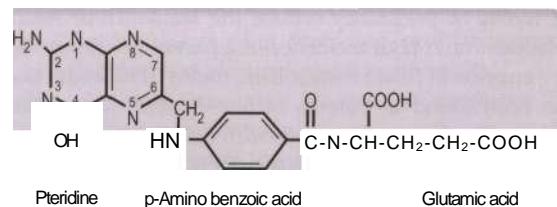
#### Treatment

See page 434.

#### Folic acid

Folic acid monoglutamate is not itself present in nature but occurs as polyglutamates (extra glutamic acid residues).

Folates are present in food as polyglutamates in the reduced dihydrofolate or tetrahydrofolate (THF) forms (Fig. 8.14), with methyl (CH<sub>3</sub>), formyl (CHO) or methylene (CH<sub>2</sub>) groups attached to the pteridine part of the molecule. Polyglutamates are broken down to monoglutamates in the upper gastrointestinal tract, and during the absorptive process these are converted to methyl THF monoglutamate, which is the main form in the serum. The methylation of homocysteine to methionine requires both methylcobalamin and methyl THF as coenzymes. This reaction is the first step in which methyl THF entering cells from the plasma is converted into folate polyglutamates. Intracellular polyglutamates are the active forms of folate and act as coenzymes in the transfer



**Fig. 8.14 Folic acid structure.** This is formed from three building blocks as shown. Tetrahydrofolate has additional hydrogen atoms at positions 5, 6, 7 and 8.

of single carbon units in amino acid metabolism and DNA synthesis (see Fig. 8.12).

#### Dietary intake

Folate is found in green vegetables such as spinach and broccoli, and offal, such as liver and kidney. Cooking causes a loss of 60–90% of the folate. The minimal daily requirement is about 100 µg.

#### Folate deficiency

The causes of folate deficiency are shown in Table 8.6. The main cause is poor intake, which may occur alone or in combination with excessive utilization or malabsorption. The body's reserves of folate, unlike vitamin B<sub>12</sub>, are low (about 10 mg). On a deficient diet, folate deficiency develops over the course of about 4 months, but folate deficiency may develop rapidly in patients who have both a poor intake and excess utilization of folate (e.g. patients in intensive care units).

There is no simple relationship between maternal folate status and fetal abnormalities but folic acid supplements at the time of conception and in the first

**Table 8.6 Causes of folate deficiency**

Nutritional (major cause)	Excess utilization
<b>Poor intake</b>	<b>Physiological</b>
Old age	Pregnancy
Poor social conditions	Lactation
Starvation	Prematurity
Alcohol excess (also causes impaired utilization)	<b>Pathological</b>
<b>Poor intake due to anorexia</b>	Haematological disease with excess red cell production, e.g. haemolysis
Gastrointestinal disease, e.g. partial gastrectomy, coeliac disease, Crohn's disease	Malignant disease with increased cell turnover
Cancer	Inflammatory disease
<b>Antifolate drugs</b>	Metabolic disease, e.g. homocystinuria
Anticonvulsants:	Haemodialysis or peritoneal dialysis
phenytoin	<b>Malabsorption</b>
primidone	Occurs in small bowel disease, but the effect is minor compared with that of anorexia
Methotrexate	
Pyrimethamine	
Trimethoprim	

## **tj^matologicaldisease**

12 weeks of pregnancy reduce the incidence of neural tube defects. A high incidence of a partial deficiency in a key enzyme in folate metabolism, methyl THF reductase, has been found in parents of fetuses with neural tube defects. The 5% of individuals with this abnormality have increased levels of homocysteine (Fig. 8.12), which may be the mechanism underlying the increased incidence of neural tube defects. Autoantibodies to the folate receptor have also been found in some women with a pregnancy complicated by a neural tube defect.

### **Clinical features**

Patients with folate deficiency may be asymptomatic or present with symptoms of anaemia or of the underlying cause. Glossitis can occur. Unlike with B<sub>12</sub> deficiency, neuropathy does not occur.

### **Investigations**

The haematological findings are those of a megaloblastic anaemia as discussed on page 430.

### **Blood measurements**

Serum and red cell folate are assayed by radioisotope dilution or immunological methods. Normal levels of serum folate are 4-18 µg/L (5-63 nmol/L). The amount of folate in the red cells is a better measure of tissue folate; the normal range is 160-640 µg/mL.

### **Further investigations**

In many cases of folate deficiency the cause is not obvious from the clinical picture or dietary history. Occult gastrointestinal disease should then be suspected and appropriate investigations, such as small bowel biopsy, should be performed (p. 300).

## **Treatment and prevention of megaloblastic anaemia**

Treatment depends on the type of deficiency. **Blood** transfusion is not indicated in chronic anaemia; indeed, it is dangerous to transfuse elderly patients, as heart failure may be precipitated. Folic acid may produce a haematological response in vitamin B<sub>12</sub> deficiency but may aggravate the neuropathy. Large doses of folic acid alone should not be used to treat megaloblastic anaemia unless the serum vitamin B<sub>12</sub> level is known to be normal. In severely ill patients, it may be necessary to treat with both folic acid and vitamin B<sub>12</sub> while awaiting serum levels.

### **Treatment of vitamin B<sub>12</sub> deficiency**

Hydroxocobalamin 1000 µg can be given intramuscularly to a total of 5-6 mg over the course of 3 weeks; 1000 µg is then necessary every 3 months for the rest of the patient's life. Alternatively, it is now recommended that oral B<sub>12</sub> 2 mg per day is given, as 1-2% of an oral dose is absorbed by diffusion and therefore does not require intrinsic factor. Compliance with an oral daily regimen may be a problem, particularly in elderly patients. The use of sublingual nuggets of B<sub>12</sub> (2 x 1000 µg daily) has been suggested to be an effective and more convenient option.

Clinical improvement may occur within 48 hours and a reticulocytosis can be seen some 2-3 days after starting therapy, peaking at 5-7 days. Improvement of the polyneuropathy may occur over 6-12 months, but long-standing spinal cord damage is irreversible. Hypokalaemia can occur and, if severe, supplements should be given. Iron deficiency often develops in the first few weeks of therapy. Hyperuricaemia also occurs but clinical gout is uncommon. In patients who have had a total gastrectomy or an ileal resection, vitamin B<sub>12</sub> should be monitored; if low levels occur, prophylactic vitamin B<sub>12</sub> should be given.

### **Treatment of folate deficiency**

Folate deficiency can be corrected by giving 5 mg of folic acid daily; the same haematological response occurs as seen after treatment of vitamin B<sub>12</sub> deficiency. Treatment should be given for about 4 months to replace body stores. Any underlying cause, e.g. coeliac disease, should be treated.

Prophylactic folic acid (400 µg daily) is recommended for all women planning a pregnancy. Many authorities also recommend prophylactic administration of folate throughout pregnancy. Whether this can be achieved by increased consumption of foods with a high folate content or whether women should take folate supplements is under debate. The US Food and Drugs Administration has introduced a requirement for the fortification with folic acid of grain products such as bread, flour and rice (p. 247).

Women who have had a child with a neural tube defect should take 5 mg folic acid daily before and during a subsequent pregnancy.

Prophylactic folic acid is also given in chronic haematological disorders where there is rapid cell turnover. A dose of 5 mg each week is probably sufficient.

## **MACROCYTOSIS WITHOUT MEGLOBLASTIC CHANGES**

A raised MCV with macrocytosis on the peripheral blood film can occur with a normoblastic rather than a megaloblastic bone marrow.

A common *physiological* cause of macrocytosis is pregnancy. Macrocytosis may also occur in the newborn.

Common *pathological* causes are:

- alcohol excess
- liver disease
- reticulocytosis
- hypothyroidism
- some haematological disorders (e.g. aplastic anaemia, sideroblastic anaemia, pure red cell aplasia)
- drugs (e.g. cytotoxics - azathioprine)
- spurious (agglutinated red cells measured on red cell counters)
- cold agglutinins due to autoagglutination of red cells (see p. 449) (the MCV decreases to normal with warming of the sample to 37°C).

In all these conditions, normal serum levels of vitamin B<sub>12</sub> and folate will be found. The exact mechanisms in each case are uncertain, but in some there is increased lipid deposition in the red cell membrane.

An increased number of reticulocytes leads to a raised MCV because they are large cells.

Alcohol is a frequent cause of a raised MCV in an otherwise normal individual. A megaloblastic anaemia can also occur in people who abuse alcohol; this is due to a toxic effect of alcohol on erythropoiesis or to dietary folate deficiency.

#### FURTHER READING

- Chanarin I, Metz J (1997) Diagnosis of cobalamin deficiency: the old and the new. *British Journal of Haematology* 97: 695-700. Kuzminski AM et al. (1998) Effective treatment of cobalamin deficiency with oral cobalamin. *Blood* 92: 1191-1198. Mills JL (2000) Fortification of foods with folic acid. *New England Journal of Medicine* 342:1442-1445. Toh BH, van Driel IR, Gleeson PA (1997) Pernicious anaemia. *New England Journal of Medicine* 337: 1441-1448. Wharton B, Booth I (2001) Fortification of flour with folic acid. *British Medical Journal* 323: 1198-1199.

## ANAEMIA DUE TO MARROW FAILURE (APLASTIC ANAEMIA!)

Aplastic anaemia is defined as pancytopenia with hypocellularity (aplasia) of the bone marrow; there are no leukaemic, cancerous or other abnormal cells in the peripheral blood or bone marrow. It is an uncommon but serious condition that may be inherited but is more commonly acquired.

Aplastic anaemia is due to a reduction in the number of pluripotential stem cells (see Fig. 8.1) together with a fault in those remaining or an immune reaction against them so that they are unable to repopulate the bone marrow. Failure of only one cell line may also occur, resulting in isolated deficiencies such as the absence of red cell precursors in pure red cell aplasia. Evolution to myelodysplasia, paroxysmal nocturnal haemoglobinuria (PNH) or acute myeloblastic leukaemia occurs in some cases, probably owing to the emergence of an abnormal clone of haemopoietic cells.

#### Causes

A list of causes of aplasia is given in Table 8.7. Immune mechanisms are probably responsible for most cases of idiopathic acquired aplastic anaemia and play a part in at least the persistence of many secondary cases. Activated cytotoxic T cells in blood and bone marrow are responsible for the bone marrow failure.

Many drugs may cause marrow aplasia, including cytotoxic drugs such as busulfan and doxorubicin, which are expected to cause transient aplasia as a consequence

**Table 8.7 Causes of aplastic anaemia**

#### Primary

Congenital, e.g. Fanconi's anaemia  
Idiopathic acquired (67% of cases)

#### Secondary

Chemicals, e.g. benzene

Drugs:

chemotherapeutic  
idiosyncratic reactions

Insecticides Ionizing

radiation Infections:

viral, e.g. hepatitis, EBV, HIV, parvovirus  
other, e.g. tuberculosis Paroxysmal

nocturnal haemoglobinuria

Miscellaneous, e.g. pregnancy

of their therapeutic use. However, some individuals develop aplasia due to sensitivity to non-cytotoxic drugs such as chloramphenicol, gold, carbimazole, chlorpromazine, phenytoin, tolbutamide, non-steroidal anti-inflammatory agents, and many others which have been reported to cause occasional cases of aplasia.

Congenital aplastic anaemias are rare. Gene mutations are being identified, e.g. the telomerase RNA component, and have also been seen in one third of aplastic anaemias. Fanconi's anaemia is inherited as an autosomal recessive and is associated with skeletal, renal and central nervous system abnormalities. It usually presents between the ages of 5 and 10 years.

#### Clinical features

The clinical manifestations of marrow failure from any cause are anaemia, bleeding and infection. Bleeding is often the predominant initial presentation of aplastic anaemia with bruising with minimal trauma or blood blisters in the mouth. Physical findings include ecchymoses, bleeding gums and epistaxis. Mouth infections are common. Lymphadenopathy and hepatosplenomegaly are rare in aplastic anaemia.

#### Investigations

- pancytopenia
- the virtual absence of reticulocytes
- a hypocellular or aplastic bone marrow with increased fat spaces (Fig. 8.15). ■

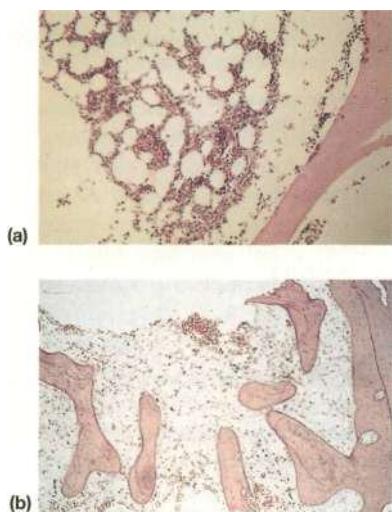
#### Differential diagnosis

This is from other causes of pancytopenia (Table 8.8). A bone marrow trephine is essential for assessment of the bone marrow cellularity.

#### Treatment and prognosis

The treatment of aplastic anaemia depends on providing supportive care while awaiting bone marrow recovery and specific treatment to accelerate marrow recovery.

The main danger is infection and stringent measures should be undertaken to avoid this (see also p. 495). Any suspicion of infection in a severely neutropenic patient



**Fig. 8.15** Bone marrow trephine biopsies in low-power view, (a) Normal cellularity. (b) Hypocellularity in aplastic anaemia.

**Table 8.8 Causes of pancytopenia**

Aplastic anaemia (see Table 8.7)
Drugs
Megaloblastic anaemia
Bone marrow infiltration or replacement
Hodgkin's and non-Hodgkin's lymphoma
Acute leukaemia
Myeloma
Secondary carcinoma
Myelofibrosis
Hypersplenism
Systemic lupus erythematosus
Disseminated tuberculosis
Paroxysmal nocturnal haemoglobinuria
Overwhelming sepsis

should lead to immediate institution of broad-spectrum parenteral antibiotics. Supportive care including transfusions of red cells and platelets should be given as necessary. The cause of the aplastic anaemia must be eliminated if possible.

The course of aplastic anaemia can be variable, ranging from a rapid spontaneous remission to a persistent increasingly severe pancytopenia, which may lead to death through haemorrhage or infection. The most reliable determinants for the prognosis are the number of neutrophils, reticulocytes, platelets, and the cellularity of the bone marrow.

A bad prognosis (i.e. severe aplastic anaemia) is associated with the presence of two of the following three features:

- neutrophil count of  $< 0.5 \times 10^9/L$
- platelet count of  $< 20 \times 10^9/L$
- reticulocyte count of  $< 40 \times 10^9/L$ .

In severe aplastic anaemia, there is a very poor outcome without treatment. Bone marrow transplantation is the

treatment of choice for patients under 40 years of age who have an HLA-identical sibling donor, which gives a 75–90% chance of long-term survival and restoring the blood count to normal. Patients over the age of 40 are not eligible for bone marrow transplantation whether an HLA-identical donor is available or not, because of the high risk of graft-versus-host disease as a complication of bone marrow transplantation. Immunosuppressive therapy is used for patients without HLA-matched siblings and those over the age of 40 years; antilymphocyte globulin (ALG) and ciclosporin in combination give a response rate of 60–80%.

For the patients under the age of 40 who fail to respond to immunosuppression, bone marrow transplantation using unrelated donors is an option, but the results are poor (5-year survival of only 30%) owing to a high incidence of graft rejection, graft-versus-host disease and viral infections.

Levels of haemopoietic growth factors (Fig. 8.1) are normal or increased in most patients with aplastic anaemia, and are ineffective as primary treatment.

Androgens (e.g. oxymethalone) are sometimes useful in patients not responding to immunosuppression and in patients with moderately severe aplastic anaemia.

Steroids have little activity in severe aplastic anaemia but are used for serum sickness due to ALG. They are also used to treat children with congenital pure red cell aplasia (Diamond-Blackfan syndrome). Adult pure red cell aplasia is associated with a thymoma in 30% of cases and thymectomy may induce a remission. It may also be associated with autoimmune disease or may be idiopathic. Steroids and ciclosporin are effective treatment in some cases.

#### FURTHER READING

- British Committee for Standards in Haematology (2003) Guidelines for the diagnosis and management of acquired aplastic anaemia. *British Journal of Haematology* **123**: 782–801.  
 Brodsky RA, Jones RJ (2005) Aplastic anaemia. *Lancet* **365**: 1647–1656.  
 Young NS (2002) Acquired aplastic anaemia. *Annals of Internal Medicine* **136**: 534–546.

## HAEMOLYTIC ANAEMIAS: AN INTRODUCTION

Haemolytic anaemias are caused by increased destruction of red cells. The red cell normally survives about 120 days, but in haemolytic anaemias the red cell survival times are considerably shortened.

Breakdown of normal red cells occurs in the macrophages of the bone marrow, liver and spleen (see Fig. 8.5).

#### Consequences of haemolysis

Shortening of red cell survival does not always cause anaemia as there is a compensatory increase in red cell

production by the bone marrow. If the red cell loss can be contained within the marrow's capacity for increased output, then a haemolytic state can exist without anaemia (*compensated haemolytic disease*). The bone marrow can increase its output by six to eight times by increasing the proportion of cells committed to erythropoiesis (*erythroid hyperplasia*) and by expanding the volume of active marrow. In addition, immature red cells (*reticulocytes*) are released prematurely. These cells are larger than mature cells and stain light blue on a peripheral blood film (the description of this appearance on the blood film is *polychromasia*). Reticulocytes may be counted accurately as a percentage of all red cells on a blood film using a supravital stain for residual RNA (e.g. new methylene blue).

### Sites of haemolysis

#### Extravascular haemolysis

In most haemolytic conditions red cell destruction is extravascular. The red cells are removed from the circulation by macrophages in the reticuloendothelial system, particularly the spleen.

#### Intravascular haemolysis

When red cells are rapidly destroyed within the circulation, haemoglobin is liberated (Fig. 8.16). This is initially bound to plasma haptoglobin but these soon become saturated.

Excess free plasma Hb is filtered by the renal glomerulus and enters the urine, although small amounts are reabsorbed by the renal tubules. In the renal tubular cell, Hb is broken down and becomes deposited in the cells as *haemosiderin*. This can be detected in the spun sediment of urine using Perls' reaction. Some of the free plasma Hb is oxidized to *methaemoglobin*, which dissociates into *ferrihaem* and globin. *Plasma haemopexin* binds ferrihaem; but if its binding capacity is exceeded, ferrihaem becomes attached to albumin, forming *methaemalbumin*. On spectrophotometry of the plasma, methaemalbumin forms a characteristic band; this is the basis of *Schumm's test*.

The liver removes Hb bound to haptoglobin and *haemopexin* and any remaining free Hb.

### Evidence for haemolysis

#### *Increased red cell breakdown leads to:*

- elevated serum bilirubin (unconjugated)
- excess urinary urobilinogen (resulting from bilirubin breakdown in the intestine, Fig. 8.5)
- reduced plasma haptoglobin
- raised serum lactic dehydrogenase (LDH).

#### *Increased red cell production leads to:*

- reticulocytosis
- erythroid hyperplasia of the bone marrow.

There may be evidence of abnormal red cells in some haemolytic anaemias:

- spherocytes (see Fig. 8.18)
- sickle cells (see Fig. 8.23)
- red cell fragments.

#### *Demonstration of shortened red cell lifespan*

Red cell survival can be estimated from  $^{51}\text{Cr}$ -labelled red cells given intravenously but is rarely performed.

#### *Intravascular haemolysis*

This is suggested by raised levels of plasma Hb, haemosiderinuria, very low or absent haptoglobins, and the presence of methaemalbumin (positive Schumm's test). Various laboratory studies will be necessary to determine the exact type of haemolytic anaemia present. The causes of haemolytic anaemias are shown in Table 8.9.

## INHERITED HAEMOLYTIC ANAEMIA

### RED CELL MEMBRANE DEFECTS

The normal red cell membrane consists of a lipid bilayer crossed by integral proteins with an underlying lattice of proteins (or cytoskeleton), including spectrin, actin, ankyrin and protein 4.1, attached to the integral proteins (Fig. 8.17).

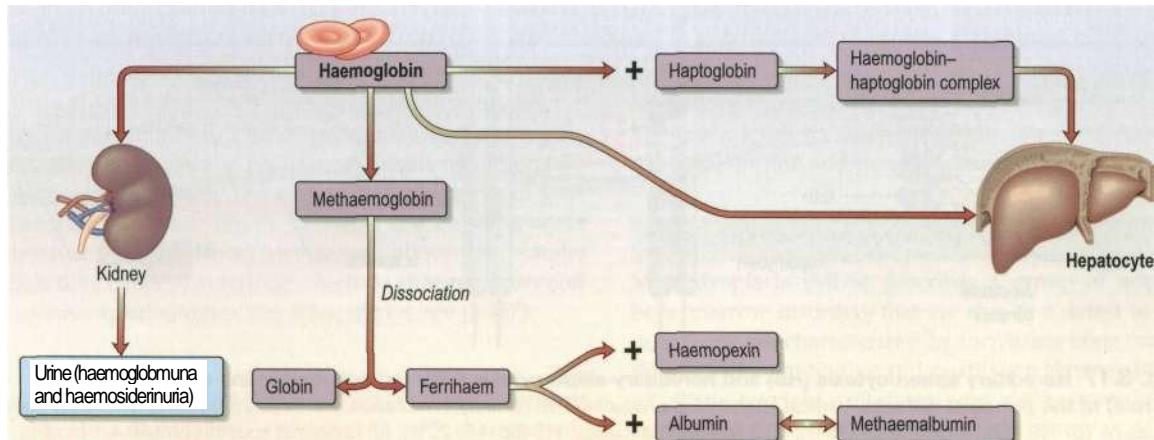


Fig. 8.16 The fate of haemoglobin in the plasma following haemolysis.

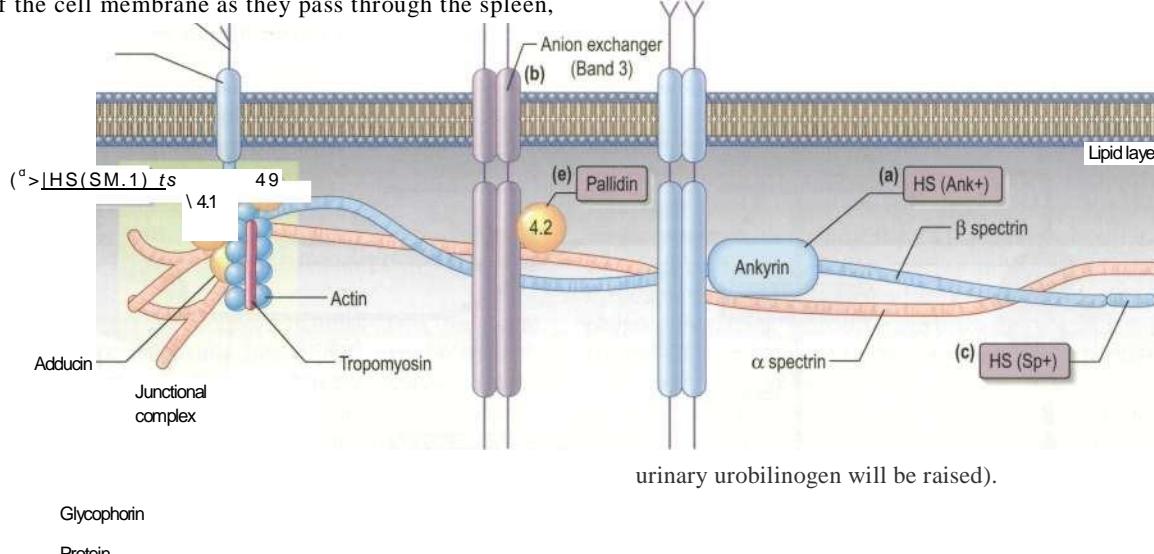
## Haematological disease

**Table 8.9 Causes of haemolytic anaemia**

Inherited red cell membrane defect	Acquired immune
Hereditary spherocytosis	Autoimmune (see Table 8.15)
Hereditary elliptocytosis	Warm Cold Alloimmune
<b>Haemoglobin abnormalities</b>	Haemolytic transfusion reactions Haemolytic disease of the newborn
Thalassaemia Sickle cell disease	After allogeneic bone marrow or organ transplantation Drug-induced
<b>Metabolic defects</b>	<b>Non-immune</b>
Glucose-6-phosphate dehydrogenase deficiency Pyruvate kinase deficiency	Acquired membrane defects Paroxysmal nocturnal haemoglobinuria
<b>Miscellaneous</b>	Mechanical Microangiopathic haemolytic anaemia
Infections, e.g. malaria, mycoplasma	Valve prosthesis March haemoglobinuria
<i>Clostridium welchii</i> , generalized sepsis	Secondary to systemic disease
Drugs and chemicals causing damage to the red cell membrane or oxidative haemolysis	Renal and liver failure
Hypersplenism	
Burns	

### Hereditary spherocytosis (HS)

HS is the most common inherited haemolytic anaemia in northern Europeans, affecting 1 in 5000. It is inherited in an autosomal dominant manner, but in 25% of patients neither parent is affected and it is presumed that HS has occurred by spontaneous mutation. HS is due to defects in the red cell membrane, resulting in the cells losing part of the cell membrane as they pass through the spleen,



possibly because the lipid bilayer is inadequately supported by the membrane skeleton. The best-characterized defect is a deficiency in the structural protein spectrin, but quantitative defects in other membrane proteins have been identified (Fig. 8.17), with ankyrin defects being the most common. The abnormal red cell membrane in HS is associated functionally with an increased permeability to sodium, and this requires an increased rate of active transport of sodium out of the cells which is dependent on ATP produced by glycolysis. The surface-to-volume ratio decreases, and the cells become spherocytic. Spherocytes are more rigid and less deformable than normal red cells. They are unable to pass through the splenic microcirculation and they die.

### Clinical features

The condition may present with jaundice at birth. However, the onset of jaundice can be delayed for many years and some patients may go through life with no symptoms and are detected only during family studies. The patient may eventually develop anaemia, splenomegaly and ulcers on the leg. As in many haemolytic anaemias, the course of the disease may be interrupted by aplastic, haemolytic and megaloblastic crises. Aplastic anaemia usually occurs after infections, particularly with parvovirus, whereas megaloblastic anaemia is the result of folate depletion owing to the hyperactivity of the bone marrow. Chronic haemolysis leads to the formation of pigment gallstones (see p. 398).

### Investigations

- **Anaemia.** This is usually mild, but occasionally can be severe.
- **Blood film.** This shows spherocytes (Fig. 8.18) and reticulocytes.
- **Haemolysis** is evident (e.g. the serum bilirubin and

urinary urobilinogen will be raised).

**Fig. 8.17** Hereditary spherocytosis (HS) and hereditary elliptocytosis (HE): red cell membrane showing the sites (purple) of the principal defects. Vertical interactions producing HS: (a) ankyrin mutation, HS (Ank+) producing deficiency (45% of cases); (b) HS band 3 deficiency (20%); (c) p spectrin deficiency, HS (Sp+) (< 20%); (d) abnormal spectrin/protein 4.1 binding, HS (Sp-4.1); (e) protein 4.2 (pallidin) deficiency (Japanese). These produce various autosomal dominant and recessive forms of the disease. Horizontal interactions producing HE: a spectrin (80%), protein 4.1 (15%), p spectrin (5%).

appears slit-like. Their presence in large numbers may occur in a hereditary haemolytic anaemia associated with a membrane defect, but excess alcohol intake is also a common cause.

...::.

## HAEMOGLOBIN ABNORMALITIES

**Fig. 8.18** Spherocytes (arrowed). This blood film also shows reticulocytes, polychromasias and a nucleated erythroblast.

- **Osmotic fragility.** When red cells are placed in solutions of increasing hypotonicity, they take in water, swell, and eventually lyse. Spherocytes tolerate hypotonic solutions less well than do normal biconcave red cells. Osmotic fragility tests are infrequently carried out in routine practice, but may be useful to confirm a suspicion of spherocytosis on a blood film.
- **Direct antiglobulin (Coombs') test** is negative in spherocytosis, virtually ruling out autoimmune haemolytic anaemia where spherocytes are also commonly present.

### Treatment

The spleen, which is the site of cell destruction, should be removed in all but the mildest cases. The decision about splenectomy in symptomless patients is difficult, but a raised bilirubin and especially the presence of gallstones should encourage splenectomy.

It is best to postpone splenectomy until after childhood, as sudden overwhelming fatal infections, usually due to encapsulated organisms such as pneumococci, may occur (see p. 457). Splenectomy should be preceded by appropriate immunization and followed by lifelong penicillin prophylaxis (see Box 2.9).

Following splenectomy, the spherocytes persist but the Hb level usually returns to normal as the red cells are no longer destroyed.

Folate deficiency often occurs in chronic haemolysis with rapid cell turnover. Folate levels should be monitored, or folic acid can be given prophylactically.

### Hereditary elliptocytosis

This disorder of the red cell membrane is inherited in an autosomal dominant manner and has a prevalence of 1 in 2500 in Caucasians. The red cells are **elliptical** due to deficiencies of protein 4.1 or the spectrin/actin/4.1 complex which leads to weakness of the horizontal protein interaction and to the membrane defect (Fig. 8.17). Clinically it is a similar condition to HS but milder. Only a minority of patients have anaemia and only occasional patients require splenectomy.

Rarely, hereditary spherocytosis or elliptocytosis may be inherited in a homozygous fashion giving rise to a severe haemolytic anaemia sometimes necessitating splenectomy in early childhood.

### Hereditary stomatocytosis

*Stomatocytes* are red cells in which the pale central area

In early embryonic life, haemoglobins Gower 1, Gower 2 and Portland predominate (Fig. 8.19). Later, fetal haemoglobin (Hb F), which has two **a** and two **y** chains, is produced. There is increasing synthesis of (3 chains from 13 weeks of gestation and at term there is 80% Hb F and 20% Hb A. The switch from Hb F to Hb A occurs after birth when the genes for **y** chain production are further suppressed and there is rapid increase in the synthesis of P chains. The exact mechanism responsible for the switch remains unknown. There is little Hb F produced (normally less than 1%) from 6 months after birth. The 5 chain is synthesized just before birth and Hb A<sub>2</sub> (oc<sub>2</sub>8<sub>2</sub>) remains at a level of about 2% throughout adult life (Table 8.10).

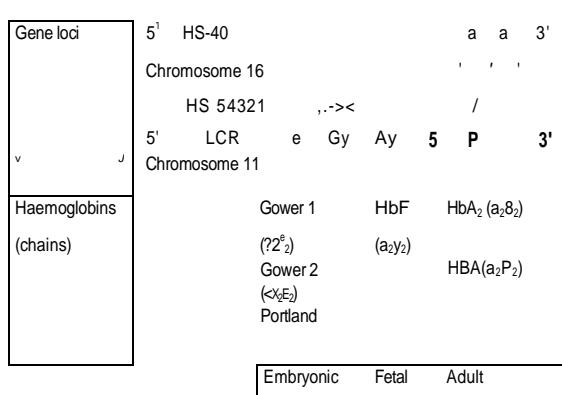
Globin chains are synthesized in the same way as any protein (see Ch. 3). Four globin chain genes are required to control oc-chain production (Fig. 8.19). Two are present on each haploid genome (genes derived from one parent). These are situated close together on chromosome 16. The genes controlling the production of e, y, 8 and fS chains are close together on chromosome 11. The globin genes are arranged on chromosomes 16 and 11 in the order in which they are expressed and combine to give different haemo globins. Normal haemoglobin synthesis is discussed on

page 422.

### Abnormal haemoglobins

Abnormalities occur in:

- globin chain production (e.g. thalassaemia)
- structure of the globin chain (e.g. sickle cell disease)
- combined defects of globin chain production and structure, e.g. sickle cell P-thalassaemia.



**Fig. 8.19** Loci of genes on chromosomes 16 and 11 and the combination of various chains to produce different haemoglobins. HS-40 (for a genes) and locus control region (LCR) HS1-5 (for p genes) are regulatory control elements.

**Table 8.10 Some types of haemoglobin**

	Haemoglobin	Structure	Comment
Normal	A A <sub>2</sub>	O2ft.  O2Y2	Comprises 97% of adult haemoglobin Comprises 2% of adult haemoglobin Elevated in p-thalassaemia Normal haemoglobin in fetus from 3rd to 9th month Increased in p-thalassaemia
<b>Abnormal chain production</b>			Comprises < 1 % of haemoglobin in adult
H		Bails	Found in a-thalassaemia Biologically useless
<b>Abnormal chain structure</b>	S	Y4  a,B, <sup>c</sup>	Comprises 100% of haemoglobin in homozygous a-thalassaemia Biologically useless Substitution of valine for glutamine acid in position 6 of p chain Substitution of lysine for glutamic acid in position 6 of p chain

Genetic defects in haemoglobin are the most common of all genetic disorders.

### THE THALASSAEMIAS

The thalassaemias (Greek *thalassa* = sea) affect people throughout the world (Fig. 8.20). Normally there is balanced (1:1) production of *a* and *p* chains. The defective synthesis of globin genes in thalassaemia leads to 'unbalanced' globin chain production, leading to precipitation of globin chains within the red cell precursors and resulting in ineffective erythropoiesis. Precipitation of globin chains in mature red cells leads to haemolysis.

### p-Thalassaemia

In *homozygous* P-thalassaemia, either no normal P chains are produced ((3°), or (3-chain production is very reduced (P<sup>+</sup>). There is an excess of *a* chains, which precipitate in erythroblasts and red cells causing ineffective erythropoiesis and haemolysis. The excess *a* chains combine

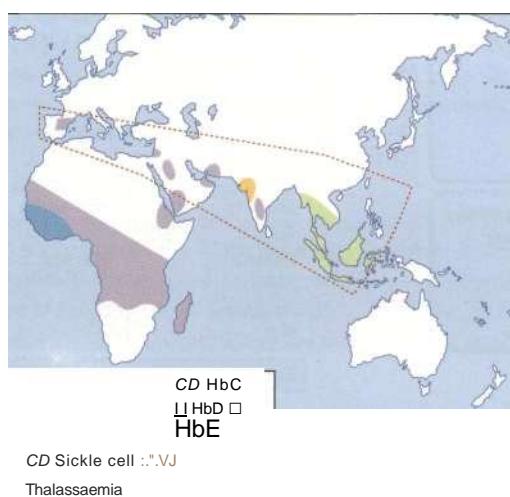
with whatever (5, 5 and *y* chains are produced, resulting in increased quantities of Hb A<sub>2</sub> and Hb F and, at best, small amounts of Hb A. In *heterozygous* p-thalassaemia there is usually symptomless microcytosis with or without mild anaemia. Table 8.11 shows the findings in the homozygote and heterozygote for the common types of (3-thalassaemia.

### Molecular genetics

The molecular errors accounting for over 200 genetic defects leading to P-thalassaemia have been characterized. Unlike in a-thalassaemia, the defects are mainly point mutations rather than gene deletions. The mutations result in defects in transcription, RNA splicing and modification, translation via frame shifts and nonsense codons producing highly unstable P-globin which cannot be utilized.

### Clinical syndromes

Clinically, p-thalassaemia can be divided into the following:



**Fig. 8.20 Major haemoglobin abnormalities: geographical distribution.**

**Table 8.11 (i-Thalassaemia: common findings**

Type of thalassaemia	Findings in homozygote	Findings in heterozygote
P <sup>+</sup>	Thalassaemia major	Thalassaemia minor
	Hb A + F + A <sub>2</sub>	Hb A <sub>2</sub> raised
P <sup>o</sup>	Thalassaemia major	Thalassaemia minor
	Hb F + A <sub>2</sub>	Hb A <sub>2</sub> raised
5p	Thalassaemia intermedia	Thalassaemia minor
	Hb F only	HbF5-15%
5p <sup>+</sup> (Lepore)	Thalassaemia major or intermedia	Hb A <sub>2</sub> normal
	Hb F and Lepore	Hb Lepore 5-15%
		Hb A <sub>2</sub> normal

Hb Lepore is a cross fusion product of 8 and p globin genes Adapted with permission from Weatherall DJ (2003) Disorders of the synthesis of function of haemoglobin. In: Weatherall DJ, Warrell DA, Cox TM, Firth JD (eds) *Oxford Textbook of Medicine*, 4th edn. Oxford: Oxford University Press.

- thalassaemia minor (or trait), the symptomless heterozygous carrier state
- thalassaemia intermedia, with moderate anaemia, rarely requiring transfusions
- thalassaemia major, with severe anaemia requiring regular transfusions.

#### *Thalassaemia minor (trait)*

This common carrier state (heterozygous ( $\beta$ -thalassaemia) is asymptomatic. Anaemia is mild or absent. The red cells are hypochromic and microcytic with a low MCV and MCH, and it may be confused with iron deficiency. However, the two are easily distinguished, as in thalassaemia trait the serum ferritin and the iron stores are normal (see Table 8.3). Hb electrophoresis usually shows a raised Hb A<sub>2</sub> and often a raised Hb F (Fig. 8.21). Iron should not be given to these patients unless they develop coincidental iron deficiency.

#### *Thalassaemia intermedia*

Thalassaemia intermedia includes patients who are symptomatic with moderate anaemia (Hb 7–10 g/dL) and who do not require regular transfusions. That is, it is more severe than in  $\beta$ S-thalassaemia trait but milder than in transfusion-dependent thalassaemia major.

Thalassaemia intermedia may be due to a combination of homozygous mild p<sup>+</sup> and oc-thalassaemia, where there is reduced cc-chain precipitation and less ineffective erythropoiesis and haemolysis. The inheritance of hereditary persistence of Hb F with homozygous P-thalassaemia also results in a milder clinical picture than unmodified P-thalassaemia major because the excess  $\alpha$  chains are partially removed by the increased production of  $\gamma$  chains.

Patients may have splenomegaly and bone deformities. Recurrent leg ulcers, gallstones and infections are also seen.

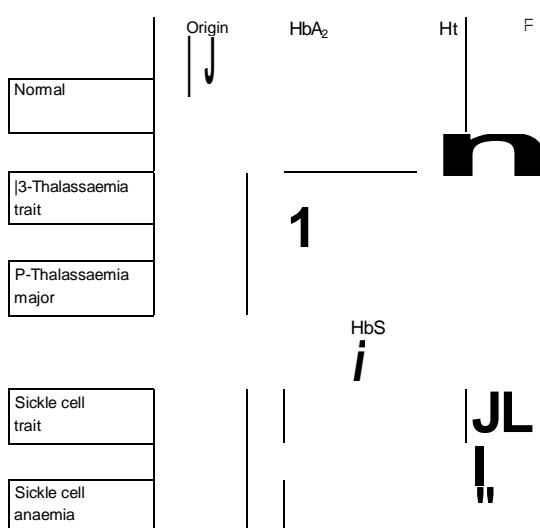


Fig. 8.21 Patterns of haemoglobin electrophoresis.

#### *Thalassaemia major (Cooley's anaemia)*

Most children affected by homozygous P-thalassaemia present during the first year of life with:

- failure to thrive and recurrent bacterial infections
- severe anaemia from 3–6 months when the switch from  $\gamma$ - to (3-chain production should normally occur
- extramedullary haemopoiesis that soon leads to hepatosplenomegaly and bone expansion, giving rise to the classical thalassaemic facies (Fig. 8.22a).

Skull X-rays in these children show the characteristic 'hair on end' appearance of bony trabeculation as a result of expansion of the bone marrow into cortical bone (Fig. 8.22b). The expansion of the bone marrow is also shown in an X-ray of the hand (Fig. 8.22c).

The classic features of untreated thalassaemia major are only observed in patients from countries without good blood transfusion support.

#### *Management*

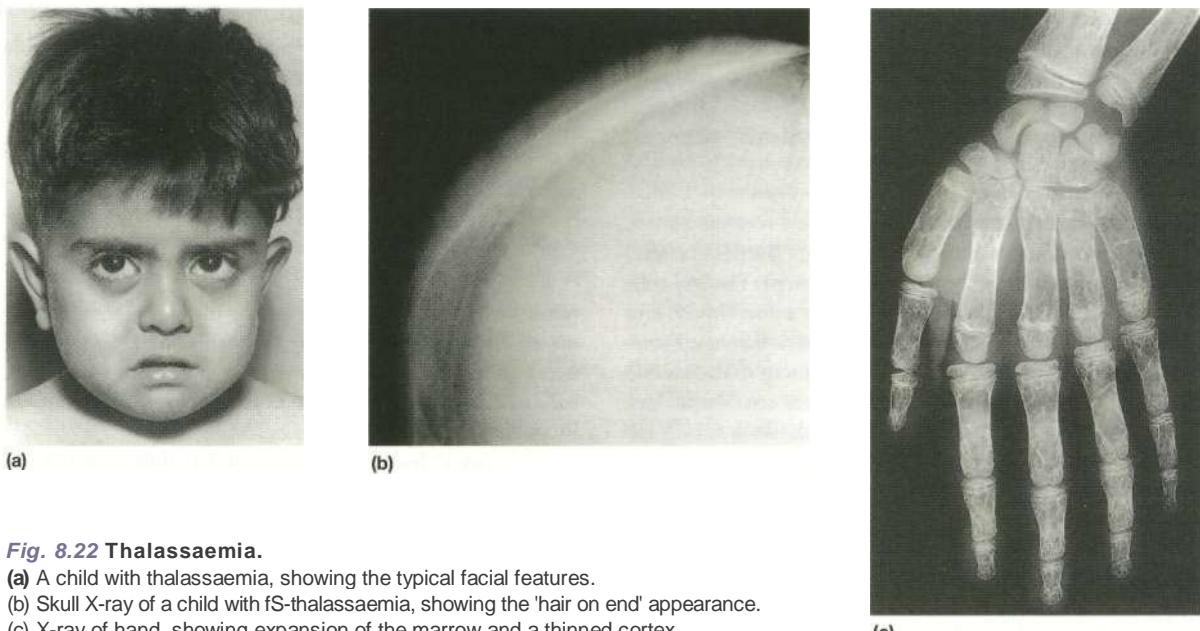
The aims of treatment are to suppress ineffective erythropoiesis, prevent bony deformities and allow normal activity and development. Long-term folic acid supplements are required, and regular transfusions should be given to keep the Hb above 10 g/dL. Blood transfusions may be required every 4–6 weeks.

If transfusion requirements increase, splenectomy should be considered, although this is usually delayed until after the age of 6 years because of the risk of infection. Prophylaxis against infection is required for patients undergoing splenectomy (see p. 457).

Iron overload caused by repeated transfusions (transfusion haemosiderosis) may lead to damage to the endocrine glands, liver, pancreas and the myocardium by the time patients reach adolescence. The iron-chelating agent of choice remains desferrioxamine, although it has to be administered parenterally. The oral iron-chelating agent ICL670 appears to be safe and effective but long-term studies are awaited. Desferrioxamine is given as an overnight subcutaneous infusion on 5–7 nights each week. Ascorbic acid 200 mg daily is given, along with desferrioxamine, as it increases the urinary excretion of iron in response to desferrioxamine.

With current therapy, normal growth and sexual development occur but compliance may be a problem, especially in teenagers. Intensive treatment with desferrioxamine has been reported to reverse damage to the heart in patients with severe iron overload, but excessive doses of desferrioxamine may cause cataracts, retinal damage and nerve deafness. Infection with *Yersinia enterocolitica* occurs in iron-loaded patients treated with desferrioxamine. Iron overload should be periodically assessed by measuring the serum ferritin and by measurement of hepatic iron stores.

Bone marrow transplantation has been used in young patients with HLA-matched siblings. It has been successful in cases in good clinical condition with a 3-year mortality of less than 5%, but there is a high mortality (> 50%) in patients in poor condition with iron overload and liver dysfunction.



**Fig. 8.22 Thalassaemia.**

- (a) A child with thalassaemia, showing the typical facial features.
- (b) Skull X-ray of a child with fS-thalassaemia, showing the 'hair on end' appearance.
- (c) X-ray of hand, showing expansion of the marrow and a thinned cortex.

Prenatal diagnosis and gene therapy are discussed on page 191.

### a-Thalassaemia

#### Molecular genetics

In contrast to p-thalassaemia, oc-thalassaemia is often caused by gene deletions, although mutations also occur. The gene for a chains is duplicated on both chromosomes 16, i.e. there are four genes. Deletion of one a-chain gene ( $\alpha^+$ ) or both a-chain genes ( $\alpha^0$ ) on each chromosome 16 may occur (Table 8.12). The former is the most common of these abnormalities.

- Four-gene deletion (deletion of both genes on both chromosomes); there is no a-chain synthesis and only Hb Barts ( $\gamma_4$ ) is present. Hb Barts cannot carry oxygen and is incompatible with life (Tables 8.10 and 8.12). Infants are either stillborn at 28<sup>10</sup> weeks or die very shortly after birth. They are pale, oedematous and

have enormous livers and spleens - a condition called hydrops fetalis.

- Three-gene deletion; there is moderate anaemia (Hb 7–10 g/dL) and splenomegaly (Hb H disease). The patients are not usually transfusion-dependent. Hb A, Hb Barts and Hb H ( $f\beta_4$ ) are present. Hb  $A_2$  is normal or reduced.
- Two-gene deletion (a-thalassaemia trait); there is microcytosis with or without mild anaemia. Hb H bodies may be seen on staining a blood film with brilliant cresyl blue.
- One-gene deletion; the blood picture is usually normal.

Globin chain synthesis studies for the detection of a reduced ratio of a to P chains may be necessary for the definitive diagnosis of a-thalassaemia trait.

Less commonly, a-thalassaemia may result from genetic defects other than deletions, for example mutations in the stop codon producing an a chain with many extra amino acids (Hb Constant Spring). (See p. 177.)

### SICKLE SYNDROMES

Sickle cell haemoglobin (Hb S) results from a single-base mutation of adenine to thymine which produces a substitution of valine for glutamine at the sixth codon of the P-globin chain ( $\alpha_2p^{6glu \wedge val}$ ). In the homozygous state (*sickle cell anaemia*) both genes are abnormal (Hb SS), whereas in the heterozygous state (*sickle cell trait*, Hb AS) only one chromosome carries the gene. As the synthesis of Hb F is normal, the disease usually does not manifest itself until the Hb F decreases to adult levels at about 6 months of age.

The disease occurs mainly in Africans (25% carry the gene) but is also found in India, the Middle East and southern Europe (see Fig. 8.20).

**Table 8.12 The a-thalassaeemias**

Gene deletion	Haemoglobin type	Clinical picture
4 genes	$\alpha^0$	Hb Barts ( $\gamma_4$ )
Hydrops fetalis		
3 genes	$\alpha^0$ - -/ - a	Hb H (04) Moderate anaemia Splenomegaly
- -/a a	Some Hb H or -oJ-a	Mild anaemia Hb A
1 gene	$\alpha^+$ - a/a a	'Normal' a-Thalassaemia trait a-Thalassaemia trait

## Pathogenesis

Deoxygenated Hb S molecules are insoluble and polymerize. The flexibility of the cells is decreased and they become rigid and take up their characteristic sickle appearance. This process is initially reversible but, with repeated sickling, the cells eventually lose their membrane flexibility and become irreversibly sickled. This is due to dehydration, partly caused by potassium leaving the red cells via calcium activated potassium channels called the Gados channel. These irreversibly sickled cells are dehydrated and dense and will not return to normal when oxygenated. Sickling can produce:

- a shortened red cell survival
- impaired passage of cells through the microcirculation, leading to obstruction of small vessels and tissue infarction.

Sickling is precipitated by infection, dehydration, cold, acidosis or hypoxia. In many cases the cause is unknown, but adhesion proteins on activated endothelial cells (VCAM-1) may play a causal role, particularly in vaso-occlusion when rigid cells are trapped, facilitating polymerization. Hb S releases its oxygen to the tissues more easily than does normal Hb (see Fig. 15.5), and patients therefore feel well despite being anaemic (except of course during crises or complications).

Depending on the type of haemoglobin chain combinations, three clinical syndromes occur:

- homozygous Hb SS have the most severe disease
- combined heterozygosity (Hb SC) for Hb S and C (see below) who suffer intermediate symptoms.
- heterozygous Hb AS (sickle cell trait) usually have no symptoms (see p. 445).

## Sickle cell anaemia

### Clinical features

#### Vaso-occlusive crises

The earliest presentation in the first few years of life is acute pain in the hands and feet (dactylitis) owing to vaso-occlusion of the small vessels. Severe pain in other bones, e.g. femur, humerus, vertebrae, ribs, pelvis, occurs in older children/adults. These attacks vary in frequency from daily to perhaps only once a year. Fever often accompanies the pain.

#### Anaemia

Chronic haemolysis produces a stable haemoglobin level, usually in the 6–8 g/dL range but an acute fall in the haemoglobin level can occur owing to:

- splenic sequestration
- bone marrow aplasia
- further haemolysis.

#### Splenic sequestration

Vaso-occlusion produces an acute painful enlargement of the spleen. There is splenic pooling of red cells and hypovolaemia, leading in some to circulatory collapse and death. The condition occurs in childhood before multiple

infarctions have occurred. The latter eventually leads to a fibrotic non-functioning spleen. Liver sequestration can also occur.

#### Bone marrow aplasia

This most commonly occurs following infection with parvovirus B<sub>19</sub>, which invades proliferating erythroid progenitors. There is a rapid fall in haemoglobin with no reticulocytes in the peripheral blood, because of the failure of erythropoiesis in the marrow.

*Haemolysis* due to drugs, acute infection or associated G6PD deficiency also occurs. Anaemia can also result from folate deficiency.

#### Long-term problems

In adults, nearly every organ is involved eventually, as patients survive longer with better treatment.

*Growth and development.* Young children are short but regain their height by adulthood. However, they remain below the normal weight. There is often delayed sexual maturation which may require hormone therapy. (Splenectomy can also reverse this.)

*Bones* are a common site for vaso-occlusive episodes, leading to chronic infarcts. Avascular necrosis of hips, shoulders, compression of vertebrae and shortening of bones in the hands and feet occur. Pain is a major problem. Osteomyelitis is commoner in sickle cell disease and is caused by *Salmonella*, *Staphylococcus aureus* and *Staph. pneumoniae*. Antibiotic treatment is necessary (p. 573). Joint replacements therapy may be required.

*Infections* are common in tissues susceptible to vaso-occlusion, e.g. bones, lungs, kidneys.

*Respiratory.* The acute sickle chest syndrome occurs in up to 30%, and pulmonary hypertension and chronic lung disease are the commonest cause of death of adults with sickle cell disease. The acute chest syndrome is caused by infection, fat embolism from necrotic bone marrow or pulmonary infarction due to sequestration of sickle cells. It comprises shortness of breath, chest pain, hypoxia, and new chest X-ray changes due to consolidation. The presentation may be gradual or very rapid, leading to death in a few hours. Initial management is with pain relief, inspired oxygen, antibiotics and exchange transfusion to reduce the amount of Hb S to < 20%; occasionally ventilation may be necessary. Infections can be due to chlamydia and mycoplasma, as well as *Streptococcus pneumoniae*.

*Leg ulcers* occur spontaneously (vaso-occlusive episodes) or following trauma and are usually over the medial or lateral malleoli. They often become infected and are quite resistant to treatment.

*Cardiac* problems occur, with cardiomegaly, arrhythmias and iron overload cardiomyopathy. Myocardial infarctions occur due to thrombotic episodes which are not secondary to atheroma.

## Haematological disease

**Neurological** complications occur in 25% of patients, with transient ischaemic attacks, fits, cerebral infarction, cerebral haemorrhage and coma. Ischaemic strokes occur in children and it has been suggested that regular transcranial Doppler ultrasonography is performed annually and patients are transfused in order to avoid brain damage.

**Cholelithiasis.** Pigment stones occur as a result of chronic haemolysis.

**Liver.** Chronic hepatomegaly and liver dysfunction are caused by trapping of sickle cells.

**Renal.** Chronic tubulo-intestinal nephritis occurs (see p. 643).

**Priapism.** An unwanted painful erection occurs from vaso-occlusion and can be recurrent. This may result in impotence. Treatment is with an α-adrenergic blocking drug, analgesia and hydration.

**Eye.** Background retinopathy, proliferative retinopathy, vitreous haemorrhages and retinal detachments all occur. Regular yearly eye checks are required.

**Pregnancy.** Impaired placental blood flow causes spontaneous abortion, intrauterine growth retardation, pre-eclampsia and fetal death. Painful episodes, infections and severe anaemia occur in the mother. Prophylactic transfusion does not improve fetal outcome. Oral contraceptives with low-dose oestrogens are safe.

### Investigations

- **Blood count.** The level of Hb is in the range 6–8 g/dL with a high reticulocyte count (10–20%).
- **Blood films** can show features of hyposplenism (see Fig. 8.28).
- **Sickling** of red cells on a blood film can be induced in the presence of sodium metabisulphite (see Fig. 8.23).
- **Sickle solubility test.** A mixture of Hb S in a reducing solution such as sodium dithionite gives a turbid appearance because of precipitation of Hb S, whereas normal Hb gives a clear solution. A number of commercial kits such as Sickledex are available for rapid screening for the presence of Hb S, for example before surgery in appropriate ethnic groups and in the A&E department.

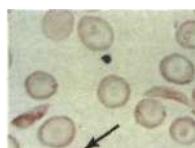


Fig. 8.23 Sickle cells (arrowed) and target cells.

- **Hb electrophoresis** (see Fig. 8.21) is always needed to confirm the diagnosis. There is no Hb A, 80–95% Hb SS, and 2–20% Hb F.
- **The parents** of the affected child will show features of sickle cell trait.

### Management

Precipitating factors (see above) should be avoided or treated quickly. The complications requiring inpatient management are shown in Table 8.13.

Acute painful attacks require supportive therapy with intravenous fluids, oxygen, antibiotics and adequate analgesia. Crises can be extremely painful and require strong, usually narcotic, analgesia. Morphine is the drug of choice. Milder pain can sometimes be relieved by codeine, paracetamol and NSAIDs.

Prophylaxis is with penicillin 500 mg daily and vaccination with polyvalent pneumococcal and *Haemophilus influenzae* type B vaccine (see p. 42). Folic acid is given to all patients with haemolysis.

### Anaemia

Transfusions should only be given for clear indications. Patient with steady state anaemia, those having minor surgery or having painful episodes without complications should not be transfused. Transfusions should be given for heart failure, TIAs, strokes, acute chest syndrome, acute splenic sequestration and aplastic crises. Before elective operations and during pregnancy, repeated transfusions may be used to reduce the proportion of circulating Hb S to less than 20% to prevent sickling. Exchange transfusions may be necessary in patients with severe or recurrent crises, or before emergency surgery. Transfusion and splenectomy may be life-saving for young children with splenic sequestration. A full compatibility screen should always be performed.

*Hydroxycarbamide* (hydroxyurea) is the first drug which has been widely used as therapy for sickle cell anaemia. It acts by increasing Hb F concentrations but the reduction in neutrophils may also help. Hydroxycarbamide has been shown in trials to reduce the episodes of pain, the acute chest syndrome, and the need for blood transfusions.

**Table 8.13 Complications requiring inpatient management**

- Pain uncontrolled by non-opiate analgesia
- Swollen painful joints
- Central nervous system deficit
- Acute sickle chest syndrome or pneumonia
- Mesenteric sickling and bowel ischaemia
- Splenic or hepatic sequestration
- Cholecystitis
- Renal papillary necrosis resulting in colic or severe haematuria
- Hyphema and retinal detachment

From Oavies SC, Oni L (1997) Management of patients with sickle cell disease. *British Medical Journal* 315: 656–660.

Inhaled nitric oxide inhibits platelet function, reduces vascular adhesion of red cells and is also a vasodilator. It has been shown to reduce opiate requirements in acute painful episodes.

*Bone marrow transplantation* has been used to treat sickle cell anaemia although in fewer numbers than for thalassaemia. Children and adolescents younger than 16 years of age who have severe complications (strokes, recurrent chest syndrome, or refractory pain) and have an HLA-matched donor are the best candidates for transplantation.

### Counselling

A multidisciplinary team should be involved, with regular clinic appointments to build up relationships. Adolescents require careful counselling over psychosocial issues, drug and birth control.

### Prognosis

Some patients with Hb SS die in the first few years of life from either infection or episodes of sequestration. However, there is marked individual variation in the severity of the disease and some patients have a relatively normal lifespan with few complications.

## Sickle cell trait

These individuals have no symptoms unless extreme circumstances cause anoxia, such as flying in non-pressurized aircraft or problems with anaesthesia. Sickle cell trait protects against *Plasmodium falciparum* malaria (see p. 97), and consequently the sickle gene has been seen as an example of a balanced polymorphism (where the advantage of the malaria protection in the heterozygote is balanced by the mortality of the homozygous condition). Typically there is 60% Hb A and 40% Hb S. The blood count and film are normal. The diagnosis is made by a positive sickle test or by Hb electrophoresis (see Fig. 8.21).

## Other structural globin chain defects

There are many Hb variants (e.g. Hb C, D), many of which are not associated with clinical manifestations.

Hb C ( $\alpha_2\beta_2^{6\text{Glu}\rightarrow\text{Lys}}$ ) disease may be associated with Hb S (Hb SC disease). The clinical course is similar to that with Hb SS, but there is an increased likelihood of thrombosis, which may lead to life-threatening episodes of thrombosis in pregnancy, and retinopathy.

## Combined defects of globin chain production and structure

Abnormalities of Hb structure (e.g. Hb S, C) can occur in combination with thalassaemia. The combination of P-thalassaemia trait and sickle cell trait (sickle cell P-thalassaemia) resembles sickle cell anaemia (Hb SS) clinically.

Hb E ( $\alpha_2\beta_2^{2\text{Glu}\rightarrow\text{Asp}}$ ) is the most common Hb variant in South East Asia, and the second most prevalent haemoglobin variant world-wide. Hb E heterozygotes are

asymptomatic; the haemoglobin level is normal, but red cells are microcytic. Homozygous Hb E causes a mild microcytic anaemia, but the combination of heterozygosity for Hb E and (3-thalassaemia produces a variable anaemia which can be as severe as P-thalassaemia major.

### Prenatal screening and diagnosis of severe haemoglobin abnormalities

Of the offspring of parents who both have either P-thalassaemia or sickle cell trait, 25% will have P-thalassaemia major or sickle cell anaemia, respectively. Recognition of these heterozygous states in parents and family counselling provide a basis for antenatal screening and diagnosis.

### Prognosis

Pregnant women with either sickle cell trait or thalassaemia trait must be identified at antenatal booking either by selective screening of high-risk groups on the basis of ethnic origin or by universal screening of all pregnant women. P-Thalassaemia trait can always be detected by a low MCV and MCH and confirmed by haemoglobin electrophoresis. However, sickle cell trait is undetectable from a blood count and the laboratory need a specific request to screen for sickle cell trait.

If a pregnant woman is found to have a haemoglobin defect, her partner should be tested. Antenatal diagnosis is offered if both are affected as there is a risk of a severe fetal Hb defect, particularly P-thalassaemia major. Fetal DNA analysis can be carried out using amniotic fluid, chorionic villus or fetal blood samples. Abortion is offered if the fetus is found to be affected. Chorionic villus biopsy has the advantage that it can be carried out in the first trimester, thus avoiding the need for second trimester abortions.

*Gene therapy* would be the ultimate corrective therapy for severe Hb abnormalities. Normal Hb genes could be inserted into the patient's haemopoietic cells in vitro and these cells could be transplanted back into the patient after ablative bone marrow treatment.

## METABOLIC DISORDERS OF THE RED CELL

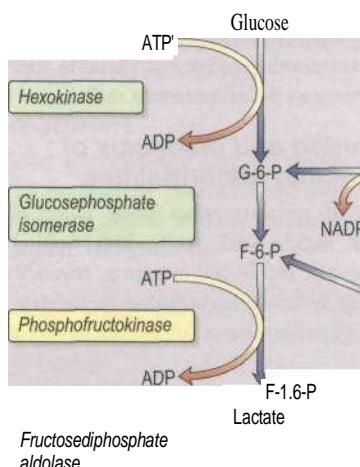
### Red cell metabolism

The mature red cell has no nucleus, mitochondria or ribosomes and is therefore unable to synthesize proteins. Red cells have only limited enzyme systems but they maintain the viability and function of the cells. In particular, energy is required in the form of ATP for the maintenance of the flexibility of the membrane and the biconcave shape of the cells to allow passage through small vessels, and for regulation of the sodium and potassium pumps to ensure osmotic equilibrium. In addition, it is essential that Hb be maintained in the reduced state.

The enzyme systems responsible for producing energy and reducing power are (Fig. 8.24):

- the glycolytic (Embden-Meyerhof) pathway, in which glucose is metabolized to pyruvate and lactic acid with production of ATP

### Embden-Meyerhof glycolytic pathway



*Pyruvate kinase  
Glyceraldehyde-3-phosphate dehydrogenase*

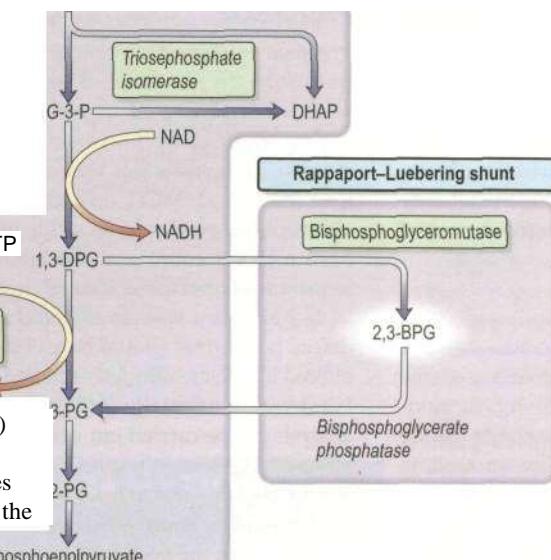
*Lactate dehydrogenase*

*Phosphoglycerate kinase*

ATP

*Phosphoglyceromutase*

*Enolase*



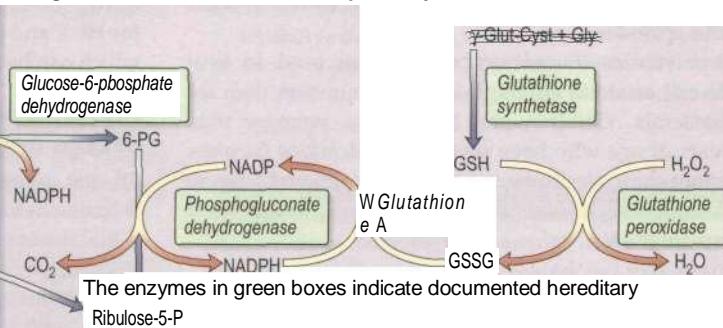
- the hexose monophosphate (pentosephosphate) pathway, which provides reducing power for the red cell in the form of NADPH.

About 90% of glucose is metabolized by the former and 10% by the latter. The hexose monophosphate shunt maintains glutathione (GSH) in a reduced state. Glutathione is necessary to combat oxidative stress to the red cell, and failure of this mechanism may result in:

- rigidity due to cross-linking of spectrin, which decreases membrane flexibility (see Fig. 8.17) and causes 'leakiness' of the red cell membrane
- oxidation of the Hb molecule, producing methaemoglobin and precipitation of globin chains as Heinz bodies localized on the inside of the membrane; these bodies are removed from circulating red cells by the spleen.

### Hexose monophosphate pathway

**Fig. 8.24 Glucose metabolism pathways in red cells.**



The enzymes in green boxes indicate documented hereditary deficiency diseases. GSSG, oxidized glutathione; DHAP, dihydroxyacetone phosphate; BPG, bisphosphoglycerate; F, fructose; G, glucose; GSH, reduced glutathione; P, phosphate; PG, phosphoglycerate.

GSSG, oxidized glutathione; DHAP, dihydroxyacetone phosphate; BPG, bisphosphoglycerate; F, fructose; G, glucose; GSH, reduced glutathione; P, phosphate; PG, phosphoglycerate.

2,3-BPG is formed from a side-arm of the glycolytic pathway (see Fig. 8.24). It binds to the central part of the Hb tetramer, fixing it in the low-affinity state (see Fig. 8.4). A decreased affinity with a shift in the oxygen dissociation curve to the right enables more oxygen to be delivered to the tissues (see Fig. 15.5).

In addition to the G6PD and pyruvate kinase deficiencies described below, there are a number of rare enzyme deficiencies that need specialist investigation.

### Glucose-6-phosphate dehydrogenase (G6PD) deficiency

The enzyme G6PD holds a vital position in the hexose monophosphate shunt (Fig. 8.24), oxidizing glucose-6-phosphate to 6-phosphoglycerate with the reduction of NADP to NADPH. The reaction is necessary in red cells

where it is the only source of NADPH, which is used via glutathione to protect the red cell from oxidative damage. G6PD deficiency is a common condition that presents with a haemolytic anaemia and affects millions of people throughout the world, particularly in Africa, around the Mediterranean, the Middle East (around 20%) and South East Asia (up to 40% in some regions).

The gene for G6PD is localized to chromosome Xq28 near the factor VIII gene. The deficiency is more common in males than in females. However, female heterozygotes can also have clinical problems due to lyonization, whereby because of random X-chromosome inactivation female heterozygotes have two populations of red cells - a normal one and a G6PD-deficient one.

There are over 400 structural types of G6PD, and mutations are mostly single amino acid substitutions. The most common types with normal activity are called type B<sup>+</sup>, which is present in almost all Caucasians and about 70% of black Africans, and type A<sup>+</sup>, which is present in about 20% of black Africans. There are many variants with reduced activity but only two are common. In the African, or A~ type, the degree of deficiency is mild and more marked in older cells. Haemolysis is self-limiting as the young red cells newly produced by the bone marrow have nearly normal enzyme activity. However, in the Mediterranean type, both young and old red cells have very low enzyme activity. After an oxidant shock the Hb level may fall precipitously; death may follow unless the condition is recognized and the patient is transfused urgently.

### Clinical syndromes

- Acute drug-induced haemolysis (Table 8.14) - usually dose related
- Favism (ingestion of fava beans)
- Chronic haemolytic anaemia
- Neonatal jaundice
- Infections and acute illnesses will also precipitate haemolysis in patients with G6PD deficiency.

Mothballs containing naphthalene can also cause haemolysis.

**Table 8.14 Drugs causing haemolysis in glucose-6-phosphate deficiency**

<b>Analgesics</b> , such as:	<b>Antibacterials</b> , such as:
Aspirin	Most sulphonamides
Phenacetin (withdrawn in the UK)	Dapsone
<b>Antimalarials</b> , such as:	Nitrofurantoin
Primaquine	Chloramphenicol
Pyrimethamine	Quinolones
Quinine	
Chloroquine	<b>Miscellaneous drugs</b> , such as:
Pamaquin	Vitamin K
	Probenecid Nalidixic acid
	Quinidine Dimercaprol
	Phenylhydrazine



**Fig. 8.25 'Blister' cells (arrowed) in G6PD deficiency.**

The clinical features are due to rapid intravascular haemolysis with symptoms of anaemia, jaundice and haemoglobinuria.

### Investigations

- **Blood count** is normal between attacks.
- **During an attack** the blood film may show irregularly contracted cells, bite cells (cells with an indentation of the membrane), blister cells (cells in which the Hb appears to have become partially detached from the cell membrane; see Fig. 8.25), Heinz bodies (best seen on films stained with methyl violet) and reticulocytosis.
- **Haemolysis** is evident (see p. 437).
- **G6PD deficiency** can be detected using several screening tests, such as demonstration of the decreased ability of G6PD-deficient cells to reduce dyes. The level of the enzyme may also be directly assayed. There are two diagnostic problems. Immediately after an attack the screening tests may be normal (because the oldest red cells with least G6PD activity are destroyed selectively). Secondly, the diagnosis of heterozygous females may be difficult because the enzyme level may range from very low to normal depending on lyonization. However, the risk of clinically significant haemolysis is minimal in patients with borderline G6PD activity.
- DNA analysis may also be performed.

### Treatment

- Any offending drugs should be stopped.
- Underlying infection should be treated.
- Blood transfusion may be life-saving.
- Splenectomy is not usually helpful.

### Pyruvate kinase deficiency

This is the most common defect of red cell metabolism after G6PD deficiency, affecting thousands rather than millions of people. The site of the defect is shown in Figure 8.24. There is reduced production of ATP, causing rigid red cells. Homozygotes have haemolytic anaemia and splenomegaly. It is inherited as an autosomal recessive.

### Investigations

- **Anaemia** of variable severity is present (Hb 5–10 g/dL). The oxygen dissociation curve is shifted to the right as a result of the rise in intracellular 2,3-BPG (Fig. 15.5), and this reduces the severity of symptoms due to anaemia.

- **Blood film** shows distorted ('prickle') cells and a reticulocytosis.
- **Pyruvate kinase activity** is low (affected homozygotes have levels of 5-20%).

### Treatment

Blood transfusions may be necessary during infections and pregnancy. Splenectomy may improve the clinical condition and is usually advised for patients requiring frequent transfusions.

### FURTHER READING

- Platt OS (2000) The acute chest syndrome of sickle cell disease. *New England Journal of Medicine* **342**: 1904-1907. Porter JB (2001) Practical management of iron overload. *British Journal of Haematology* **115**: 239-252. Steinberg MH et al. (2003) Effect of hydroxyurea on mortality and morbidity in adult sickle cell anaemia; risks and benefits up to 9 years of treatment. *Journal of the American Medical Association* **289**: 1645-1651. Stuart M, Nagel RL (2004) Sickle cell disease. *Lancet* **364**: 1343-1360. Vichinsky E (2002) New therapies in sickle cell disease. *Lancet* **360**: 629-631. Weatherall DJ, Clegg JB (eds) (2001) The thalassaemic syndromes, 4th edn. Oxford: Oxford Blackwell Science. Weatherall DJ, Provan AB (2000) Red cells I: inherited anaemias. *Lancet* **355**: 1169-1175.

## ACQUIRED HAEMOLYTIC ANAEMIA

These anaemias may be divided into those due to immune, non-immune, or other causes (see Table 8.9).

### Causes of immune destruction of red cells

- Autoantibodies
- Drug-induced antibodies
- Alloantibodies.

### Causes of non-immune destruction of red cells

- Acquired membrane defects (e.g. paroxysmal nocturnal haemoglobinuria; see p. 452).
- Mechanical factors (e.g. prosthetic heart valves, or microangiopathic haemolytic anaemia; see p. 453).
- Secondary to systemic disease (e.g. renal and liver disease).

### Miscellaneous causes

- Various toxic substances can disrupt the red cell membrane and cause haemolysis (e.g. arsenic, and products of *Clostridium welchii*).
- Malaria frequently causes anaemia owing to a combination of a reduction in red cell survival and reduced production of red cells.
- Hypersplenism (p. 457) results in a reduced red cell survival, which may also contribute to the anaemia seen in malaria.

- Extensive burns result in denaturation of red cell membrane proteins and reduced red cell survival.
- Some drugs (e.g. dapsone, sulfasalazine) cause oxidative haemolysis with Heinz bodies.
- Some ingested chemicals (e.g. weedkillers such as sodium chlorate) cause severe oxidative haemolysis leading to acute renal failure.

## AUTOIMMUNE HAEMOLYTIC ANAEMIAS

Autoimmune haemolytic anaemias (AIHA) are acquired disorders resulting from increased red cell destruction due to red cell autoantibodies. These anaemias are characterized by the presence of a positive direct antiglobulin (Coombs') test, which detects the autoantibody on the surface of the patient's red cells (Fig. 8.26).

AIHA is divided into 'warm' and 'cold' types, depending on whether the antibody attaches better to the red cells at body temperature (37°C) or at lower temperatures. The major features and the causes of these two forms of AIHA are shown in Table 8.15. In warm AIHA, IgG antibodies predominate and the direct antiglobulin test is positive with IgG alone, IgG and complement, or complement only. In cold AIHA, the antibodies are usually IgM. They easily elute off red cells, leaving complement which is detected as C3d.

### immune destruction of red cells

IgM or IgG red cell antibodies which fully activate the complement cascade cause lysis of red cells in the circulation (intravascular haemolysis).

IgG antibodies frequently do not activate complement and the coated red cells undergo extravascular haemolysis (Fig. 8.27). They are either completely phagocytosed in the spleen through an interaction with Fc receptors on macrophages, or they lose part of the cell membrane through partial phagocytosis and circulate as spherocytes until they too become sequestered in the spleen. Some IgG antibodies partially activate complement, leading to deposition of C3b on the red cell surface, and this may enhance phagocytosis as macrophages also have receptors for C3b.

Non-complement-binding IgM antibodies are rare and have little or no effect on red cell survival. IgM antibodies which partially rather than fully activate complement cause adherence of red cells to C3b receptors on macrophages, particularly in the liver, although this is an ineffective mechanism of haemolysis. Most of the red cells are released from the macrophages when C3b is cleaved to C3d and then circulate with C3d on their surface.

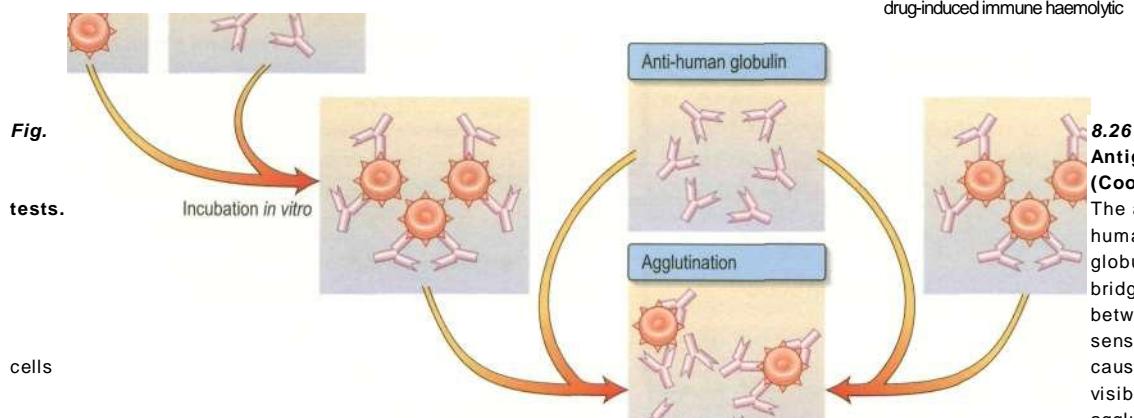
## 'Warm' autoimmune haemolytic anaemias

### Clinical features

These anaemias may occur at all ages and in both sexes, although they are most frequent in middle-aged females. They can present as a short episode of anaemia and

## Acquired haemolytic anaemia

Indirect antiglobulin test
Normal cells sensitized <i>in vitro</i> e.g. antibody screening, crossmatching
Normal RBC Patient's serum/plasma



The direct test detects patients' cells sensitized *in vivo*. The indirect test detects normal cells sensitized *in vitro*. HDN, haemolytic disease of newborn.

**Table 8.15 Causes and major features of autoimmune haemolytic anaemias**

	Warm	Cold
Temperature at which antibody attaches best to red cells	37°C	Lower than 37°C
Type of antibody	IgG	IgM
Direct Coombs' test	Strongly positive	Positive
Causes of primary conditions	Idiopathic	Idiopathic
Causes of secondary condition	Autoimmune disorders, e.g. systemic lupus erythematosus, Chronic lymphocytic leukaemia, Lymphomas, Hodgkin's lymphoma, Carcinomas, Drugs, e.g. methyldopa	Infections, e.g. infectious mononucleosis, <i>Mycoplasma pneumoniae</i> , other viral infections (rare), Lymphomas, Paroxysmal cold haemoglobinuria (IgG)

jaundice but they often remit and relapse and may progress to an intermittent chronic pattern. The spleen is often palpable. Infections or folate deficiency may provoke a profound fall in the haemoglobin level.

In more than 30% of cases, the cause remains unknown. These anaemias may be associated with lymphoid malignancies or diseases such as rheumatoid arthritis and SLE or drugs (Table 8.15).

### Investigations

- **Haemolytic anaemia** is evident (see p. 437).
- **Spherocytosis** is present as a result of red cell damage.
- **Direct antiglobulin test** is positive, with either IgG alone (67%), IgG and complement (20%), or complement alone (13%) being found on the surface of the red cells.
- **Autoantibodies** may have specificity for the Rh blood group system (e.g. for the e antigen).

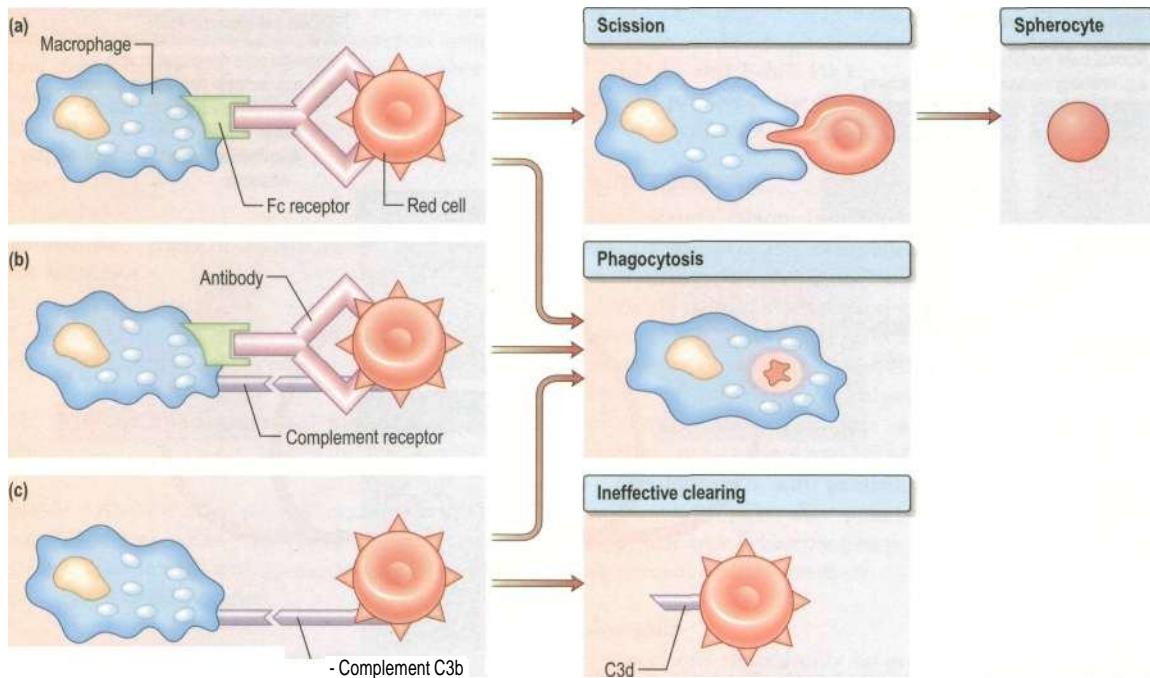
- **Autoimmune thrombocytopenia** and/or neutropenia may also be present (Evans' syndrome).

### Treatment and prognosis

Corticosteroids (e.g. prednisolone in doses of 1 mg/kg daily) are effective in inducing a remission in about 80% of patients. Steroids reduce both production of the red cell autoantibody and destruction of antibody-coated cells. Splenectomy may be necessary if there is no response to steroids or if the remission is not maintained when the dose of prednisolone is reduced. Other immunosuppressive drugs, such as azathioprine and cyclophosphamide, may be effective in patients who fail to respond to steroids and splenectomy.

### 'Cold' autoimmune haemolytic anaemias

Normally, low titres of IgM cold agglutinins reacting at



**Fig. 8.27 Extravascular haemolysis is due to interaction of antibody-coated cells with cells in the reticuloendothelial system, predominantly in the spleen.** (a) Spherocytosis results from partial phagocytosis, (b) Complete phagocytosis may occur and this is enhanced if there is complement as well as antibody on the cell surface, (c) Cells coated with complement only are ineffectively removed and circulate with C3d or C3b on their surface.

4°C are present in plasma and are harmless. At low temperatures these antibodies can attach to red cells and cause their agglutination in the cold peripheries of the body. In addition, activation of complement may cause intravascular haemolysis when the cells return to the higher temperatures in the core of the body.

After certain infections (such as *Mycoplasma*, cytomegalovirus, Epstein-Barr virus (EBV)) there is increased synthesis of polyclonal cold agglutinins producing a mild to moderate transient haemolysis.

#### Chronic cold haemagglutinin disease (CHAD)

This usually occurs in the elderly with a gradual onset of haemolytic anaemia owing to the production of monoclonal IgM cold agglutinins. After exposure to cold, the patient develops an acrocyanosis similar to Raynaud's (see p. 869) as a result of red cell autoagglutination.

#### Investigations

- Red cells agglutinate in the cold or at room temperature. Agglutination is sometimes seen in the sample tube after cooling but is more easily seen on the peripheral blood film made at room temperature. The agglutination is reversible after warming the sample. The agglutination may cause a spurious increase in the MCV (see p. 1425).
- Direct antiglobulin test is positive with complement alone.
- Monoclonal IgM antibodies with specificity for the Ii blood group system, usually for the I antigen but occasionally for the i antigen.

#### Treatment

The underlying cause should be treated, if possible. Patients should avoid exposure to cold. Treatment with steroids, alkylating agents and splenectomy is usually ineffective. Treatment with anti-CD20 (rituximab) has been successful in some cases.

#### Paroxysmal cold haemoglobinuria (PCH)

This is a rare condition associated with common childhood infections, such as measles, mumps and chickenpox. Intravascular haemolysis is associated with polyclonal IgG complement-fixing antibodies. These antibodies are biphasic, reacting with red cells in the cold in the peripheral circulation, with lysis occurring due to complement activation when the cells return to the central circulation. The antibodies have specificity for the P red cell antigen. The lytic reaction is demonstrated in vitro by incubating the patient's red cells and serum at 4°C and then warming the mixture to 37°C (Donath-Landsteiner test). Haemolysis is self-limiting but supportive transfusions of warmed blood may be necessary.

#### DRUG-INDUCED IMMUNE HAEMOLYTIC ANAEMIA

The interaction between a drug and red cell membrane produces a composite antigenic structure (or neoantigen), provoking two types of antibodies:

- Drug-dependent antibodies, which bind to both the drug and the cell membrane but not to either separately.

Clinically there is usually severe complement-mediated intravascular haemolysis, which resolves quickly after withdrawal of the drug. ■ *Drug-independent antibodies*, which are induced by a subtle alteration of the red cell membrane. Such antibodies react with red cells *in vitro* in the absence of the drug and are indistinguishable from 'true' autoantibodies. There is extravascular haemolysis and the clinical course tends to be more protracted.

This concept for drug-induced immune haemolytic anaemia probably also applies to drug-induced thrombocytopenia and neutropenia.

### ALLOIMMUNE HAEMOLYTIC ANAEMIA

Antibodies produced in one individual react with the red cells of another. This situation occurs in haemolytic disease of the newborn, haemolytic transfusion reactions (see p. 461) and after allogeneic bone marrow, renal, liver or cardiac transplantation when donor lymphocytes transferred in the allograft ('passenger lymphocytes') may produce red cell antibodies against the recipient and cause haemolytic anaemia.

#### Haemolytic disease of the newborn (HDN)

HDN is due to fetomaternal incompatibility for **red** cell antigens. Maternal alloantibodies against fetal red cell antigens pass from the maternal circulation via the placenta into the fetus, where they destroy the fetal red cells. Only IgG antibodies are capable of transplacental passage from mother to fetus.

The most common type of HDN is that due to ABO incompatibility, where the mother is usually group O and the fetus group A.

HDN due to ABO incompatibility is usually mild and exchange transfusion is rarely needed. HDN due to RhD incompatibility has become much less common in developed countries following the introduction of anti-D prophylaxis (see below). HDN may be caused by antibodies against antigens in many blood group systems (e.g. other Rh antigens such as c and E, and Kell, Duffy and Kidd; see p. 458).

Sensitization occurs as a result of passage of fetal red cells into the maternal circulation (which most readily occurs at the time of delivery), so that first pregnancies are rarely affected. However, sensitization may occur at other times, for example after a miscarriage, ectopic pregnancy or blood transfusion, or due to episodes during pregnancy which cause transplacental bleeding such as amniocentesis, chorionic villus sampling and threatened miscarriage.

#### Clinical features

These vary from a mild haemolytic anaemia of the newborn to intrauterine death from 18 weeks' gestation with the characteristic appearance of hydrops fetalis (hepatosplenomegaly, oedema and cardiac failure).

Kernicterus occurs owing to severe jaundice in the neonatal period, where the unconjugated (lipid-soluble) bilirubin exceeds 250( $\mu$ mol/L and bile pigment deposition occurs in the basal ganglia. This can result in permanent brain damage, choreoathetosis, and spasticity. In mild cases it may present as deafness.

### Investigations

#### Routine antenatal serology

All mothers should have their ABO and RhD groups determined and their serum tested for atypical antibodies after attending the antenatal booking clinic. Tests for red cell antibodies should be repeated at 28 weeks' gestation. If an antibody is detected, its blood group specificity should be determined and the mother should be retested at least monthly. A rising antibody titre of IgG antibodies or a history of HDN in a previous pregnancy is an indication for referral to a specialist unit to determine the need for amniocentesis (to assess the level of bilirubin in the amniotic fluid) or fetal blood sampling to determine the severity of HDN and to guide further management.

#### Ultrasound

This shows changes in the fetal blood flow and cardiac function caused by compensated anaemia and can be demonstrated *in utero* before hydrops develops. Fetal DNA may be obtained by amniocentesis, chorionic villous sampling, or fetal blood sampling. Soluble fetal DNA in maternal plasma can also be used for this purpose, avoiding an invasive procedure.

#### At the birth of an affected infant

A sample of cord blood is obtained. This shows: - . . . - '

- anaemia with a high reticulocyte count
- a positive direct antiglobulin test
- a raised serum bilirubin.

### Treatment

#### Management of the baby

In mild cases, phototherapy may be used to convert bilirubin to water-soluble biliverdin. Biliverdin can be excreted by the kidneys and this therefore reduces the chance of kernicterus.

In more severely affected cases, exchange transfusion may be necessary to replace the infant's red cells and to remove bilirubin. Indications for exchange transfusion include:

- a cord Hb of < 12 g/dL (normal cord Hb is 13.6–19.6 g/dL)
- a cord bilirubin of > 60  $\mu$ mol/L
- a later serum bilirubin of > 300  $\mu$ mol/L
- a rapidly rising serum bilirubin level.

Further exchange transfusions may be necessary to remove the unconjugated bilirubin.

The blood used for exchange transfusions should be ABO-compatible with the mother and infant, lack the antigen against which the maternal antibody is directed, be fresh (no more than 5 days from the day of collection), and be CMV-seronegative to prevent transmission of cytomegalovirus.

A severely affected fetus may need intrauterine blood transfusions carried out in a special unit.

An advance in the antenatal management of RhD alloimmunized women is the development of molecular methods for fetal RhD blood grouping in women with partners heterozygous for RhD. RhD-negative fetuses, who will be unaffected, can be distinguished from RhD-positive fetuses, who may be severely affected and require intensive monitoring.

#### *Prevention of RhD immunization in the mother*

Anti-D should be given after delivery when all of the following are present:

- the mother is RhD negative
- the fetus is RhD positive
- there is no maternal anti-D detectable in the mother's serum; i.e. the mother is not already immunized.

The dose is 500 i.u. of IgG anti-D intramuscularly within 48 hours of delivery. *The Kleihauer test* is used to assess the number of fetal cells in the maternal circulation. A blood film prepared from maternal blood is treated with acid, which elutes Hb A. Hb F is resistant to this treatment and can be seen when the film is stained with eosin. If large numbers of fetal red cells are present in the maternal circulation, a higher or additional dose of anti-D will be necessary.

It may be necessary to give prophylaxis to RhD-negative women at other times when sensitization may occur, for example after an ectopic pregnancy, threatened miscarriage or amniocentesis. The dose of anti-D is 250 i.u. before 20 weeks' gestation and 500 i.u. after 20 weeks.

Of previously non-immunized RhD-negative women carrying RhD-positive fetuses, 1-2% are immunized by the time of delivery. *Antenatal prophylaxis* with administration of 500 i.u. anti-D to RhD-negative women at both 28 and 34 weeks' gestation has been shown to reduce the incidence of immunization, and its routine use is being implemented in the UK. Monoclonal anti-D could in principle replace polyclonal anti-D, which is collected from RhD-negative women immunized in pregnancy and deliberately immunized RhD-negative males, but it is likely to be some years before trials have been completed and it is available in sufficient quantity.

#### FURTHER READING

- Joint Working Party of the British Blood Transfusion Society and the Royal College of Obstetricians and Gynaecologists (1999) Guidelines for the use of anti-D immunoglobulin for Rh prophylaxis. *Transfusion Medicine* 9: 93-97.  
 Urbaniak SJ, Greiss MA (2000) RhD haemolytic disease of the newborn. *Blood Reviews* 14: 44-61.

#### NON-IMMUNE HAEMOLYTIC ANAEMIA

##### **Paroxysmal nocturnal haemoglobinuria (PNH)**

This is a rare acquired red cell defect in which a clone of red cells is particularly sensitive to destruction by activated complement. These cells are continually haemolysed intravascularly. Platelets and granulocytes are also affected and there may be thrombocytopenia and neutropenia.

The underlying defect is an inability of PNH cells to make glycosylphosphatidylinositol (GPI), which anchors surface proteins such as decay accelerating factor (DAF; CD55) and membrane inhibitor of reactive lysis (MIRL; CD59) to cell membranes. CD55 and CD59 and other proteins are involved in complement degradation (at the C3 and C5 levels), and in their absence the haemolytic action of complement continues. The molecular basis of PNH has been found to be mutations in the *pig-A* (phosphatidylinositol glycan protein A) gene responsible for synthesis of the GPI anchor.

##### *Clinical features*

The major clinical signs are intravascular haemolysis, venous thrombosis and haemoglobinuria. Haemolysis may be precipitated by infection, iron therapy or surgery. Characteristically only the urine voided at night and in the morning on waking is dark in colour, although the reason for this phenomenon is not clear. In severe cases all urine samples are dark. Urinary iron loss may be sufficient to cause iron deficiency.

Some patients present insidiously with signs of anaemia and recurrent abdominal pains.

Venous thrombotic episodes are very common in unusual places and severe thromboses may occur, for example in hepatic (Budd-Chiari syndrome), mesenteric or cerebral veins. The cause of the increased predisposition to thrombosis is not known, but may be due to complement-mediated activation of platelets deficient in CD55 and CD59.

##### *Investigations*

- **Intravascular haemolysis** is evident (see p. 437).
- **Flow cytometric analysis** of red cells with anti-CD55 and anti-CD59 has replaced the Ham's test.
- **Bone marrow** is sometimes hypoplastic (or even aplastic) despite haemolysis.

##### *Treatment and prognosis*

PNH is a chronic disorder requiring supportive measures such as blood transfusions, which are necessary for patients with severe anaemia. Leucocyte-depleted blood should be used in order to prevent transfusion reactions resulting in complement activation and acceleration of the haemolysis. Recently a recombinant humanized monoclonal antibody (eculizumab) that prevents the cleavage of C5 (and therefore the formation of the membrane attack complex) has been shown to reduce intravascular haemolysis, haemoglobinuria and the need for transfusion in PNH.

Long-term anticoagulation may be necessary for patients with recurrent thrombotic episodes. In patients with bone marrow failure, treatment options include immunosuppression with antilymphocyte globulin, ciclosporin, or bone marrow transplantation. Bone marrow transplantation has been successfully carried out using either HLA-matched sibling donors in patients under the age of 50 or matched unrelated donors in patients under the age of 25.

The course of PNH is variable. PNH may transform into aplastic anaemia or acute leukaemia, but it may remain stable for many years and the PNH clone may even disappear, which must be taken into account if considering potentially dangerous treatments such as bone marrow transplantation. The median survival is 10–15 years.

Gene therapy will perhaps be possible in the future.

^

Red cells may be injured by physical trauma in the circulation. Direct injury may cause immediate cell lysis or be followed by resealing of the cell membrane with the formation of distorted red cells or 'fragments'. These cells may circulate for a short period before being destroyed prematurely in the reticuloendothelial system.

The causes of mechanical haemolytic anaemia include:

- damaged artificial heart valves
- march haemoglobinuria, where there is damage to red cells in the feet associated with prolonged marching or running
- microangiopathic haemolytic anaemia (MAHA), where fragmentation of red cells occurs in an abnormal microcirculation caused by malignant hypertension, eclampsia, haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, vasculitis or disseminated intravascular coagulation.

#### FURTHER READING

- Rosse WF, Nishimura J (2003) Clinical manifestations of paroxysmal nocturnal haemoglobinuria. *International Journal of Haematology* 77:113–120.  
 Schwartz RS (2004) Paroxysmal nocturnal haemoglobinuria. *New England Journal of Medicine* 350: 537–538.

## MYELOPROLIFERATIVE DISORDERS

In these disorders there is uncontrolled clonal proliferation of one or more of the cell lines in the bone marrow, namely erythroid, myeloid and megakaryocyte lines. Myeloproliferative disorders include polycythaemia vera (PV), essential thrombocythaemia (ET), myelofibrosis and chronic myeloid leukaemia (CML). These disorders are grouped together as there can be transition from one disease to another; for example PV can lead to myelofibrosis. They may also transform to acute

**Table 8.16 Causes of polycythaemia**

Primary		
Polycythaemia vera		
<b>Secondary</b>		
	<b>Due to an appropriate increase in erythropoietin</b>	<b>Due to an inappropriate increase in erythropoietin</b>
High altitude	Lung disease	Congenital: Chuvash polycythaemia
	Cardiovascular disease	Renal disease: tumours, cysts
	(right-to-left shunt)	Liver disease: hepatocellular carcinoma, cirrhosis
Heavy smoking	Mutant high oxygen affinity haemoglobin, e.g. congenital polycythaemia	Endocrine: adrenal tumours
		Tumours: cerebellar haemangioblastoma, massive uterine fibroma, bronchial carcinoma
<b>Relative</b>		
'Apparent' polycythaemia	Drugs: erythropoietin, androgens	
Dehydration		
Burns		

myeloblastic leukaemia. The non-leukaemic myeloproliferative disorders (PV, ET and myelofibrosis) will be discussed in this section. Chronic myeloid leukaemia is described on page 505.

## POLCYTHAEMIA

Polycythaemia (or erythrocytosis) is defined as an increase in haemoglobin, PCV and red cell count. PCV is a more reliable indicator of polycythaemia than is Hb, which may be disproportionately low in iron deficiency. Polycythaemia can be divided into absolute erythrocytosis where there is a true increase in red cell volume, or relative erythrocytosis where the red cell volume is normal but there is a decrease in the plasma volume (see Fig. 8.6).

Absolute erythrocytosis is due to primary polycythaemia (PV) or secondary polycythaemia. Secondary polycythaemia is due to either an appropriate increase in red cells in response to anoxia, or an inappropriate increase associated with tumours, such as a renal carcinoma. The causes of polycythaemia are given in Table 8.16.

### Primary polycythaemia: polycythaemia vera (PV)

PV is a clonal stem cell disorder in which there is an alteration in the pluripotent progenitor cell leading to excessive proliferation of erythroid, myeloid and megakaryocytic progenitor cells. This is partly due to a failure of apoptosis as a result of deregulation of the *Bcl-x* gene (opposes programmed cell death, p. 162), in addition a mutation in the *JAK2* protein has been found; this stimulates low grade erythropoiesis.

#### Clinical features

The onset is insidious. It usually presents in patients aged over 60 years with tiredness, depression, vertigo, tinnitus and visual disturbance. It should be noted that these symptoms are also common in the normal population

## Haematological disease

over the age of 60 and consequently PV is easily missed. These features, together with hypertension, angina, intermittent claudication and a tendency to bleed, are suggestive of PV.

Severe itching after a hot bath or when the patient is warm is common. Gout due to increased cell turnover may be a feature, and peptic ulceration occurs in a minority of patients. Thrombosis and haemorrhage are the major complications of PV.

The patient is usually plethoric and has a deep dusky cyanosis. Injection of the conjunctivae is commonly seen. The spleen is palpable in 70% and is useful in distinguishing PV from secondary polycythaemia. The liver is enlarged in 50% of patients.

### Investigations

**m Hb and PCV** are increased. The WBC is raised in about 70% of cases of PV and the platelet count is elevated in about 50%.

- **Bone marrow** shows erythroid hyperplasia and increased numbers of megakaryocytes.
- **Red cell volume** measured using  $^{51}\text{Cr}$ -labelled red cells is increased ( $> 36 \text{ mL/kg}$  in males and  $32 \text{ mL/kg}$  in females).
- **Plasma volume** shows normal or increased values (normal range is  $45 \pm 5 \text{ mL/kg}$ ).
- **Serum uric acid** levels may be raised.
- **Leucocyte alkaline phosphatase (LAP)** score is usually high.
- **Serum vitamin B<sub>12</sub> and vitamin B<sub>12</sub>-binding protein** transcobalamin I (TC I) levels may be high, although these are not routinely measured.

### Differential diagnosis

An increase in the red cell volume should be established. Raised WBC and platelet counts with splenomegaly makes a diagnosis of PV very likely. The principal secondary causes can often be excluded by the history and examination, but a renal ultrasound, an arterial  $P_{\text{O}_2}$  and carboxyhaemoglobin levels are of additional help.

The serum erythropoietin level is not diagnostic but may be helpful in distinguishing PV from secondary polycythaemia. In PV the level is low or normal, whereas in secondary polycythaemia the level may be raised, as expected, but also can be normal.

### Course and management

Treatment is designed to maintain a normal blood count and to prevent the complications of the disease, particularly thromboses and haemorrhage. *Treatment* is aimed at keeping the PCV below 0.45 L/L and the platelet count below  $400 \times 10^9/\text{L}$ . There are three types of specific treatment:

- **Venesection.** This will successfully relieve many of the symptoms of PV. Iron deficiency limits erythropoiesis. Venesection is often used as the sole treatment and other therapy is reserved to control the thrombocytosis.

■ **Chemotherapy.** Continuous or intermittent treatment with hydroxycarbamide (hydroxyurea) is used frequently because of the ease of controlling thrombocytosis and general safety in comparison to the alkylating agents such as busulfan, which carry an increased risk of acute leukaemia. Low-dose intermittent busulfan may be more convenient for elderly people, and this must be weighed against the potential risk of long-term complications.

■ **Radioactive  $^{32}\text{P}$ .** One dose may give control for up to 18 months, but the administration of  $^{32}\text{P}$  carries an increased risk of transformation to acute leukaemia.  $^{32}\text{P}$  is confined to the over-70 years age group.

### General treatment

Allopurinol is given to block uric acid production. Low-dose aspirin (100 mg daily) can safely prevent thrombotic complications in treated patients. The pruritus is lessened by avoiding very hot baths. H<sub>2</sub>-receptor antagonists have largely proved unsuccessful in relieving distressing pruritus, but H<sub>2</sub>-receptor antagonists such as cimetidine are occasionally effective.

Patients with uncontrolled PV have a high operative risk; 75% of patients have severe haemorrhage following surgery and 30% of these patients die. Polycythaemia should be controlled before surgery. In an emergency, reduction of the haematocrit by venesection and appropriate fluid replacement must be carried out.

### Prognosis

PV develops into myelofibrosis in 30% of cases and into acute myeloblastic leukaemia in 5% as part of the natural history of the disease.

### Secondary polycythaemias

Many high-oxygen affinity haemoglobin mutants (HOAHM) have been described which lead to increased oxygen affinity but decreased oxygen delivery to the tissues, resulting in compensatory polycythaemia. A congenital (autosomal recessive disorder - Chuvasch polycythaemia) is due to a defect in the oxygen-sensing erythropoietin production pathway caused by a mutation of the von Hippel-Lindau (VHL) gene, resulting in an increased production of erythropoietin.

The causes of secondary polycythaemias are shown in Table 8.16.

The treatment is that of the precipitating factor; for example, renal or posterior fossa tumours need to be resected. Heavy smoking can produce as much as 10% carboxyhaemoglobin and this can produce polycythaemia because of a reduction in the oxygen-carrying capacity of the blood. Complications of secondary polycythaemia are similar to those seen in PV, including thrombosis, haemorrhage and cardiac failure, but the complications due to myeloproliferative disease such as progression to myelofibrosis or acute leukaemia do not develop. Venesection may be symptomatically helpful in the hypoxic patient, particularly if the PCV is above 0.55 L/L.

### 'Relative' or 'apparent' polycythaemia (Gaisbock's syndrome)

This condition was originally thought to be stress-induced. The red cell volume is normal, but as the result of a decreased plasma volume, there is a relative polycythaemia. 'Relative' polycythaemia is more common than PV and occurs in middle-aged men, particularly in smokers who are obese and hypertensive. The condition may present with cardiovascular problems such as myocardial or cerebral ischaemia. For this reason, it may be justifiable to venesection of the patient. Smoking should be stopped.

### Essential thrombocythaemia (ET)

ET is closely related to PV. The platelet count is usually  $> 1000 \times 10^9/L$ . It presents with bruising, bleeding and cerebrovascular symptoms. Initially splenic hypertrophy may be seen but, as the condition progresses, recurrent thromboses owing to the increased number of platelets reduce the size of the spleen and it may atrophy.

ET should be distinguished from secondary thrombocytosis that is seen in haemorrhage, connective tissue disorders, malignancy, after splenectomy and in other myeloproliferative disorders.

*Treatment* is with hydroxycarbamide (hydroxyurea), anagrelide or busulfan to control the platelet count to less than  $400 \times 10^9/L$ .

a-Interferon is also effective but it is expensive and is administered by subcutaneous injection. ET may eventually transform into PV, myelofibrosis or acute leukaemia, but the disease may not progress for many years.

### MYELOFIBROSIS (MYELOSCLEROSIS)

The terms myelosclerosis and myelofibrosis are interchangeable. There is clonal proliferation of stem cells and myeloid metaplasia in the liver, spleen and other organs. Increased fibrosis in the bone marrow is caused by hyperplasia of abnormal megakaryocytes which release fibroblast-stimulating factors such as platelet-derived growth factor. In about 25% of cases there is a preceding history of PV.

#### Clinical features

The disease presents insidiously with lethargy, weakness and weight loss. Patients often complain of a 'fullness' in the upper abdomen due to splenomegaly. Severe pain related to respiration may indicate perisplenitis secondary to splenic infarction, and bone pain and attacks of gout can complicate the illness. Bruising and bleeding occur because of thrombocytopenia or abnormal platelet function. Other physical signs include anaemia, fever and massive splenomegaly (for other causes, see p. 457).

#### Investigations

- **Anaemia** with leucoerythroblastic features is present (p. 464). Poikilocytes and red cells with characteristic tear-drop forms are seen. The WBC count may be over  $100 \times 10^9/L$ , and the differential WBC count may be

very similar to that seen in chronic myeloid leukaemia (CML); later leucopenia may develop.

- **The platelet count** may be very high, but in later stages, thrombocytopenia occurs.
- **Bone marrow aspiration** is often unsuccessful and this gives a clue to the presence of the condition. A bone marrow trephine is necessary to show the markedly increased fibrosis. Increased numbers of megakaryocytes may be seen.
- **The Philadelphia chromosome** is absent; this helps to distinguish myelofibrosis from most cases of CML.
- **The leucocyte alkaline phosphatase (LAP) score** is normal or high.
- **A high serum urate** is present.
- **Low serum folate** levels may occur owing to the increased haemopoietic activity.

#### Differential diagnosis

The major diagnostic difficulty is the differentiation of myelofibrosis from CML as in both conditions there may be marked splenomegaly and a raised WBC count with many granulocyte precursors seen in the peripheral blood. The main distinguishing features are the appearance of the bone marrow and the absence of the Philadelphia chromosome in myelofibrosis.

Fibrosis of the marrow, often with a leucoerythroblastic anaemia, can also occur secondarily to leukaemia or lymphoma, tuberculosis or malignant infiltration with metastatic carcinoma, or to irradiation.

#### Treatment

This consists of general supportive measures such as blood transfusion, folic acid, analgesics and allopurinol. Drugs such as hydroxycarbamide (hydroxyurea) and busulfan are used to reduce metabolic activity and high WBC count and platelet levels; hydroxycarbamide is the most common drug used. Chemotherapy and radiotherapy are used to reduce splenic size. If the spleen becomes very large and painful, and transfusion requirements are high, it may be advisable to perform splenectomy. Splenectomy may also result in relief of severe thrombocytopenia.

#### Prognosis

Patients may survive for 10 years or more; median survival is 3 years. Death may occur in 10-20% of cases from transformation to acute myeloblastic leukaemia. The most common causes of death are cardiovascular disease, infection and gastrointestinal bleeding.

### MYELODYSPLASIA (MDS)

Myelodysplasia (MDS) describes a group of acquired bone marrow disorders that are due to a defect in stem cells. They are characterized by increasing bone marrow failure with quantitative and qualitative abnormalities of all three myeloid cell lines (red cells, granulocyte/monocytes and platelets). The natural history of MDS is variable, but there is a high morbidity and mortality owing to bone marrow failure, and transformation into

**Table 8.17 Classification of myelodysplasia (based on WHO and FAB)**

Category		Peripheral blasts (%)	Bone marrow (%)	Median prognosis (months)
<b>1a</b>	Refractory anaemia (RA)	<1	Blasts < 5	50
<b>2a</b>	RA and ring sideroblasts (RARS)	<1	RS<15 Blasts < 5	= 50
<b>3a</b>	RA with excess blasts (RAEB) I	1-4	RS> 15	
<b>3b</b>	(RAEB-t) II	5-19	Blasts 5-10 Blasts 11-19	10 5

In all categories, monocytes in the peripheral blood  $< 1.0 \times 10^9/L$

RS, ring sideroblasts; RAEB-t (in transformation) also includes the 5q syndrome (deletion of 5q-) - usually elderly females but with good prognosis

acute myeloblastic leukaemia occurs in about 30% of cases. A classification of the myelodysplastic syndrome is shown in Table 8.17.

### Clinical and laboratory features

MDS occurs mainly in the elderly, and presents with symptoms of anaemia, infection or bleeding due to pancytopenia. Serial blood counts show evidence of increasing bone marrow failure with anaemia, neutropenia, monocytosis and thrombocytopenia, either alone or in combination. In contrast, in chronic myelomonocytic leukaemias (CMML), monocytes are  $> 1 \times 10^9/L$  and the WBC count may be  $> 100 \times 10^9/L$ .

The bone marrow usually shows increased cellularity despite the pancytopenia. Dyserythropoiesis is present, and granulocyte precursors and megakaryocytes also have abnormal morphology. Ring sideroblasts are present in all types. In RAEB and RAEB-t, the number of blasts in the bone marrow is increased, and the prognosis is worse than in those types with a low number of blast cells (<5%).

### Management

Patients with < 5% blasts in the bone marrow are usually managed conservatively with red cell and platelet transfusions and antibiotics for infections, as they are needed. Haemopoietic growth factors (e.g. erythropoietin, G-CSF) may be useful in some patients.

Patients with > 5% blasts have a less favourable prognosis, and a number of treatment options are available:

- **Supportive care** only is suitable for elderly patients with other medical problems.
- '**Gentle' chemotherapy** (low-dose or single-agent, e.g. azacytidine) may be useful in patients with high WBC counts.
- **Intensive chemotherapy** schedules used for acute myeloblastic leukaemia (see p. 504) may be tried in patients under the age of 60, but the remission rate is less, and prolonged pancytopenia may occur owing to poor haemopoietic regeneration because of the defect in stem cells.
- **Bone marrow transplantation** offers the hope of cure in the small proportion of MDS patients who are under the age of 50 and who have an HLA-identical sibling or an unrelated HLA-matched donor.

### FURTHER READING

- Cazzola M, Malcovati L (2005) Myelodysplastic syndromes. *New England Journal of Medicine* **352**: 536-538. Provan D, Weatherall D (2000) Red cells II: acquired anaemias. *Lancet* **355**: 1260-1268. Schwartz RC (1998) Polycythaemia vera - chance, death and mutability. *New England Journal of Medicine* **338**: 613-615. Spirak J (2004) Daily aspirin - only half the answer (in polycythaemia vera). *New England Journal of Medicine* **350**: 99-101.

## THE SPLEEN

The spleen is the largest lymphoid organ in the body and is situated in the left hypochondrium. There are two anatomical components:

- the red pulp, consisting of sinuses lined by endothelial macrophages and cords (spaces)
- the white pulp, which has a structure similar to lymphoid follicles.

Blood enters via the splenic artery and is delivered to the red and white pulp. During the flow the blood is 'skimmed', with leucocytes and plasma preferentially passing to white pulp. Some red cells pass rapidly through into the venous system while others are held up in the red pulp.

### Functions

**Sequestration and phagocytosis.** Normal red cells, which are flexible, pass through the red pulp into the venous system without difficulty. Old or abnormal cells are damaged by the hypoxia, low glucose and low pH found in the sinuses of the red pulp and are therefore removed by phagocytosis along with other circulating foreign matter. Howell-Jolly and Heinz bodies and sideroblastic granules have their particles removed by 'pitting' and are then returned to the circulation. IgG-coated red cells are removed through their Fc receptors by macrophages.

**Extramedullary haemopoiesis.** Pluripotential stem cells are present in the spleen and proliferate during severe haematological stress, such as in haemolytic anaemia or thalassaemia major.

**Immunological function.** About 25% of the body's T lymphocytes and 15% of B lymphocytes are present in the spleen. The spleen shares the function of production of antibodies with other lymphoid tissues.

**Blood pooling.** Up to one-third of the platelets are sequestered in the spleen and can be rapidly mobilized. Enlarged spleens pool a significant percentage (up to 40%) of the red cell mass.

## SPLENOMEGALY

### Causes

A clinically palpable spleen can have many causes.

- Infection:
  - (a) acute - e.g. septic shock, infective endocarditis, typhoid, infectious mononucleosis
  - (b) chronic - e.g. tuberculosis and brucellosis
  - (c) parasitic - e.g. malaria, kala-azar and schistosomiasis.
- Inflammation: rheumatoid arthritis, sarcoidosis, SLE.
- Haematological: haemolytic anaemia, haemoglobinopathies and the leukaemias, lymphomas and myeloproliferative disorders.
- Portal hypertension: liver disease.
- Miscellaneous: storage diseases, amyloid, primary and secondary neoplasias, tropical splenomegaly.

*Massive splenomegaly* is seen in myelofibrosis, chronic myeloid leukaemia, chronic malaria, kala-azar or, rarely, Gaucher's disease.

## Hypersplenism

This can result from splenomegaly due to any cause. It is commonly seen with splenomegaly due to haematological disorders, portal hypertension, rheumatoid arthritis (Felty's syndrome) and lymphoma. Hypersplenism produces:

- pancytopenia
- haemolysis due to sequestration and destruction of red cells in the spleen
- increased plasma volume.

*Treatment* is often dependent on the underlying cause, but splenectomy is sometimes required for severe anaemia or thrombocytopenia.

## Splenectomy

Splenectomy is performed mainly for:

- trauma
- idiopathic thrombocytopenic purpura (p. 470)
- haemolytic anaemias (p. 441)
- hypersplenism.

### Problems after splenectomy

An immediate problem is an increased platelet count (usually  $600\text{--}1000 \times 10^9/\text{L}$ ) for 2-3 weeks. Thromboembolic phenomena may occur. In the longer term there

### Box 8.1 Prophylaxis against infection after splenectomy or splenic dysfunction

Vaccinate 2-3 weeks before elective splenectomy.

A 23-valent unconjugated pneumococcal polysaccharide vaccine repeated every 5 years Meningococcal group C conjugate vaccine Annual influenza vaccine *Haemophilus influenzae* type b (Hib) vaccine e Long-term penicillin V 500 mg 12-hourly (if sensitive, use erythromycin)

Meningococcal polysaccharide vaccine (ACWY) for travellers to Africa/Saudi Arabia, e.g. during Hajj and Umrah pilgrimages.

is an increased risk of overwhelming infections, particularly pneumococcal infections.

**Prophylaxis against infection after splenectomy or splenic dysfunction (Box 8.1)** All patients should be educated about the risk of infection and the importance of its early recognition and treatment. They should be given an information leaflet and should carry a card or bracelet to alert health professionals to their risk of overwhelming infection.

### Postsplenectomy haematological features

- *Thrombocytosis* persists in about 30% of cases.
- *The WBC count* is usually normal but there may be a mild lymphocytosis and monocytosis.
- *Abnormalities in red cell morphology* are the most prominent changes and include Howell-Jolly bodies, Pappenheimer bodies (contain sideroblastic granules), target cells and irregular contracted red cells (see Fig. 8.28). Pitted red cells can be counted.

### Splenic atrophy

This is seen in sickle cell disease due to infarction. It is also seen in coeliac disease, in dermatitis herpetiformis, and occasionally in ulcerative colitis and essential thrombocythaemia. Postsplenectomy haematological features are seen.

K A\_O t!MP 3 0 •

**Fig. 8.28 Postsplenectomy film with Howell-Jolly bodies (arrowed), target cells and irregularly contracted cells.**

### FURTHER READING

Davies JM, Barnes R, Milligan D (2003) Update of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen. *Clinical Medicine* 2: 440-443.

## BLOOD TRANSFUSION

The cells and proteins in the blood express antigens which are controlled by polymorphic genes; that is, a specific antigen may be present in some individuals but not in others. A blood transfusion may immunize the recipient against donor antigens that the recipient lacks (alloimmunization), and repeated transfusions increase the risk of the occurrence of alloimmunization. Similarly, the transplacental passage of fetal blood cells during pregnancy may alloimmunize the mother against fetal antigens inherited from the father. Antibodies stimulated by blood transfusion or pregnancy, such as Rhesus antibodies, are termed *immune* antibodies and are usually IgG, in contrast to *naturally occurring* antibodies, such as ABO antibodies, which are made in response to environmental antigens present in food and bacteria and which are usually IgM.

## BLOOD GROUPS

The blood groups are determined by antigens on the surface of red cells; more than 400 blood groups have been found. The ABO and Rh systems are the two major blood groups, but incompatibilities involving many other blood groups (e.g. Kell, Duffy, Kidd) may cause haemolytic transfusion reactions and/or haemolytic disease of the newborn (HDN).

### ABO system

This blood group system involves naturally occurring IgM anti-A and anti-B antibodies which are capable of producing rapid and severe intravascular haemolysis of incompatible red cells.

The ABO system is under the control of a pair of allelic genes, *H* and *h*, and also three allelic genes, *A*, *B* and *O*, producing the genotypes and phenotypes shown in Table 8.18. The A, B and H antigens are very similar in structure; differences in the terminal sugars determine their

**Table 8.18** The ABO system: antigens and antibodies

	Phenotype	Genotype	Antigens	Antibodies	Frequency UK (%)
0	00	None	Anti-A and anti-B	Anti-A and anti-B	44
A	AA or AO	A	Anti-B	Anti-B	45
B	BB or BO	B	Anti-A	Anti-A	8
AB	AB	A and B	None	None	3

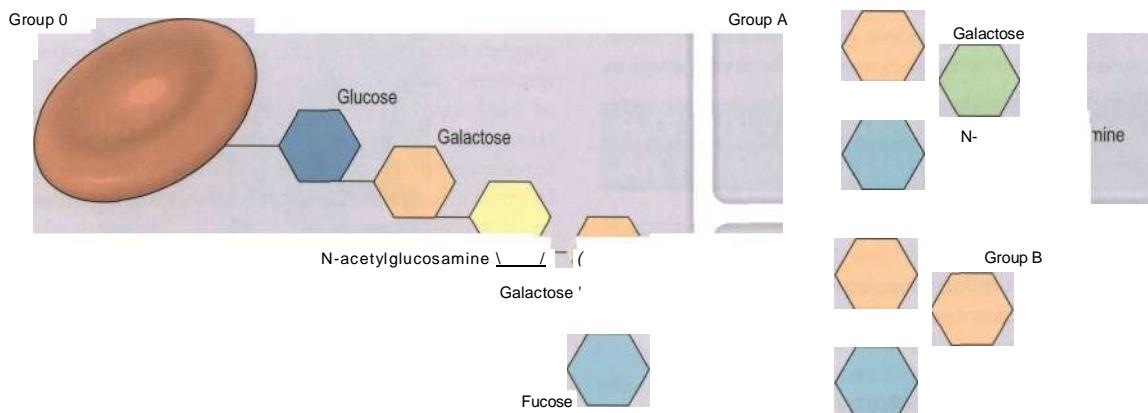
specificity. The *H* gene codes for enzyme *H*, which attaches fucose to the basic glycoprotein backbone to form H substance, which is the precursor for A and B antigens (Fig. 8.29).

The *A* and *B* genes control specific enzymes responsible for the addition to H substance of N-acetylgalactosamine for Group A and D-galactose for Group B. The *O* gene is amorphic and does not transform H substance and therefore O is not antigenic. The A, B and H antigens are present on most body cells. These antigens are also found in soluble form in tissue fluids such as saliva and gastric juice in the 80% of the population who possess secretor genes.

### Rh system

There is a high frequency of development of IgG RhD antibodies in RhD-negative individuals after exposure to RhD-positive red cells. The antibodies formed are of major importance in causing HDN and haemolytic transfusion reactions.

This system is coded by allelic genes, *C* and *c*, *E* and *e*, *D* and no *D*, which is signified as *d*; they are inherited as triplets on each chromosome, one from each pair of genes (i.e. *CDE/cde*). The presence of the *d* antigen has not been demonstrated and the presence or absence of the *D* antigen determines whether an individual is characterized as RhD positive or negative.



**Fig. 8.29** Sugar chains in the ABO blood group system. Reproduced from Fricker J (1996) Conversion of red blood cells to group 0. *Lancet* 347: 680, © The Lancet Ltd. 1996.

## PROCEDURE FOR BLOOD TRANSFUSION

The safety of blood transfusion depends on meticulous attention to detail at each stage leading to and during the transfusion. Avoidance of simple errors involving patient and blood sample identification at the time of collection of the sample for crossmatching and at the time of transfusion would avoid most serious haemolytic transfusion reactions, almost all of which involve the ABO system.

### Pretransfusion compatibility testing

This involves a number of steps, outlined below.

#### **Blood grouping**

The ABO and RhD groups of the patient are determined.

#### **Antibody screening**

The patient's serum or plasma is screened for atypical antibodies that may cause a significant reduction in the survival of the transfused red cells. The patient's serum or plasma is tested against red cells from at least two group O donors, expressing a wide range of red cell antigens, for detection of IgM red cell alloantibodies (using a direct agglutination test of cells suspended in saline) and IgG antibodies (using an indirect antiglobulin test, see p. 449). About 10% of patients have a positive antibody screening result; in which case, further testing is carried out using a comprehensive panel of typed red cells to determine the blood group specificity of the antibody (clinically significant red cell antibodies are detected in about 20% of patients with positive antibody screens).

#### **Selection of donor blood and crossmatching**

Donor blood of the same ABO and RhD group as the patient is selected. Matching for additional blood groups is carried out for patients with clinically significant red cell antibodies (see below), for patients who are likely to be multitransfused and at high risk of developing antibodies, e.g. sickle cell disease, and many centres routinely provide c-negative and Kell-negative blood for women of child-bearing age to minimize the risk of alloimmunization and subsequent HDN.

#### **Crossmatching procedures**

*Patients without atypical red cell antibodies.* The full crossmatch involves testing the patient's serum or plasma against the donor red cells suspended in saline in a direct agglutination test, and also using an indirect antiglobulin test. In some hospitals the *serological crossmatch* has been omitted as the negative antibody screen makes it highly unlikely that there will be any incompatibility with the donor units. A greater risk is that of a transfusion error involving the collection of the patient sample or a mix-up of samples in the laboratory. Laboratories can use the blood bank computer to check its records of the patient and the donor units and authorize the release of the donor units if a number of criteria are met (*computer or electronic crossmatching*), including:

- The system is automated for ABO and RhD grouping and antibody screening including positive sample identification and electronic transfer of results.
- The antibody screening procedure conforms to national recommendations.
- The patient's serum or plasma does not contain clinically significant red cell antibodies.
- The release of ABO incompatible blood must be prevented by conformation of laboratory computer software to the following requirements:
  - (a) the issue of blood is not allowed if the patient has only been grouped once
  - (b) the issue of blood is not allowed if the current group does not match the historical record
  - (c) the system must not allow the reservation and release of units which are ABO incompatible with the patient.
- The laboratory must assure the validity of the ABO and RhD group of the donor blood either by written verification from the National Blood Service or confirmatory testing in the laboratory; the UK National Blood Service guarantees that the blood group information is correct.

Alternatively, the crossmatch can be shortened to an immediate spin crossmatch where the patient's serum or plasma is briefly incubated with the donor red cells, followed by centrifugation and examination for agglutination; this rapid crossmatch is an acceptable method of excluding ABO incompatibility in patients known to have a negative antibody screen.

*Patients with atypical red cell antibodies.* Donor blood should be selected that lacks the relevant red cell antigen(s), as well as being the same ABO and RhD group as the patient. A full crossmatch should always be carried out.

Several other systems for blood grouping, antibody screening and crossmatching are available to hospital transfusion laboratories. They do not depend on agglutination of red cells in suspension, but rather on the differential passage of agglutinated and unagglutinated red cells through a column of dextran gel matrix (e.g. DiaMed, and Ortho BioVue systems), or on the capture of antibodies by red cells immobilized on the surface of a microplate well (e.g. Capture-R solid phase system).

### **Blood ordering**

#### **Elective surgery**

Many hospitals have guidelines for the ordering of blood for elective surgery (maximum surgical blood ordering schedules). These are aimed at reducing unnecessary crossmatching and the amount of blood that eventually becomes outdated. Many operations in which blood is required only occasionally for unexpectedly high blood loss can be classified as 'group and save'; this means that, where the antibody screen is negative, blood is not reserved in advance but can be made available quickly if necessary, using serum or plasma saved in the laboratory.

If a patient has atypical antibodies, compatible blood should always be reserved in advance.

### Emergencies

There may be insufficient time for full pretransfusion testing. The options include:

- Blood required immediately - use of 2 units of O RhD negative blood ('emergency stock'), to allow additional time for the laboratory to group the patient.
- Blood required in 10-15 minutes - use of blood of the same ABO and RhD groups as the patient.
- Blood required in 45 minutes - most laboratories will be able to provide fully crossmatched blood within this time.

### COMPLICATIONS OF BLOOD TRANSFUSION

(see Table 8.19)

In the United States, it has been mandatory to report transfusion-associated deaths to the Food and Drug Administration since 1975; such reports have provided useful data which have contributed to efforts to improve the safety of blood transfusion. Similar reporting schemes under the term '*haemovigilance*' have been set up in other countries, including the Serious Hazards of Transfusion (SHOT) scheme which produced its first report in the UK in 1997. Figure 8.30 shows the reports to SHOT in 2002/03, indicating that '*incorrect blood component transfused*' was the most frequent type of serious incident. Errors in collection and administration of blood were the commonest source of error followed by laboratory errors and mistakes in the prescription of blood or the collection of blood samples for compatibility testing. Death or serious morbidity can also be attributed to other complications of blood transfusion including transfusion-associated lung injury (TRALI), transfusion-associated graft-versus-host disease (TA-GvHD), and bacterial infection of blood components.

**Table 8.19 Complications of blood transfusion**

#### Immunological Non-immunological

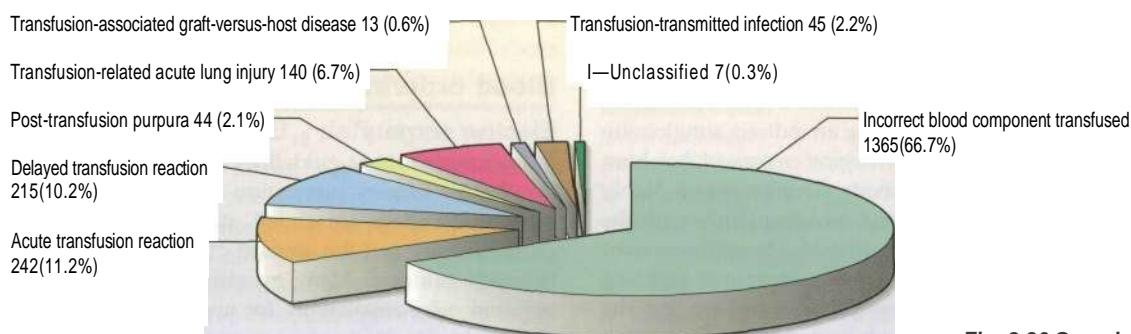
<b>Alloimmunization and incompatibility</b>	<b>Transmission of infection</b>
<b>Red cells</b>	Viruses: HAV, HBV, HCV HIV CMV, EBV, HTLV-1, West Nile virus
Immediate haemolytic transfusion reactions	
Delayed haemolytic transfusion reactions	
<b>Leucocytes and platelets</b>	Parasites: malaria, trypanosomiasis toxoplasmosis Bacteria
Non-haemolytic (febrile) transfusion reactions	Prion - vCJD <b>Circulatory failure due to</b> volume overload <b>Iron overload</b> due to multiple transfusions (see p. 441)
Post-transfusion purpura	<b>Massive transfusion of</b> stored blood may cause bleeding reactions and electrolyte changes
Poor survival of transfused platelets and granulocytes	<b>Physical damage due to</b> freezing or heating
Graft-versus-host disease	<b>Thrombophlebitis Air embolism</b>
Lung injury (TRALI)	
Plasma proteins	
Urticarial and anaphylactic reactions	

### Immunological complications

#### Alloimmunization and incompatibility

Blood transfusion carries a risk of alloimmunization to the many 'foreign' antigens present on red cells, leucocytes, platelets and plasma proteins. Alloimmunization may also occur during pregnancy - to fetal antigens inherited from the father and not shared by the mother (p. 451).

Alloimmunization does not usually cause clinical problems with the first transfusion but these may occur with subsequent transfusions. There may also be delayed consequences of alloimmunization, such as HDN and rejection of tissue transplants.



**Fig. 8.30 Overview of 2191 cases reported to the Serious Hazards of Transfusion (SHOT) scheme between 1996 and 2003.**

### **Incompatibility**

This may result in poor survival of transfused cells, such as red cells and platelets, and also in the harmful effects of antigen-antibody reaction.

### **Red cells**

#### *Haemolytic transfusion reactions*

**Immediate reaction.** This is the most serious complication of blood transfusion and is usually due to ABO incompatibility. There is complement activation by the antigen-antibody reaction, usually caused by IgM antibodies, leading to rigors, lumbar pain, dyspnoea, hypotension, haemoglobinuria and renal failure. The initial symptoms may occur a few minutes after starting the transfusion. Activation of coagulation may also occur and bleeding due to disseminated intravascular coagulation (DIC) is a bad prognostic sign. Emergency treatment may be needed to maintain the blood pressure and renal function.

#### **Diagnosis**

This is confirmed by finding evidence of haemolysis (e.g. haemoglobinuria), and incompatibility between donor and recipient. All documentation should be checked to detect errors such as:

- failure to check the identity of the patient when taking the sample for compatibility testing (i.e. sample from the wrong patient)
- mislabelling the blood sample with the wrong patient's name
- simple labelling or handling errors in the laboratory
- errors in the collection of blood, leading to delivery of the wrong blood to the ward/theatre
- failure to perform proper identity checks before the blood is transfused (i.e. blood transfused to the wrong patient).

The serious consequences of such failures emphasize the need for meticulous checks at all stages in the procedure of blood transfusion.

#### **Investigations**

To confirm where the error occurred, blood grouping should be carried out on:

- the patient's original sample (used for the compatibility testing)
- a new sample taken from the patient after the reaction
- the donor units.

At the first suspicion of any serious transfusion reaction, the transfusion should always be stopped and the donor units returned to the blood transfusion laboratory with a new blood sample from the patient to exclude a haemolytic transfusion reaction.

**Delayed reaction.** This may occur in patients alloimmunized by previous transfusions or pregnancies. The antibody level is too low to be detected by pretransfusion compatibility testing, but a secondary immune response occurs after transfusion, resulting in destruction of the transfused cells, usually by IgG antibodies.

Haemolysis is usually extravascular as the antibodies are IgG, and the patient may develop anaemia and jaundice about a week after the transfusion, although most of these episodes are clinically silent. The blood film shows spherocytosis and reticulocytosis. The direct antiglobulin test is positive and detection of the antibody is usually straightforward.

### **Leucocytes and platelets**

#### *Non-haemolytic (febrile) transfusion reactions*

Febrile reactions are a common complication of blood transfusion in patients who have previously been transfused or pregnant. The usual causes are the presence of leucocyte antibodies in an alloimmunized recipient acting against donor leucocytes in red cell concentrates leading to release of pyrogens, or the release of cytokines from donor leucocytes in platelet concentrates. Typical signs are flushing and tachycardia, fever ( $> 38^{\circ}\text{C}$ ), chills and rigors. Aspirin may be used to reduce the fever, although it should not be used in patients with thrombocytopenia. The introduction of leucocyte-depleted blood in the UK, to minimize the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood transfusion (see below), has reduced the incidence of febrile reactions.

Potent leucocyte antibodies in the plasma of donors, who are usually multiparous women, may cause severe pulmonary reactions (called transfusion-related acute lung injury or TRALI) characterized by dyspnoea, fever, cough, and shadowing in the perihilar and lower lung fields on the chest X-ray.

### **Plasma proteins**

#### *Urticaria and anaphylaxis*

Urticular reactions are often attributed to plasma protein incompatibility, but in most cases, they are unexplained. They are common but rarely severe; stopping or slowing the transfusion and administration of chlorphenamine (chlorpheniramine) 10 mg i.v. are usually sufficient treatment.

Anaphylactic reactions (see p. 997) occasionally occur; severe reactions are seen in patients lacking IgA who produce anti-IgA that reacts with IgA in the transfused blood. The transfusion should be stopped and epinephrine (adrenaline) 0.5 mg i.m. and chlorphenamine 10 mg i.v. should be given immediately; endotracheal intubation may be required. Patients who have had severe urticarial or anaphylactic reactions should receive either washed red cells, autologous blood, or blood from IgA-deficient donors for patients with IgA deficiency.

### **Non-immunological complications**

#### **Transmission of infection**

**Viral contamination:** donor blood in the UK is currently tested for HBV, HCV, HIV-1 and HTLV-1. CMV-seronegative tested blood is given to immunosuppressed patients who are susceptible to acquiring CMV. Blood Services continue a vigilant search for new infectious

## Haematological disease

agents. Donor questionnaires now record recent travel to exclude possible risks of West Nile virus (WNV) and severe acute respiratory syndrome (SARS). Recently, WNV has been the causal agent of meningoencephalitis transmitted by transfusion or transplantation in the USA.

The incidence of transmission of HBV is about 1 in 900 000 units transfused, and it is less than 1 in 30 million units transfused for HCV since the introduction of testing minipools of donor plasma (48 donations per pool) for viral DNA.

In the UK the incidence of transmission of HIV by blood transfusion is extremely low - under 1 in 8 million units transfused. Prevention is based on self-exclusion of donors in 'high-risk' groups and testing each donation for anti-HIV.

There is still a potential risk of viral transmission from coagulation factor concentrates prepared from large pools of plasma. Measures for inactivating viruses such as treatment with solvents and detergents are undertaken. Viral transmission via blood transfusion is still a major issue in the developing world.

*Bacterial contamination* of blood components is rare but it is one of the most frequent causes of death associated with transfusion. Some organisms such as *Yersinia enterocolitica* can proliferate in red cell concentrates stored at 4°C, but platelet concentrates stored at 22°C are a more frequent cause of this problem. Systems to avoid bacterial contamination include automated culture systems and bacterial antigen detection systems, but none are currently in routine use in the UK.

*Transfusion-transmitted syphilis* is very rare in the UK. Spirochaetes do not survive for more than 72 hours in blood stored at 4°C, and each donation is tested using the *Treponema pallidum haemagglutination assay* (TPHA).

There continues to be concern about the risk of transmitting the prion protein causing vCJD (p. 60) by transfusion: a possible transmission occurred following a transfusion in 2003. A number of measures have been taken in the UK, including universal leucocyte depletion of blood components (in 1999) because the prion protein is thought to be primarily associated with lymphocytes. UK donor plasma is not used for the manufacture of blood products; imported plasma from the US is used instead. For children born after January 1 1996, fresh frozen plasma (FFP) is sourced from plasma (from unremunerated donors) imported from the USA, on the basis that exposure to bovine spongiform encephalitis (BSE) from food was eliminated by 1 January 1996. FFP for this group is treated with methylthioninium chloride (methylene blue) to inactivate viruses. Blood donors must not have had a blood transfusion since 1980.

While stringent measures are being taken to minimize the risk of transfusion-transmitted infection in the UK, it may never be possible to guarantee that donor blood is absolutely 'safe'. The current approach to the safety of blood components and plasma in the UK is extremely cautious, but it is not an absolute guarantee of safety. Clinicians should always carefully consider the patient's requirement for transfusion, and only transfuse if clinically appropriate.

### Immunosuppression

Ever since observations were made of the favourable effect of transfusion on survival of subsequent renal allografts, the basis of transfusion-induced immunomodulation has been the subject of debate. It is assumed that allogeneic leucocytes are required to cause transfusion-induced immunosuppression, but the underlying mechanisms remain uncertain. There has been considerable interest in other clinical effects caused by transfusion-induced immunosuppression, such as postoperative infection and tumour recurrence.

Strategies for avoiding or reducing the use of blood transfusion should be used. These include stopping drug therapy that may cause bleeding and treatment of anaemia prior to surgery.

Strict criteria for the use of blood components and blood products must be in place.

Artificial haemoglobin solutions and other blood substitutes are in clinical trial. They have a short intravascular half-life, and are likely to find their initial clinical application in trauma and surgery.

### Autologous transfusion

An alternative to using blood from volunteer donors is to use the patient's own blood. There are three types of autologous transfusion:

- *Predeposit.* The patient donates 2-5 units of blood at approximately weekly intervals before elective surgery.
- *Preoperative haemodilution.* One or two units of blood are removed from the patient immediately before surgery and retransfused to replace operative losses.
- *Blood salvage.* Blood lost during or after surgery may be collected and retransfused. Several techniques of varying levels of sophistication are available. The operative site must be free of bacteria, bowel contents and tumour cells.

There is little demand for pre-deposit autologous transfusion in the UK as blood is generally perceived as being 'safe'. Blood salvage is increasingly being used as a way of avoiding the use of donor blood. In developing countries, autologous blood and blood from relatives are commonly used.

## BLOOD, BLOOD COMPONENTS AND BLOOD PRODUCTS

Most blood collected from donors is processed as follows:

- **Blood components**, such as red cell and platelet concentrates, fresh frozen plasma (FFP) and cryoprecipitate, are prepared from a single donation of blood by simple separation methods such as centrifugation and are transfused without further processing.
- **Blood products**, such as coagulation factor concentrates, albumin and immunoglobulin solutions, are prepared by complex processes using the plasma from many donors as the starting material (UK donor plasma is not used, see above).

In most circumstances it is preferable to transfuse only the blood component or product required by the patient (component therapy) rather than use whole blood. This is the most effective way of using donor blood, which is a scarce resource, and reduces the risk of complications from transfusion of unnecessary components of the blood.

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### **Whole blood**

The average volume of blood withdrawn is 470 mL taken into 63 mL of anticoagulant. Blood stored at 4°C has a 'shelf-life' of 5 weeks when at least 70% of the transfused red cells should survive normally. Whole blood is rarely used; packed cells or red cell concentrates plus crystalloid or colloid solutions are acceptable alternatives even for the management of acute blood loss.

### **Red cell concentrates**

Virtually all the plasma is removed and is replaced by about 100 mL of an optimal additive solution, such as SAG-M, which contains sodium chloride, adenine, glucose and mannitol. The mean volume is about 330 mL. The PCV is about 0.57 L/L, but the viscosity is low as there are no plasma proteins in the additive solution, and this allows fast administration if necessary. All blood components (red cell and platelet concentrates, and plasma) are leucocyte-depleted in the UK by filtration within 48 hours of collection of the donor blood.

### **Washed red cell concentrates**

These are preparations of red cells suspended in saline, produced by cell separators to remove all but traces of plasma proteins. They are used in patients who have had severe recurrent urticarial or anaphylactic reactions.

### **Platelet concentrates**

These are prepared either from whole blood by centrifugation or by plateletpheresis of single donors using cell separators. They may be stored for up to 5 days at 22°C. They are used to treat bleeding in patients with severe thrombocytopenia, and prophylactically to prevent bleeding in patients with bone marrow failure.

### **Granulocyte concentrates**

These are prepared from single donors using cell separators and are used for patients with severe neutropenia with definite evidence of bacterial infection. The numbers of granulocytes are increased by treating donors with G-CSF and steroids.

### **Fresh frozen plasma**

FFP is prepared by freezing the plasma from 1 unit of blood at -30°C within 6 hours of donation. The volume is approximately 200 mL. FFP contains all the coagulation factors present in fresh plasma and is used mostly for replacement of coagulation factors in acquired coagulation factor deficiencies. For children, see p. 462.

### **Cryoprecipitate**

This is obtained by allowing the frozen plasma from a single donation to thaw at 4–8°C and removing the supernatant. The volume is about 20 mL and it is stored at -30°C. It contains factor VIII:C, von Willebrand factor (vWF) and fibrinogen, and may be useful in DIC and other conditions where the fibrinogen level is very low. It is no longer used for the treatment of haemophilia A and von Willebrand's disease because of the greater risk of virus transmission compared with virus-inactivated coagulation factor concentrates.

### **Factor VIII and IX concentrates**

These are freeze-dried preparations of specific coagulation factors prepared from large pools of plasma. They are used for treating patients with haemophilia and von Willebrand's disease, where recombinant coagulation factor concentrates are unavailable. Recombinant coagulation factor concentrates, where they are available, are the treatment of choice for patients with inherited coagulation factor deficiencies (see p. 472).

### **Albumin**

There are two preparations:

- *Human albumin solution 4.5%*, previously called plasma protein fraction (PPF), contains 45 g/L albumin and 160 mmol/L sodium. It is available in 50, 100, 250 and 500 mL bottles.
- *Human albumin solution 20%*, previously called 'salt-poor' albumin, contains approximately 200 g/L albumin and 130 mmol/L sodium and is available in 50 and 100 mL bottles.

Human albumin solutions are generally considered to be inappropriate fluids for acute volume replacement or for the treatment of shock because they are no more effective in these situations than synthetic colloid solutions such as polygelatins (Gelofusine) or hydroxyethyl starch (Haemaccel). However, albumin solutions are indicated for treatment of acute severe hypoalbuminaemia and as the replacement fluid for plasma exchange. The 20% albumin solution is particularly useful for patients with nephrotic syndrome or liver disease who are fluid overloaded and resistant to diuretics. Albumin solutions should not be used to treat patients with malnutrition or chronic renal or liver disease with low serum albumin.

### **Normal immunoglobulin**

This is prepared from normal plasma. It is used in patients with hypogammaglobulinaemia, to prevent infections, and in patients with, e.g. idiopathic thrombocytopenia (p. 470).

### **Specific immunoglobulins**

These are obtained from donors with high titres of antibodies. Many preparations are available, such as anti-D, anti-hepatitis B, and anti-varicella zoster.

## Haematological disease

### FURTHER READING

- Dodd RY (2003) Emerging infections, transfusion safety, and epidemiology. *New England Journal of Medicine* **349**:1205-1206. Goodnough LT, Shander A, Brecher ME (2003) Transfusion medicine: looking to the future. *Lancet* **361**:161-169. Klein HG (2000) The prospect for red cell substitutes. *New England Journal of Medicine* **342**:1666-1668. Murphy MF, Pamphilon D (eds) (2001) *Practical Transfusion Medicine*. Oxford: Blackwell Science. Turner M (1999) The impact of new-variant Creutzfeldt-Jakob disease on blood transfusion practice. *British Journal of Haematology* **106**: 842-850.

## THE WHITE CELL IsoCh.41

The five types of leucocytes found in peripheral blood are neutrophils, eosinophils and basophils (which are all called *granulocytes*) and lymphocytes and monocytes. The development of these cells is shown in Figure 8.1.

### NEUTROPHILS

The earliest morphologically **identifiable** precursors of neutrophils in the bone marrow are myeloblasts, which are large cells constituting up to 3.5% of the nucleated cells in the marrow. The nucleus is large and contains 2-5 nucleoli. The cytoplasm is scanty and contains no granules. Promyelocytes are similar to myeloblasts but have some primary cytoplasmic granules, containing enzymes such as myeloperoxidase. Myelocytes are smaller cells without nucleoli but with more abundant cytoplasm and both primary and secondary granules. Indentation of the nucleus marks the change from myelocyte to metamyelocyte. The mature neutrophil is a smaller cell with a nucleus with 2-5 lobes, with predominantly secondary granules in the cytoplasm which contain lysozyme, collagenase and lactoferrin.

Peripheral blood neutrophils are equally distributed into a circulating pool and a marginating pool lying along the endothelium of blood vessels. In contrast to the prolonged maturation time of about 10 days for neutrophils in the bone marrow, their half-life in the peripheral blood is extremely short, only 6-8 hours. In response to stimuli (e.g. infection, corticosteroid therapy) neutrophils are released into the circulating pool from both the marginating pool and the marrow. Immature white cells are released from the marrow when a rapid response (within hours) occurs in acute infection (described as a 'shift to the left' on a blood film).

#### Function

The prime function of neutrophils is to ingest and kill bacteria, fungi and damaged cells. Neutrophils are attracted to sites of infection or inflammation by chemotaxins. Recognition of foreign or dead material is

aided by coating of particles with immunoglobulin and complement (opsonization) as neutrophils have Fc and C3b receptors (see p. 201). The material is ingested into vacuoles where it is subjected to enzymic destruction, which is either oxygen-dependent with the generation of hydrogen peroxide (myeloperoxidase) or oxygen-independent (lysosomal enzymes and lactoferrin). Leucocyte alkaline phosphatase (LAP) is an enzyme found in leucocytes. It is raised when there is a neutrophilia due to an acute illness. It is also raised in the polycythaemia and myelofibrosis and reduced in CML.

### Neutrophil leucocytosis

A rise in the number of circulating neutrophils to  $> 10 \times 10^9/L$  occurs in bacterial infections or as a result of tissue damage. This may also be seen in pregnancy, during exercise and after corticosteroid administration (Table 8.20). With any tissue necrosis there is a release of various soluble factors, causing a leucocytosis. Interleukin-1 is also released in tissue necrosis and causes a pyrexia. The pyrexia and leucocytosis accompanying a myocardial infarction are a good example of this and may be wrongly attributed to infection.

A *leukaemoid reaction* (an overproduction of white cells, with many immature cells) may occur in severe infections, tuberculosis, malignant infiltration of the bone marrow and occasionally after haemorrhage or haemolysis.

In *leucoerythroblastic anaemia*, nucleated red cells and white cell precursors are found in the peripheral blood. Causes include marrow infiltration with metastatic carcinoma, myelofibrosis, osteopetrosis, myeloma, lymphoma, and occasionally severe haemolytic or megaloblastic anaemia.

### Neutropenia and agranulocytosis

Neutropenia is defined as a circulatory neutrophil count below  $1.5 \times 10^9/L$ . A virtual absence of neutrophils is called agranulocytosis. The causes are given in Table 8.21. It should be noted that black patients may have somewhat lower neutrophil counts. Neutropenia caused by viruses is probably the most common type. Chemotherapy

**Table 8.20 Neutrophil leucocytosis**

- Bacterial infections
- Tissue necrosis, e.g. myocardial infarction, trauma
- Inflammation, e.g. gout, rheumatoid arthritis
- Drugs, e.g. corticosteroids, lithium
- Haematological:
  - myeloproliferative disease
  - leukaemoid reaction
  - leucoerythroblastic anaemia
  - Physiological, e.g. pregnancy, exercise
  - Malignant disease, e.g. bronchial, breast, gastric
  - Metabolic, e.g. renal failure, acidosis
  - Congenital, e.g. leucocyte adhesion deficiency, hereditary neutrophilia

**Table 8.21 Causes of neutropenia**

Acquired
Viral infection
Severe bacterial infection, e.g. typhoid
Felty's syndrome
Immune neutropenia - autoimmune, autoimmune neonatal neutropenia
Pancytopenia from any cause, including drug-induced marrow aplasia (see p. 435)
Pure white cell aplasia
Inherited
Ethnic (neutropenia is common in black races) Kostmann's syndrome (severe infantile agranulocytosis) Cyclical (genetic defect with neutropenia every 2-3 weeks) Others, e.g. Schwachman-Diamond syndrome, dyskeratosis congenita, Chediak-Higashi syndrome

and radiotherapy predictably produce neutropenia; many other drugs have been known to produce an idiosyncratic cytopenia and a drug cause should always be considered.

### Clinical features

Infections may be frequent, often serious, and are more likely as the neutrophil count falls. An absolute neutrophil count of less than  $0.5 \times 10^9/L$  is regarded as 'severe' neutropenia and may be associated with life-threatening infections such as pneumonia and septicaemia. A characteristic glazed mucositis occurs in the mouth, and ulceration is common.

### Investigations

The blood film shows marked neutropenia. The appearance of the bone marrow will indicate whether the neutropenia is due to depressed production or increased destruction of neutrophils. Neutrophil antibody studies are performed if an immune mechanism is suspected.

### Treatment

Antibiotics should be given as necessary to patients with acute severe neutropenia (see p. 495).

If the neutropenia seems likely to have been caused by a drug, all current drug therapy should be stopped. Recovery of the neutrophil count usually occurs after about 10 days. G-CSF (see p. 421) is used to decrease the period of neutropenia after chemotherapy and haemopoietic transplantation. It is also used successfully in the treatment of chronic neutropenia.

Steroids and high-dose intravenous immunoglobulin are used to treat patients with severe autoimmune neutropenia and recurrent infections, and G-CSF has produced responses in some cases.

## EOSINOPHILS^

Eosinophils are slightly larger than neutrophils and are characterized by a nucleus with usually two lobes and large cytoplasmic granules that stain deeply red. The eosinophil plays a part in allergic responses (p. 200) and

**Table 8.22 Causes of eosinophilia**

Parasitic infestations, such as:	Pulmonary disorders, such as:
Ascaris	Bronchial asthma
Hookworm	Tropical pulmonary eosinophilia Allergic bronchopulmonary aspergillosis Churg-Strauss syndrome
Strongyloides	
Allergic disorders, such as:	Malignant disorders, such as:
Hayfever (allergic rhinitis)	Hodgkin's lymphoma
Other hypersensitivity reactions, including drug reactions	Carcinoma Eosinophilic leukaemia
Skin disorders, such as:	Miscellaneous, such as:
Urticaria	Hypereosinophilic syndrome
Pemphigus	Sarcoidosis Hypoadrenalinism
Eczema	Eosinophilic gastroenteritis

in the defence against infections with helminths and protozoa.

Eosinophilia is  $> 0.4 \times 10^9/L$  eosinophils in the peripheral blood. The causes of eosinophilia are listed in Table 8.22.

### BASOPHILS

The nucleus of basophils is similar to that of neutrophils but the cytoplasm is filled with large black granules. The granules contain histamine, heparin and enzymes such as myeloperoxidase. The physiological role of the basophil is not known. Binding of IgE causes the cells to degranulate and release histamine and other contents involved in acute hypersensitivity reactions (p. 220).

Basophils are usually few in number ( $< 1 \times 10^9/L$ ) but are significantly increased in myeloproliferative disorders.

### MONOCYTES

Monocytes are slightly larger than neutrophils. The nucleus has a variable shape and may be round, indented or lobulated. The cytoplasm contains fewer granules than neutrophils. Monocytes are precursors of tissue macrophages and spend only a few hours in the blood but can continue to proliferate in the tissues for many years.

A monocytosis ( $> 0.8 \times 10^9/L$ ) may be seen in chronic bacterial infections such as tuberculosis or infective endocarditis, chronic neutropenia and patients with myelodysplasia, particularly chronic myelomonocytic leukaemia.

### LYMPHOCYTES

Lymphocytes form nearly half the circulating white cells. They descend from pluripotential stem cells (see Fig. 8.1).

# 8

## Haematological disease

Circulating lymphocytes are small cells, a little larger than red cells, with a dark-staining central nucleus. There are two main types: T and B lymphocytes (see p. 205).

Lymphocytosis (lymphocyte count  $> 5 \times 10^9/L$ ) occurs in response to viral infections, particularly EBV, CMV and HIV, and chronic infections such as tuberculosis and toxoplasmosis. It also occurs in chronic lymphocytic leukaemia and in some lymphomas.

### FURTHER READING

Stock W, Hoffman R (2000) White blood cells 1: non-malignant disorders. *Lancet* 355: 1351-1356.

## HAEMOSTASIS AND THROMBOSIS

The integrity of the circulation is maintained by blood flowing through intact vessels lined by endothelial cells. Injury to the vessel wall exposes collagen and together with tissue injury sets in motion a series of events leading to haemostasis.

### HAEMOSTASIS

Haemostasis is a complex process depending on interactions between the vessel wall, platelets and coagulation and fibrinolytic mechanisms. The formation of the haemostatic plug is shown in Figure 8.31.

#### Vessel wall

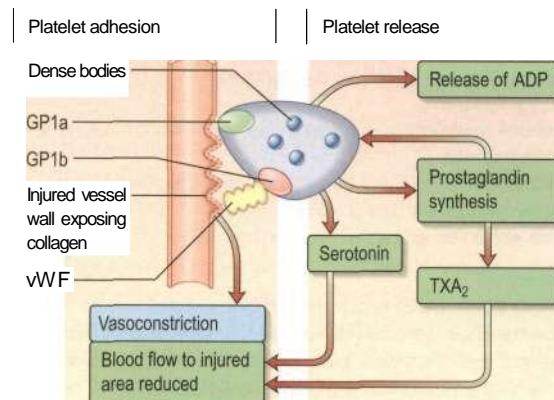
The vessel wall is lined by endothelium which, in normal conditions, prevents platelet adhesion and thrombus formation. This property is partly due to its negative charge but also to:

- thrombomodulin and heparan sulphate expression
- synthesis of prostacyclin ( $\text{PGI}_2$ ) and nitric oxide (NO), which cause vasodilatation and inhibit platelet aggregation
- production of plasminogen activator.

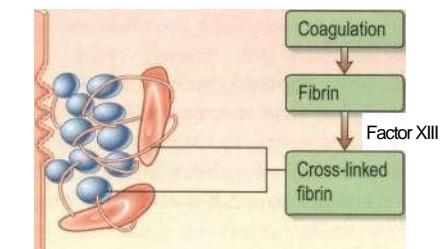
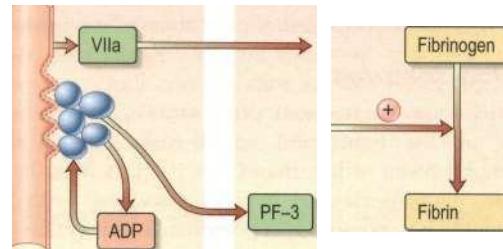
Injury to vessels causes reflex vasoconstriction, while endothelial damage results in loss of antithrombotic properties, activation of platelets and coagulation and inhibition of fibrinolysis (Fig. 8.31).

#### Platelets

*Platelet adhesion* (Fig. 8.31a) to collagen is dependent on platelet membrane receptors: glycoprotein Ia (GPIa), which binds directly to collagen; and glycoprotein Ib (GPIb), which binds to von Willebrand factor (vWF) in the plasma, and vWF in turn adheres to collagen. Following adhesion, platelets undergo a shape change from a disc to a sphere, spread along the subendothelium and release the contents of their cytoplasmic granules, i.e. the dense bodies (containing ADP and serotonin) and the a-granules (containing platelet-derived growth factor, heparin antagonist (platelet factor 4), f5-thromboglobulin, fibrinogen, vWF, fibronectin, thrombospondin and other factors).



#### Platelet aggregation

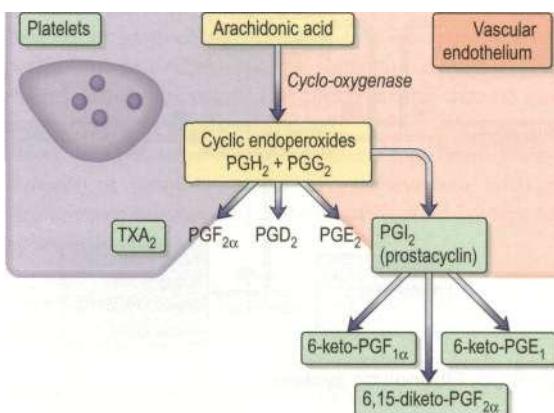


L-

**Fig. 8.31 Formation of the haemostatic plug: sequential interactions between the vessel wall, platelets and coagulation factors.**

- Contact of platelets with collagen, either via the platelet receptor GPIb and factor vWF in plasma, or directly via GPIa, activates platelet prostaglandin synthesis which stimulates release of ADP from the dense bodies. Vasoconstriction of the vessel occurs as a reflex and by release of serotonin and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) from platelets.
- Release of ADP from platelets induces platelet aggregation and formation of the platelet plug. The coagulation pathway is stimulated leading to formation of fibrin.
- Fibrin strands are cross-linked by factor XIII and stabilize the haemostatic plug by binding platelets and red cells. PF-3, platelet factor-3 (platelet phospholipid).

*Platelet release:* the release of ADP leads to a conformational change in the fibrinogen receptor, the glycoprotein IIb-IIIa complex (GPIIb-IIIa), on the surfaces of adherent platelets allowing it to bind to fibrinogen (see also Fig. 8.39).



**Fig. 8.32 Prostaglandin synthesis.**

**Platelet aggregation** (Fig. 8.31b): fibrinogen then binds platelets into activated aggregates (platelet aggregation) and further platelet release occurs. A self-perpetuating cycle of events is set up leading to formation of a platelet plug at the site of the injury.

**Coagulation:** further platelet membrane receptors, e.g. P<sub>2Y</sub><sub>12</sub> are exposed during aggregation, providing a surface for the interaction of coagulation factors; this platelet phospholipid activity is referred to as platelet factor 3 (PF-3). The presence of thrombin encourages fusion of platelets, and fibrin formation reinforces the stability of the platelet plug.

Central to normal platelet function is platelet *prostaglandin synthesis*, which is induced by platelet activation and leads to the formation of TXA<sub>2</sub> in platelets (Fig. 8.32). Thromboxane (TXA<sub>2</sub>) is a powerful vasoconstrictor and also lowers cyclic AMP levels and initiates the platelet release reaction.

Prostacyclin (PGI<sub>2</sub>) is synthesized in vascular endothelial cells and opposes the actions of TXA<sub>2</sub>. It produces vasodilatation and increases the level of cyclic AMP, preventing platelet aggregation on the normal vessel wall as well as limiting the extent of the initial platelet plug after injury.

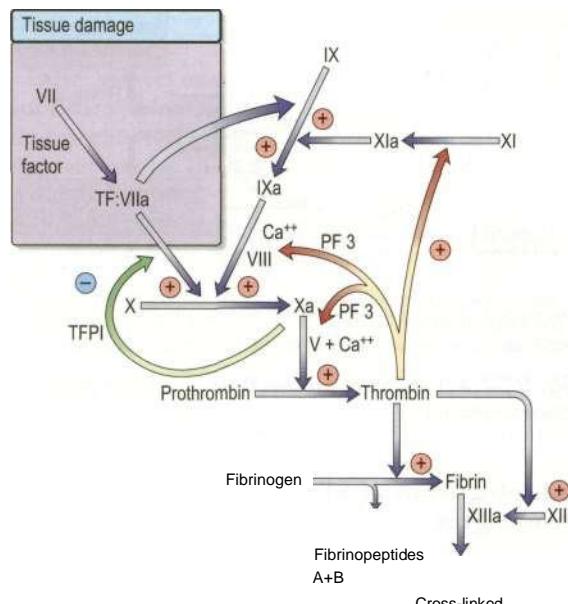
### Coagulation and fibrinolysis

Coagulation involves a series of enzymatic reactions leading to the conversion of soluble plasma fibrinogen to fibrin clot (Fig. 8.33). Roman numerals are used for most of the factors, but I and II are referred to as fibrinogen and prothrombin respectively; III, IV and VI are redundant. The active forms are denoted by 'a'.

The coagulation factors are primarily synthesized in the liver and are either enzyme precursors (factors XII, XI, X, IX and thrombin) or cofactors (V and VIII), except for fibrinogen, which is degraded to form fibrin.

### Coagulation pathway

This enzymatic amplification system is traditionally divided into 'extrinsic' and 'intrinsic' pathways. This concept remains very useful for the interpretation of clinical laboratory tests (see p. 469) but is an over-



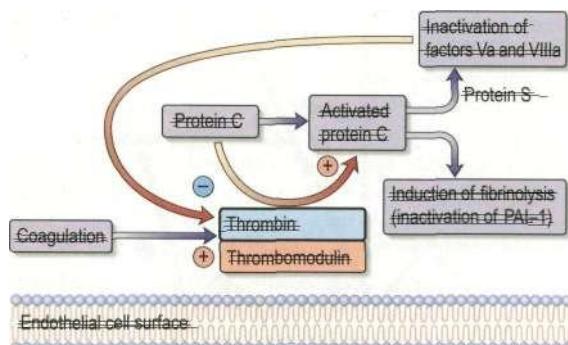
**Fig. 8.33 Coagulation mechanism.** The *in vivo* pathway begins with the activation of factor IX by factor VIIa. Factor XI is activated by thrombin. TFPI, tissue factor pathway inhibitor.

simplification. Coagulation is initiated by binding of activated factor VII (VIIa) in plasma to tissue factor (TF), a glycoprotein which is expressed on the surface of cells which are exposed after injury. The complex of VIIa and tissue factor (TF:VIIa) activates factor X but this reaction is opposed by tissue factor pathway inhibitor (TFPI), and the main role of TF:VIIa *in vivo* is to activate factor IX (Fig. 8.33). Activated factor IX then works with factor VIII to initiate the crucial activation of factor X. (It is this reaction which amplifies the generation of thrombin and which fails in haemophilia A and B. Factor XI is activated *in vivo* by thrombin and makes a limited contribution to haemostasis so that factor XI deficiency results in a rather mild bleeding disorder.)

**Activated factor X** induces the conversion of prothrombin to thrombin. Thrombin hydrolyses the peptide bonds of fibrinogen, releasing fibrinopeptides A and B, and allowing polymerization between fibrinogen molecules to form fibrin. At the same time, thrombin, in the presence of calcium ions, activates factor XIII, which stabilizes the fibrin clot by cross-linking adjacent fibrin molecules. The presence of thrombin helps in the activation of factors XI, V, VIII and XIII (and protein C; see limitation of coagulation below and Fig. 8.34).

**Factor VIII** consists of a molecule with coagulant activity (VIII:C) associated with von Willebrand factor. VWF's function here is to stabilize factor VIII:C and to promote platelet-endothelial interaction. VIII:C is a single-chain protein with a molecular weight of about 350 000.

**Von Willebrand Factor** (vWF) is a glycoprotein with a molecular weight of about 200 000 which readily forms multimers in the circulation with molecular weights of up to  $20 \times 10^6$ . It is synthesized by endothelial cells and megakaryocytes and stored in platelet and granules as well as the endothelial cells. The high-molecular-weight



**Fig. 8.34 Activation of protein C. PAI-1, plasminogen activator inhibitor 1.**

multimeric forms of vWF are the most effective in promoting platelet function (see p. 467 and Fig. 8.37).

#### Limitation of coagulation

**Antithrombin.** Antithrombin (AT), a member of the serine protease inhibitor (serpin) superfamily, is a potent inhibitor of coagulation. It inactivates the serine proteases by forming stable complexes with them, and its action is greatly potentiated by heparin.

**Activated protein C.** This is generated from its vitamin K-dependent precursor by the action of thrombin; thrombin activation of protein C is enhanced when thrombin is bound to thrombomodulin, which is an endothelial cell receptor (Fig. 8.34). Activated protein C destroys factor V and factor VIII, reducing **further thrombin generation**.

**Protein S.** This is a cofactor for protein C which acts by enhancing binding of activated protein C to **the phospholipid surface**. It circulates bound to C4b binding protein but some 30-40% remains unbound and active (free protein S).

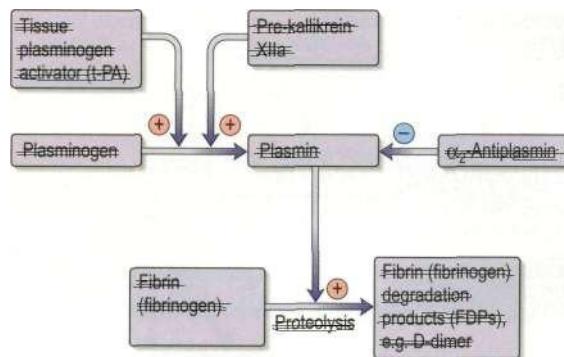
**Other inhibitors.** Other natural inhibitors of coagulation include  $\alpha_2$ -macroglobulin,  $\alpha^1$ -antitrypsin and  $\alpha_2$ -antiplasmin.

#### Fibrinolysis

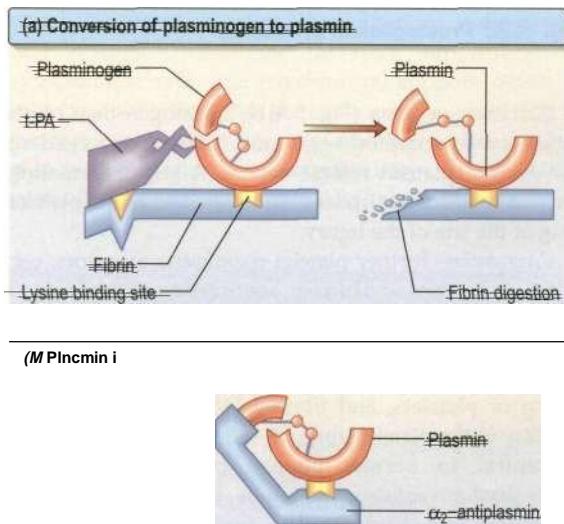
Fibrinolysis, which helps to restore vessel patency, also occurs in response to vascular damage. In this system (Fig. 8.35), an inactive plasma protein - plasminogen - is converted to plasmin by plasminogen activators derived from the plasma or blood cells (intrinsic activation) or the tissues (extrinsic activation).

Plasmin is a serine protease which breaks down fibrinogen and fibrin into fragments X, Y, D and E, collectively known as fibrin (and fibrinogen) degradation products (FDPs). D-dimer is produced when cross-linked fibrin is degraded. Its presence in the plasma indicates that the coagulation mechanism has been activated (see p. 467). Plasmin is also capable of breaking down coagulation factors such as factors V and VIII.

The fibrinolytic system is activated by the presence of fibrin. Plasminogen is specifically adsorbed to fibrin and



**Fig. 8.35 Fibrinolytic system.**



**Fig. 8.36 Fibrinolysis.**

(a) The conversion of plasminogen to plasmin by plasminogen activator (t-PA) occurs most efficiently on the surface of fibrin, which has binding sites for both plasminogen and t-PA.  
 (b) Free plasmin in the blood is rapidly inactivated by  $\alpha_2$ -antiplasmin. Plasmin generated on the fibrin surface is partially protected from inactivation. The lysine-binding sites on plasminogen are necessary for the interaction between plasmin(ogen) and fibrin and between plasmin and  $\alpha_2$ -antiplasmin.

fibrinogen by lysine-binding sites. However, little plasminogen activation occurs in the absence of fibrin, as fibrin also has a specific binding site for plasminogen activators, whereas fibrinogen does not (Fig. 8.36).

The major plasminogen activator is tissue-type plasminogen activator (t-PA); vascular endothelium is the major source of t-PA in plasma. Its release is stimulated by a number of mediators, including thrombin. Another plasminogen activator is urokinase, synthesized in the kidney and released into the urogenital tract. Intrinsic plasminogen activators such as factor XII and pre-kallikrein are of minor physiological importance.

t-PA is inactivated by plasminogen activator inhibitor-1 (PAI-1). Activated protein C inactivates PAI-1 and therefore induces fibrinolysis (Fig. 8.34). Inactivators of plasmin such as  $\alpha_2$ -antiplasmin (Fig. 8.36) and thrombin-

activatable fibrinolysis inhibitor (TAFI) also contribute to the regulation of fibrinolysis.

## Investigation of bleeding disorders

Although the precise diagnosis of a bleeding disorder may depend on laboratory tests, much information may be obtained from the history and physical examination:

- **Is there a generalized haemostatic defect?** Supportive evidence for this includes bleeding from multiple sites, spontaneous bleeding, and excessive bleeding after injury.
- **Is the defect inherited or acquired?** A family history of a bleeding disorder should be sought. Severe inherited defects usually become apparent in infancy, while mild inherited defects may only come to attention later in life, for example with excessive bleeding after surgery, childbirth, dental extractions or trauma. Some defects are revealed by routine coagulation screens which are performed before surgical procedures.
- **Is the bleeding suggestive of a vascular/platelet defect or a coagulation defect?**

*Vascular/platelet bleeding* is characterized by easy bruising and spontaneous bleeding from small vessels. There is often bleeding into the skin. The term purpura includes both petechiae, which are small skin haemorrhages varying from pinpoint size to a few millimetres in diameter and which do not blanch on pressure, and ecchymoses, which are larger areas of bleeding into the skin. Bleeding also occurs from mucous membranes especially the nose and mouth.

*Coagulation disorders* are typically associated with haemarthroses and muscle haematomas, and bleeding after injury or surgery. There is often a short delay between the precipitating event and overt haemorrhage or haematoma formation.

### Laboratory investigations

■ **Blood count and film** show the number and morphology of platelets and any blood disorder such as leukaemia or lymphoma. The normal range for the platelet count is  $150-100 \times 10^9/L$ .

■ **Bleeding time** measures platelet plug formation *in vivo*. It is determined by applying a sphygmomanometer cuff to the arm and inflating it to 40 mmHg. Two 1 mm deep, 1 cm long incisions are made in the forearm with a template. Each wound is blotted every 30 s and the time taken for bleeding to stop is recorded, normally between 3 and 10 minutes. Prolonged bleeding times are found in patients with platelet function defects, and there is a progressive prolongation with platelet counts less than  $80 \times 10^9/L$ . The bleeding time should not be performed at low platelet counts.

■ **Coagulation tests** are performed using blood collected into citrate, which neutralizes calcium ions and prevents clotting.

The *prothrombin time* (PT) (also see p. 480) is measured by adding tissue thromboplastin in the form of animal brain

extract, or a recombinant equivalent, and calcium to the patient's plasma ('extrinsic' system). The normal PT is 16-18 s, and it is prolonged with abnormalities of factors VII, X, V, II or I, liver disease, or if the patient is on warfarin.

The *activated partial thromboplastin time* (APTT) is also sometimes known as the PTT with kaolin (PTTK). It is performed by adding a surface activator (such as kaolin), phospholipid (as platelet substitute) and calcium to the patient's plasma ('intrinsic' system). The normal APTT is 30-50 s depending on the exact methodology, and it is prolonged with deficiencies or inhibitors of one or more of the following factors: XII, XI, IX, VIII, X, V, II or I (but not factor VII) (see Fig. 8.33).

The *thrombin time* (TT) is performed by adding thrombin to the patient's plasma. The normal TT is about 12 s, and it is prolonged with fibrinogen deficiency, dysfibrinogenaemia (normal level of fibrinogen but abnormal function) or inhibitors such as heparin or FDPs.

*Correction tests* can be used to differentiate prolonged times in the PT, APTT and TT due to various coagulation factor deficiencies and inhibitors of coagulation. Prolonged PT, APTT or TT because of coagulation factor deficiencies are corrected by addition of normal plasma to the patient's plasma; no correction of an abnormal result after the addition of normal plasma is suggestive of the presence of an inhibitor of coagulation.

*Factor assays* are used to confirm coagulation defects, especially where a single inherited disorder is suspected.

*Special tests of coagulation* will often be required to confirm the precise haemostatic defect. Such tests include estimation of fibrinogen and FDPs, platelet function tests such as platelet aggregation and tests of the fibrinolytic pathway which include the euglobulin clot lysis time (ELT) and assays of plasminogen, t-PA and PAI-1. The ELT involves precipitation by acidification of the euglobulin fraction of plasma, which contains fibrinogen, plasminogen and plasminogen activators but excluding  $\alpha_2$ -antiplasmin. The euglobulin fraction is clotted with thrombin and the time taken for lysis of the fibrin clot is a measure of fibrinolytic activity; the normal range is 60-270 minutes. Factor XIII can be estimated by a clot stability screening test or by direct assay.

## VASCULAR DISORDERS

The vascular disorders (Table 8.23) are characterized by easy bruising and bleeding into the skin. Bleeding from mucous membranes sometimes occurs but the bleeding is rarely severe. Laboratory investigations including the bleeding time are normal. The vascular disorders include the following.

*Hereditary haemorrhagic telangiectasia* is a rare disorder with autosomal dominant inheritance. Dilatation of capillaries and small arterioles produces characteristic small red spots that blanch on pressure in the skin and mucous membranes, particularly the nose and gastrointestinal tract. Recurrent epistaxis and chronic gastrointestinal bleeding are the major problems and may cause chronic iron deficiency anaemia.

**Table 8.23 Vascular disorders**

Congenital	Allergic
Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu disease)	Henoch-Schonlein purpura
Connective tissue disorders (Ehlers-Danlos syndrome, osteogenesis imperfecta, pseudoxanthoma elasticum, Marfan's syndrome)	<b>Drugs</b>
<b>Acquired</b>	Steroids
Severe infections: septicaemia meningococcal infections measles typhoid	Sulphonamides
	<b>Others</b>
	Senile purpura
	Easy bruising syndrome
	Scurvy
	Factitious purpura

*Easy bruising syndrome* is a common benign disorder occurring in otherwise healthy women. It is characterized by bruises on the arms, legs and trunk with minor trauma, possibly because of skin vessel fragility. It may give rise to the suspicion of a serious bleeding disorder.

*Senile purpura and purpura due to steroids* are both due to atrophy of the vascular supporting tissue.

*Purpura due to infections* is mainly caused by damage to the vascular endothelium. The rash of meningococcal septicaemia is particularly characteristic (p. 75).

*Henoch-Schonlein purpura* (p. 629) occurs mainly in children. It is a type III hypersensitivity reaction that is often preceded by an acute upper respiratory tract infection. Purpura is mainly seen on the legs and buttocks. Abdominal pain, arthritis, haematuria and glomerulonephritis also occur. Recovery is usually spontaneous, but some patients develop renal failure.

*Episodes of inexplicable bleeding or bruising* may represent abuse, either self-inflicted or caused by others. These various forms of artificial or factitious purpura are expressions of severe emotional or psychiatric disturbances.

## PLATELET DISORDERS

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Bleeding due to thrombocytopenia or abnormal platelet function is characterized by purpura and bleeding from mucous membranes. Bleeding is uncommon with platelet counts above  $50 \times 10^9/L$ , and severe spontaneous bleeding is unusual with platelet counts above  $20 \times 10^9/L$

(Table 8.24). ■■■, ■■■, M, V, ■■■, ■■■, ■■■

## Thrombocytopenia

This is caused by reduced platelet production in the bone marrow or excessive peripheral destruction of platelets (Table 8.25). The underlying cause may be revealed by history and examination but a bone marrow examination will show whether the numbers of megakaryocytes are reduced, normal or increased, and will provide essential information on morphology. Specific laboratory tests may be useful to confirm the presence of such conditions as paroxysmal nocturnal haemoglobinuria (PNH) or systemic lupus erythematosus (SLE).

**Table 8.24 Clinical effects caused by different levels of platelet count**

Platelet count ( $\times 10^9/L$ )	Clinical defect
>500	Haemorrhage or thrombosis
500-100	No clinical effect
100-50	Moderate haemorrhage after injury
<b>50-20</b>	Purpura may occur
<b>&lt;20</b>	Haemorrhage after injury
	Purpura common Spontaneous haemorrhage from mucous membranes
	<b>Impaired production</b>
	Intracranial haemorrhage (rare)

By permission of Colvin BT (2004) *Medicine* 32(5): 27-33  
Bone marrow failure

**Table 8.25 Causes of thrombocytopenia**

Megaloblastic anaemia	<b>Excessive destruction</b>
Leukaemia	Immune
Myeloma	ITP
Myelofibrosis	Secondary immune (SLE, CLL, viruses, drugs, e.g. heparin) Alloimmune
Solid tumour infiltration	neonatal
Aplastic anaemia:	thrombocytopenia Post-transfusion purpura
drugs	
chemicals	
viruses	
paroxysmal nocturnal haemoglobinuria	<b>Sequestration</b>
	Hypersplenism
	<b>Dilutional</b>
	Massive transfusion
	<b>Other</b>
	Disseminated intravascular coagulation
	Thrombotic
	thrombocytopenic purpura

In patients with thrombocytopenia due to failure of production, no specific treatment may be necessary but the underlying condition should be treated if possible. Where the platelet count is very low or the risk of Weeding is very high, then platelet concentrate administration is indicated.

### Idiopathic thrombocytopenic purpura (ITP)

Thrombocytopenia is due to immune destruction of platelets. The antibody-coated platelets are removed following binding to Fc receptors on macrophages.

#### ITP in children

The condition is usually acute but self-limiting and may follow a viral infection or immunization. Bone marrow examination is not usually performed unless treatment becomes necessary on clinical grounds.

#### ITP in adults

The presentation is usually less acute than in children. Adult ITP is characteristically seen in women and may be associated with other autoimmune disorders such as SLE, thyroid disease and autoimmune haemolytic anaemia (Evans' syndrome), in patients with chronic lymphocytic

leukaemia and solid tumours, and after infections with viruses such as HIV. Platelet autoantibodies are detected in about 60-70% of patients, and are presumed to be present, although not detectable, in the remaining patients; the antibodies often have specificity for platelet membrane glycoproteins lib/Ilia and/ or Ib.

### Clinical features

Major haemorrhage is rare and is seen only in patients with severe thrombocytopenia. Easy bruising, purpura, epistaxis and menorrhagia are common. Physical examination is normal except for evidence of bleeding. Splenomegaly is rare.

### Investigation

The only blood count abnormality is thrombocytopenia. Normal or increased numbers of megakaryocytes are found in the bone marrow (if examination is performed), which is otherwise normal. The detection of platelet autoantibodies is not essential for confirmation of the diagnosis, which often depends on exclusion of other causes of excessive destruction of platelets.

### Treatment

#### Children

Children do not usually require treatment. Where this is necessary on clinical grounds, high-dose prednisolone is effective, given for a very short course. Intravenous immunoglobulin (i.v. IgG) should be reserved for very serious bleeding or urgent surgery. Chronic ITP is rare and requires specialist management, avoiding cytotoxic agents.

#### Adults

Patients with platelet counts greater than  $30 \times 10^9/L$  require no treatment unless they are about to undergo a surgical procedure.

*First-line therapy* consists of oral corticosteroids 1 mg/kg body weight but i.v. IgG is useful where a rapid rise in platelet count is desired, especially before surgery. There are also advocates for high-dose corticosteroids as initial therapy.

*Second-line therapy* involves splenectomy, to which the majority of patients respond, but a wide range of treatments is available in chronic ITP. These include high-dose corticosteroids, high-dose i.v. IgG, intravenous anti-D, vinca alkaloids, danazol, immunosuppressive agents such as azathioprine, ciclosporine and dapsone. There is also interest in the use of specific monoclonal antibodies such as rituximab, as well as recombinant thrombopoietin.

Platelet transfusions are reserved for intracranial or other extreme haemorrhage, where emergency splenectomy may be justified.

### Other immune thrombocytopenias

Drugs cause immune thrombocytopenia by the same mechanisms as described for drug-induced immune haemolytic anaemia (p. 450). The same drugs can be responsible for immune haemolytic anaemia, thrombocytopenia or neutropenia in different patients.

*Heparin-induced thrombocytopenia.* See page 480.

*Neonatal alloimmune thrombocytopenia* is due to foeto-maternal incompatibility for platelet-specific antigens, usually for HPA-1a (human platelet alloantigen) and is the platelet equivalent of haemolytic disease of the newborn (HDN). The mother is HPA-1a-negative and produces antibodies which destroy the HPA-1a-positive fetal platelets.

Thrombocytopenia is self-limiting after delivery, but platelet transfusions may be required initially to prevent or treat bleeding associated with severe thrombocytopenia; platelets are prepared from HPA-1a-negative volunteers or the mother herself. Severe bleeding such as intracranial haemorrhage may also occur in utero. Antenatal treatment of the mother - with platelet transfusions given directly to the fetus by ultrasound-guided needling of the umbilical vessels - has been effective in preventing haemorrhage in severely affected cases.

*Post-transfusion purpura* (PTP) is rare, occurring 2-12 days after a blood transfusion. PTP is associated with a platelet-specific alloantibody, usually anti-HPA-1a in an HPA-1a-negative individual. PTP almost invariably occurs in females who have been previously immunized by pregnancy or blood transfusion. The cause of the destruction of the patient's own platelets is not well understood, but they may be destroyed as 'bystanders' during the acute immune response to HPA-1a. PTP is self-limiting, but high-dose intravenous immunoglobulin may limit the period of thrombocytopenia.

### Thrombotic thrombocytopenic purpura (TTP) (p. 636)

TTP is a rare, but very serious condition, in which platelet destruction leads to profound thrombocytopenia. There is a characteristic symptom complex of florid purpura, fever, fluctuating cerebral dysfunction and haemolytic anaemia with red cell fragmentation, often accompanied by renal failure. The coagulation screen is usually normal but lactic dehydrogenase (LDH) levels are markedly raised as a result of haemolysis.

The underlying cause is not fully understood but TTP seems to be due to endothelial damage associated with the presence in the circulation of very-high-molecular-weight multimers of von Willebrand factor (vWF) which accumulate owing to the absence of a protease which is normally responsible for vWF degradation. This absence is due to mutations in the *ADAMTS 13* gene. In some cases there is a true deficiency of the protease, while in others an immune response appears to reduce protease activity temporarily. TTP is associated with pregnancy, oral contraceptives, systemic lupus erythematosus, infection and drug treatment, including the use of ticlopidine and clopidogrel, but many cases have no obvious cause.

*Treatment* consists of plasma exchange, using cryoprecipitate-depleted FFP (cryo-poor supernatant) or solvent detergent-treated FFP, both of which contain reduced amounts of high-molecular-weight vWF multimers. It is also thought that FFP supplies the missing I

protease. Most patients are also treated with prednisone 1 mg/kg daily and low-dose aspirin 75 mg daily is often given as the platelet count rises above  $50 \times 10^9/\text{L}$ . Disease activity is monitored by measuring the platelet count and serum LDH. Platelet concentrates are contraindicated.

The untreated condition has a mortality of up to 90% but modern management has reduced this figure to about 10%. Recurrent and relapsing TTP occurs, often associated with a persistent lack of vWF protease.

### **Platelet function disorders**

These are usually associated with excessive bruising and bleeding and, in some of the acquired forms, with thrombosis. The platelet count is normal or increased and the bleeding time is prolonged. The rare inherited defects of platelet function require more detailed investigations such as platelet aggregation studies and factor VIII:C and vWF assays, if von Willebrand's disease is suspected.

#### **Inherited types of platelet dysfunction**

- *Glanzmann's thrombasthenia* - lack of the platelet membrane glycoprotein lib/Ilia complex resulting in defective fibrinogen binding and failure of platelet aggregation.
- *Bernard-Soulier syndrome* - lack of platelet membrane glycoprotein Ib, the binding site for factor vWF. This causes a failure of platelet adhesion and moderate thrombocytopenia.
- *Storage pool disease* - lack of the storage pool of platelet dense bodies, causing poor platelet function.

#### **Acquired types of platelet dysfunction**

- Myeloproliferative disorders
- Renal and liver disease
- Paraproteinaemias
- Drug-induced, such as by aspirin or other platelet inhibitory drugs.

If there is serious bleeding or if the patient is about to undergo surgery, drugs with antiplatelet activity should be withdrawn and any underlying condition should be corrected if possible. In patients with renal failure, the haematocrit should be increased to greater than 0.30 and the use of desmopressin may be helpful. Platelet transfusions may be required if these measures are unsuccessful or if the risk of bleeding is high.

### **Thrombocytosis**

The platelet count may rise above  $400 \times 10^9/\text{L}$  as a result of:

- splenectomy
- Hodgkin's lymphoma and other malignancies
- inflammatory disorders such as rheumatoid arthritis, ulcerative colitis and Crohn's disease
- major surgery.

Thus thrombocytosis is part of the acute-phase reaction, although following splenectomy platelet numbers are

also elevated because of the loss of a major site of platelet destruction.

*Essential thrombocythaemia*, a myeloproliferative disorder which is described on page 455, and other myeloproliferative conditions such as polycythaemia vera (PV) and chronic myeloid leukaemia (CML) may also be associated with a high platelet count.

A persistently elevated platelet count can lead to arterial or venous thrombosis. It is usual to treat the underlying cause of the thrombocytosis but a small dose of aspirin (75 mg) is also sometimes given. In myeloproliferative disease there is also a paradoxical risk of abnormal bleeding and specific action to reduce the platelet count, usually with hydroxycarbamide (hydroxyurea) is often taken.

### **INHERITED COAGULATION DISORDERS**

Coagulation disorders may be inherited or acquired. The inherited disorders are uncommon and usually involve deficiency of one factor only. The acquired disorders occur more frequently and almost always involve several coagulation factors; they are considered in the next subsection.

In inherited coagulation disorders, deficiencies of all factors have been described. Those leading to abnormal bleeding are rare, apart from haemophilia A (factor VIII deficiency), haemophilia B (factor IX deficiency) and von Willebrand's disease.

### **Haemophilia A**

In haemophilia A, the level of factor VIII:C is reduced but the level of factor vWF is normal (see Fig. 8.37). The prevalence of haemophilia A is about 1 in 5000 of the male population. It is inherited as an X-linked disorder. If a female carrier has a son, he has a 50% chance of having haemophilia, and a daughter has a 50% chance of being a carrier. All daughters of men with haemophilia are carriers and the sons are normal.

The human factor VIII gene is enormous, constituting about 0.1% of the X chromosome, encompassing 186 kilobases of DNA. Various genetic defects have been found, including deletions, duplications, frameshift mutations and insertions. In approximately 50% of families with severe disease, the defect is an inversion. There is a high mutation rate, with one-third of cases being apparently sporadic with no family history of haemophilia.

#### **Clinical and laboratory features**

The clinical features depend on the level of factor VIII:C.

- *Levels of less than 1%* are associated with frequent spontaneous bleeding from early life. Haemarthroses are common and may lead to joint deformity and crippling if adequate treatment is not given. Bleeding into muscles is also common, and intramuscular injections should be avoided.

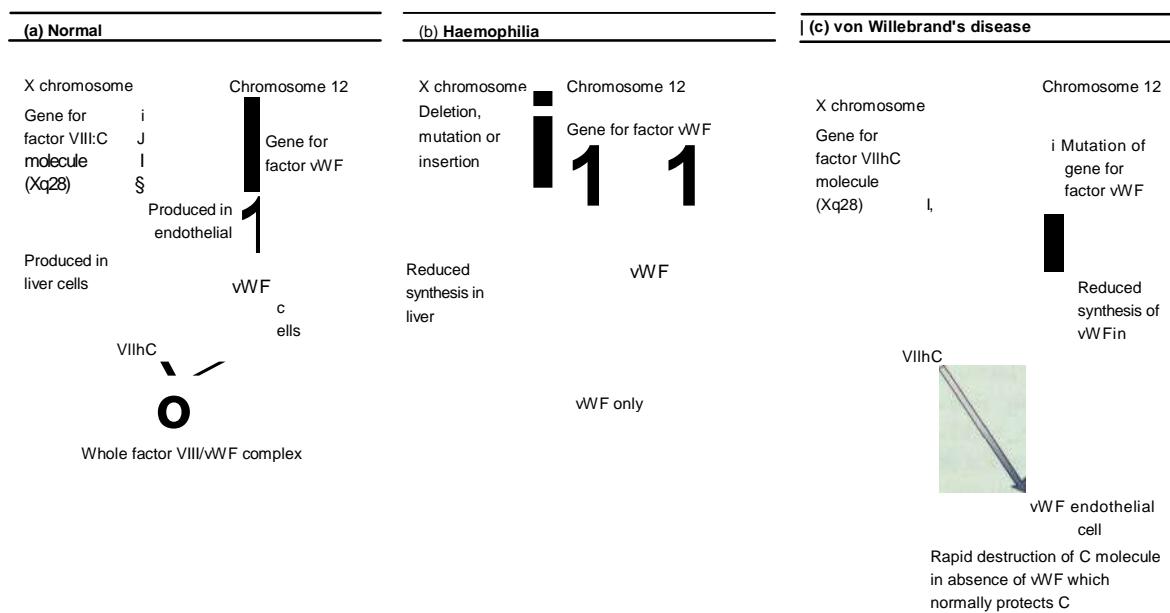


Fig. 8.37 (a) Normal factor VIII synthesis, (b) Haemophilia A showing defective synthesis of factor VIIIC. (c) von Willebrand's disease showing reduced synthesis of vWF.

- *Levels of 1-5% are associated with severe bleeding following injury and occasional apparently spontaneous episodes.*
- *Levels above 5% produce mild disease, usually with bleeding only after injury or surgery. It should be noted that patients with mild haemophilia can still bleed badly once haemostasis has failed.*
- *Diagnosis in this group is often delayed until quite late in life.*

The most common causes of death in people with haemophilia are cancer and heart disease, as for the general population. Cerebral haemorrhage is much more frequent however, and in recent years, HIV infection and liver disease (due to hepatitis C) have become a more common cause of death.

The main laboratory features of haemophilia A are shown in Table 8.26. The abnormal findings are a prolonged APTT and a reduced level of factor VIIIC. The PT, bleeding time and vWF level are normal.

### Treatment

Bleeding is treated by administration of factor VIII concentrate by intravenous infusion.

- *Minor bleeding:* the factor VIII:C level should be raised to 20-30%.

PT	Normal	Normal	t
APTT	T+	t±	T
VIIIC	4++	i	Normal
vWF	Normal	i	Normal

Table 8.26 Blood changes in haemophilia A, von Willebrand's disease and vitamin K deficiency

	Haemophilia A	von Willebrand's disease	Vitamin K deficiency
Bleeding time	Normal	t	Normal

- *Severe bleeding*: the factor VIII:C should be raised to at least 50%.
- *Major surgery*: the factor VIII:C should be raised to 100% preoperatively and maintained above 50% until healing has occurred.

Factor VIII has a half-life of 12 hours and therefore must be administered at least twice daily to maintain the required therapeutic level. Continuous infusion is sometimes used to cover surgery. Factor VIII concentrate is freeze-dried and may be stored in domestic refrigerators at 4°C. This allows it to be administered by the patient immediately after bleeding has started, reducing the likelihood of chronic damage to joints and the need for inpatient care.

Recombinant factor VIII concentrate is well established as the treatment of choice for people with haemophilia, but economic constraints and limited production capacity for recombinant factors

have resulted in many previously treated patients still being offered treatment with plasma-derived concentrates, particularly in developing countries.

The majority of severely affected patients are given prophylaxis three times per week from early childhood in an attempt to prevent permanent joint damage.

*Synthetic vasopressin* (Desmopressin - an analogue of vasopressin) - intravenous, subcutaneous or intranasal - produces a rise in factor VIII:C proportional to the initial level of factor VIII. It avoids the complications associated with blood products and is useful for treating bleeding episodes in mild haemophilia and as prophylaxis before minor surgery. It is ineffective in severe haemophilia.

People with haemophilia should be registered at comprehensive care centres (CCC), which take responsibility for their full medical care, including social and psychological support. Each person with haemophilia carries a special medical card giving details of the disorder and its treatment.

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### Complications

Up to 30% of people with severe haemophilia will develop antibodies to factor VIII:C during their lifetime, usually after the first few treatment doses of factor VIII. The prevalence of antibodies in the UK haemophilia population is 5-10% because they uncommonly develop in more mildly affected patients and often disappear spontaneously or with continued treatment.

Management of such patients may be very difficult, and even extremely high doses of factor VIII may not produce a rise in the plasma level of factor VIII:C. Purified porcine factor VIII may not cross-react with the patient's antibody. Some factor IX concentrates contain activated factors, which may 'bypass' the inhibitor and stop the bleeding. Recombinant factor VIIa also has this bypassing potential and shows great promise as an agent for treating patients with inhibitors. There is a growing interest in immune tolerance induction, especially in the management of recently developed inhibitors in young people. Similar strategies, including immunosuppression and immunoabsorption, have been described.

The risk of viral transmission has been virtually eliminated in developed countries by excluding high-risk blood donors, testing all donations for HBsAg, HCV and HIV antibodies, and by including steps to inactivate viruses during the preparation of plasma-derived concentrate.

Hepatitis A and B vaccination is offered routinely to all patients with haemophilia and von Willebrand's disease. The clinical consequences of haemophilia patients infected with HIV are similar to other HIV-infected patients (see p. 132), except that Kaposi's sarcoma does not occur. A number of patients with hepatitis C will progress to develop chronic liver disease and cirrhosis (see p. 372).

The use of recombinant factor VIII eliminates any residual risk of transfusion-transmitted infection, and it is safe and effective; but there is a similar incidence of inhibitor development as with plasma-derived factor VIII.

### Carrier detection and antenatal diagnosis

Determination of carrier status in females begins with a family history and coagulation factor assays. Female carriers usually have a factor VIII level of about 50% of normal, but the exact value is very variable, partly because of lyonization. Owing to this process early in embryonic life (that is, random inactivation of one chromosome; see p. 172), some carriers have very low levels of factor VIII while others will have normal levels. Carriers could be diagnosed with reasonable confidence if the level of factor VIII:C was 50% or less of that expected from the level of factor vWF measured at the same time, but often no clear-cut answer was provided by this method. Carrier detection can be carried out using molecular genetic testing, either by direct detection of mutations within the factor VIII gene or by tracking of the abnormal gene using DNA polymorphisms as markers.

Antenatal diagnosis may be carried out by molecular analysis of fetal tissue obtained by chorionic villus biopsy at 11-12 weeks' gestation.

### **Haemophilia B (Christmas disease)**

Haemophilia B is caused by a deficiency of factor IX. The inheritance and clinical features are identical to haemophilia A, but the incidence is only about 1 in 30 000 males. The gene is smaller at 34 kilobases and the half-life of the factor is longer at 18 hours. Haemophilia B is treated with factor IX concentrates, recombinant factor IX now being available, and prophylactic doses are given twice a week. Desmopressin is ineffective.

### **Von Willebrand's disease (vWD)**

In vWD, there is defective platelet function as well as factor VIII:C deficiency, and both are due to a deficiency or abnormality of vWF (see Fig. 8.37). vWF plays a role in platelet adhesion to damaged subendothelium as well as stabilizing factor VIII:C in plasma (see p. 467).

The vWF gene is located on chromosome 12 and numerous mutations of the gene have been identified. vWD has been classified into three types:

- Type 1 is characterized by a mild reduction in vWF and is usually inherited as an autosomal dominant.
- Type 2 is due to a decrease in the proportion of high-molecular-weight multimers, and it too is usually inherited as an autosomal dominant.
- Type 3 is recessively inherited and patients have barely detectable levels of factor vWF (and therefore factor VIII:C). Their parents are often phenotypically normal.

Many subtypes have also been described, such as type 2B where increased vWF avidity for platelets causes mild thrombocytopenia, and type 2N where there is an abnormal vWF binding site for VIII:C.

**Clinical features.** These are variable. Type 1 and type 2 patients usually have mild clinical features. Bleeding follows minor trauma or surgery, and epistaxis and menorrhagia often occur. Haemarthroses are rare. Type 3 patients have more severe bleeding but rarely experience the joint and muscle bleeds seen in haemophilia A.

Characteristic laboratory findings are shown in Table 8.26. These also include defective platelet aggregation with ristocetin.

**Treatment** depends on the severity of the condition and may be similar to that of mild haemophilia, including the use of Desmopressin where possible. Intermediate purity factor VIII or von Willebrand factor concentrates should be used to treat bleeding or to cover surgery in patients who require replacement therapy, especially in type 3

(severe disease. Cryoprecipitate should be avoided  
because of the greater risk of transfusion-

transmitted infection, since cryoprecipitate is not a  
virally inactivated product.

## **ACQUIRED COAGULATION DISORDERS**

### **Vitamin K deficiency (see also p. 242)**

Vitamin K is necessary for the  $\gamma$ -carboxylation of glutamic acid residues on coagulation factors II, VII, IX and X and on proteins C and S. Without it, these factors cannot bind calcium.

Deficiency of vitamin K may be due to:

- *inadequate stores*, as in haemorrhagic disease of the newborn and severe malnutrition (especially when combined with antibiotic treatment) (see p. 352)
- *malabsorption of vitamin K*, a fat-soluble vitamin, which occurs in cholestatic jaundice owing to the lack of intraluminal bile salts
- *oral anticoagulant drugs*, which are vitamin K antagonists.

The PT and APTT are prolonged (see Table 8.26) and there may be bruising, haematuria and gastrointestinal or cerebral bleeding. Minor bleeding is treated with phytomenadione (vitamin K<sub>1</sub>) 10 mg intravenously. Some correction of the PT is usual within 6 hours but it may not return to normal for 2 days.

Newborn babies have low levels of vitamin K, and this may cause minor bleeding in the first week of life (*classical haemorrhagic disease of the newborn*). Vitamin K deficiency may also cause *late haemorrhagic disease of the newborn*, which occurs 2–26 weeks after birth and may result in severe bleeding such as intracranial haemorrhage. Most infants with these syndromes have been exclusively breast-fed, and both conditions may be prevented by administering 1 mg i.m. vitamin K to all neonates (p. 242). Concerns about the safety of this are unfounded.

## **Liver disease**

Liver disease may result in a number of defects in haemostasis:

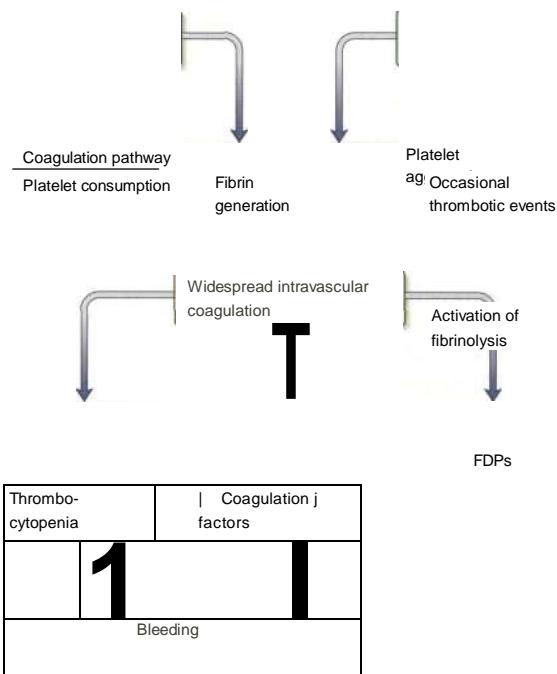
- *Vitamin K deficiency*. This occurs owing to intrahepatic or extrahepatic cholestasis.
- *Reduced synthesis*. Reduced synthesis of coagulation factors may be the result of severe hepatocellular damage. The use of vitamin K does not improve the results of abnormal coagulation tests, but it is generally given to ensure that a treatable cause of failure of haemostasis has not been missed.
- *Thrombocytopenia*. This results from hypersplenism due to splenomegaly associated with portal hypertension or from folic acid deficiency.
- *Functional abnormalities*. Functional abnormalities

of platelets and fibrinogen are found in many patients with liver failure.

- *Disseminated intravascular coagulation*. DIC (see below) may occur in acute liver failure.

### **Disseminated intravascular coagulation (DIC)**

There is widespread generation of **fibrin within** blood vessels, owing to activation of coagulation by release of



**Fig. 8.38 Disseminated intravascular coagulation.**  
FDR fibrin degradation products.

procoagulant material, and by diffuse endothelial damage or generalized platelet aggregation. Activation of leucocytes, particularly monocytes causing expression of tissue factor and the release of cytokines, may play a role in the development of DIC.

There is consumption of platelets and coagulation factors and secondary activation of

fibrinolysis leading to production of fibrin degradation products (FDPs), which may contribute to the coagulation defect by inhibiting fibrin polymerization (Fig. 8.38). The consequences of these changes are a mixture of initial thrombosis followed by a bleeding tendency due to consumption of coagulation factors and fibrinolytic activation.

### Causes of DIC

These include:

- malignant disease
- septicaemia (e.g. Gram-negative and meningococcal)
- haemolytic transfusion reactions
- obstetric causes (e.g. abruptio placentae, amniotic fluid embolism)
- trauma, burns, surgery
- other infections (e.g. falciparum malaria)
- liver disease
- snake bite.

### Clinical features

The underlying disorder is usually obvious. The patient is often acutely ill and shocked. The clinical presentation of DIC varies from no bleeding at all to profound haemostatic failure with widespread haemorrhage. Bleeding may occur from the mouth, nose and venepuncture sites and there may be widespread ecchymoses.

Thrombotic events may occur as a result of vessel occlusion by fibrin and platelets. Any organ may be

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## Haematological disease

involved, but the skin, brain and kidneys are most often affected.

### Investigations

The diagnosis is often suggested by the underlying condition of the patient.

#### Severe cases with haemorrhage

- The PT, APTT and TT are usually very prolonged and the fibrinogen level markedly reduced.
- High levels of FDPs, including D-dimer are found owing to the intense fibrinolytic activity stimulated by the presence of fibrin in the circulation.
- There is severe thrombocytopenia.
- The blood film may show fragmented red blood cells.

#### Mild cases without bleeding

- Increased synthesis of coagulation factors and platelets
- Normal PT, APTT, TT and platelet counts
- FDPs are raised.

### Treatment

The underlying condition is treated and this may be all that is necessary in patients who are not bleeding. Maintenance of blood volume and tissue perfusion is essential. Transfusions of platelet concentrates, FFP, cryoprecipitate and red cell concentrates is indicated in patients who are bleeding. The use of heparin to prevent intravascular coagulation is rarely indicated. Inhibitors of fibrinolysis such as tranexamic acid should not be used in DIC as dangerous fibrin deposition may result. Antithrombin and/or activated protein C concentrates have been used in selected cases.

### Excessive fibrinolysis

Excessive fibrinolysis may occur during surgery involving tumours of the prostate, breast, pancreas and uterus owing to release of tissue plasminogen activators.

Primary hyperfibrinolysis is very rare but activation of fibrinolysis occurs in DIC as a secondary event in response to intravascular deposition of fibrin.

The clinical picture is similar to DIC with widespread bleeding. Laboratory investigations are also similar with a prolonged PT, APTT and TT, a low fibrinogen level, and increased FDPs, although fragmented red cells and thrombocytopenia are not seen, since disseminated coagulation is not present.

If the diagnosis is certain, fibrinolytic inhibitors such as e-aminocaproic acid (EACA) or tranexamic acid should be considered. If DIC cannot be excluded, it is safer to treat as for DIC.

### Massive transfusion

Few platelets and reduced levels of factors V and VIII are

found in stored blood, although there are adequate amounts of the other coagulation factors. During massive transfusion (defined as transfusion of a volume of blood equal to the patient's own blood volume within 24 hours,

e.g. approximately 10 units in an adult), the platelet count and PT and APTT should be checked at intervals. Transfusion of platelet concentrates and FFP should be given if thrombocytopenia or defective coagulation are thought to be contributing to continued blood loss. Other problems of massive transfusion are described on page 974.

### **Inhibitors of coagulation**

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In addition to the factor VIII:C alloantibodies that are found in 5-10% of people with severe haemophilia A, factor VIIIC autoantibodies arise occasionally in patients with autoimmune disorders such as SLE, in elderly patients, and sometimes after childbirth. There can be severe bleeding. The antibodies sometimes disappear spontaneously, but treatment with plasma exchange, high-dose intravenous immunoglobulin and immuno-suppressive drugs may be required in addition to any replacement therapy with factor concentrates (see above). Lupus anticoagulant antibodies (p. 534) are autoantibodies directed against phospholipids (anti-phospholipid antibodies). They are found in about 10% of patients with SLE and also occur in otherwise healthy individuals. They lead to prolongation of phospholipid-dependent coagulation tests, particularly the APTT, but do not inhibit coagulation factor activity. Bleeding does not occur unless there is coexistent severe thrombocytopenia. The main clinical problems are thrombosis and recurrent

miscarriages (p. 577).

### **FURTHER READING**

Bolton-Maggs PHB, Pasi KJ (2003) Haemophilias A and B. *Lancet* 361:1801-1809. Levi M, ten Cate H (1999) Disseminated intravascular coagulation. *New England Journal of Medicine* 341: 586-592. Moake JL (2002) Thrombotic microangiopathies. *New England Journal of Medicine* 347: 589-600. Schafer AI (2004) Thrombocytosis. *New England Journal of Medicine* 350: 1211-1219.

### **THROMBOSIS**

A thrombus is defined as a solid mass formed in the circulation from the constituents of the blood during life. Fragments of thrombi (emboli) may break off and block vessels downstream. Thromboembolic disease is much more common than abnormal bleeding; nearly half of adult deaths in England and Wales are due to coronary artery thrombosis, cerebral artery thrombosis or pulmonary embolism.

A thrombus results from a complex series of events involving coagulation factors, platelets, red blood cells and the vessel wall.

### **Arterial thrombosis**

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This usually occurs in association with atheroma, which tends to form at areas of turbulent blood flow such as the

bifurcation of arteries. Platelets adhere to the damaged vascular endothelium and aggregate in response to ADP and TXA<sub>2</sub> to form a 'white thrombus'. The growth of the platelet thrombus is limited at its margins by PGI<sub>2</sub> and NO. Plaque rupture leads to the exposure of blood containing factor VIIa to tissue factor within the plaque which may trigger blood coagulation and lead to thrombus formation. This results in complete occlusion of the vessel or embolization that produces distal obstruction. The risk factors for arterial thrombosis are related to the development of atherosclerosis (see p. 799).

Arterial thrombi may also form in the heart, as mural thrombi in the left ventricle after myocardial infarction, in the left atrium in mitral valve disease, or on the surfaces of prosthetic valves.

### Venous thrombosis

Unlike arterial thrombosis, venous thrombosis often occurs in normal vessels. Major causes are stasis and hypercoagulability. The majority of venous thrombi occur in the deep veins of the leg, originating around the valves as 'red thrombi' consisting mainly of red cells and fibrin. The propagating thrombus is formed of fibrin and platelets and is particularly liable to embolize. Chronic venous obstruction following thrombosis in the deep veins of the leg frequently results in a permanently swollen limb and may lead to ulceration (post-phlebitic syndrome).

Risk factors for venous thrombosis are shown in Table 8.27. Venous thrombosis may occur with changes in blood cells such as polycythaemia and thrombocythaemia, and with coagulation abnormalities (thrombophilia; see below).

**Table 8.27 Risk factors of venous thromboembolism**

Patient factors	Disease or surgical procedure
Age	
Obesity	
Varicose veins	
Long air travel	Trauma or surgery, especially of pelvis, hip or lower limb
Immobility	
(bed rest	Malignancy
> 4 days)	Cardiac failure
Pregnancy and puerperium	Recent myocardial infarction
Previous deep vein thrombosis or pulmonary embolism	Infection
	Inflammatory bowel disease
Thrombophilia	Nephrotic syndrome
Antithrombin deficiency	Polycythaemia
Protein C or S deficiency	Thrombocythaemia
Resistance to activated protein C (caused by factor V Leiden variant)	Paroxysmal nocturnal haemoglobinuria
Prothrombin gene variant	Sickle cell anaemia
Homocysteinaemia	
Antiphospholipid antibody/lupus anticoagulant	
Oestrogen therapy including HRT	

The clinical features and diagnosis of venous thrombosis are discussed on page 870.

### Thrombophilia

Thrombophilia is a term describing inherited or acquired defects of haemostasis leading to a predisposition to venous or arterial thrombosis. It should be considered in patients with:

- recurrent venous thrombosis
- venous thrombosis for the first time under age 40 years
- an unusual venous thrombosis such as mesenteric or cerebral vein thrombosis
- unexplained neonatal thrombosis
- recurrent miscarriages
- arterial thrombosis in the absence of arterial disease.

### Coagulation abnormalities

#### Factor V Leiden

Factor V Leiden is formed by a single nucleotide substitution (Arg506Gln) in the factor V gene (factor V Leiden mutation). This makes factor V less likely to be cleaved by activated protein C. Factor V is a cofactor for thrombin generation (see Fig. 8.33) and the failure of activated protein C to inactivate factor V (see Fig. 8.34) results in a tendency to thrombosis. Factor V Leiden is found in 3–5% of healthy individuals in the western world and in about 20% of patients with venous thrombosis.

The risk of venous thrombosis is increased in women with factor V Leiden who are pregnant or taking oral contraceptives. Screening for the defect before prescribing oral contraceptives or during pregnancy would be costly and might deny oral contraception to a substantial number of women who would then be at an increased risk of pregnancy, and therefore thrombosis. In addition, the use of oral anticoagulants in pregnancy carries a risk of fatal maternal bleeding, which may equal the risk of death due to postpartum thrombosis, and the fetus is also at risk of complications from the use of oral anticoagulants (see p. 481).

#### Prothrombin variant

A mutation in the 3' untranslated region of the prothrombin gene has been described (G20210A). This variant is associated with elevated levels of prothrombin and a two- to threefold increase in the risk of venous thrombosis. There is an interaction with factor V Leiden and contraceptive pill use or pregnancy. The prevalence is 2% in Caucasian populations, 6% in unselected patients with thrombosis and about 18% in families with unexplained thrombophilia.

#### Antithrombin (AT) deficiency

This deficiency can be inherited as an autosomal dominant. Many variations have been described that lead to a conformational change in

the protein. It can also be acquired following trauma, with major surgery and with the contraceptive pill. Low levels are also seen in

severe proteinuria (e.g. the nephrotic syndrome). Recurrent

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## Haematological disease

thrombotic episodes occur starting at a young age in the inherited variety. Patients are relatively resistant to heparin as antithrombin is required for its action. Anti-thrombin III concentrates are available.

### *Protein C and S deficiency*

These autosomal dominant conditions result in an increased risk of venous thrombosis, often before the age of 40 years. Homozygous protein C or S deficiency causes neonatal purpura fulminans, which is fatal without immediate replacement therapy. Protein C concentrate and a recombinant activated protein C are available.

### *Antiphospholipid antibody*

See pages 534 and 577.

### *Investigations*

#### *Haemostatic screening tests*

- **Full blood count** including platelet count
- **Coagulation screen** including a fibrinogen level.

These tests will detect erythrocytosis, thrombocytosis, and dysfibrinogenaemia and the possible presence of a lupus anticoagulant.

#### *Testing for specific causes of thrombophilia*

- **Assays** for naturally occurring anticoagulants such as AT, protein C and protein S
- **Assay** for activated protein C resistance and molecular testing for factor V Leiden and the prothrombin variant
- **Screen for a coagulation factor inhibitor** including a lupus anticoagulant (and anticardiolipin antibodies) (see p. 534)
- **Fibrinolytic pathway tests** (see p. 469).

### *Prevention and treatment of arterial thrombosis*

Attempts to prevent or reduce arterial thrombosis are directed mainly at minimizing factors predisposing to atherosclerosis. Treatment of established arterial thrombosis includes the use of antiplatelet drugs and thrombolytic therapy.

### **Antiplatelet drugs**

Platelet activation at the site of vascular damage is crucial to the development of arterial thrombosis, and this can be altered by the following drugs (Table 8.28):

- **Aspirin** irreversibly inhibits the enzyme cyclooxygenase (COX), resulting in reduced platelet production of TXA<sub>2</sub> (see Fig. 8.32). At the low doses used in cardiovascular disease prevention or treatment, there is selective inhibition of the isoform COX-1 found within platelets.

This inhibition cannot be repaired and is effective for the life of the circulating platelet, which is about 1 week.

- **Dipyridamole** - which inhibits platelet phosphodiesterase, causing an increase in cyclic AMP with potentiation of the action of PGI<sub>2</sub> - has been used widely as an anti-thrombotic agent, but there is little evidence that it is effective.

**Table 8.28 Drugs used in the treatment of thrombotic disorders**

Antiplatelet	Anticoagulant
Aspirin	Heparin:
Dipyridamole	unfractionated (or standard) low molecular weight
Clopidogrel	Hirudins, e.g. lepirudin
Gp, IIb/IIIa inhibitors, e.g. abciximab, eptifibatide, tirofiban	Fondaparinux
Epoprostenol	Warfarin
	Ximelagatran
<b>Thrombolytic</b>	
Streptokinase Tissue-type plasminogen activator (t-PA or alteplase)	
Reteplase (r-PA) Tenecteplase (TNK-tPA)	

- *Clopidogrel* - affects the ADP-dependent activation of the glycoprotein IIb/IIIa complex. It is similar to ticlopidine but has fewer side-effects. Trials support its use in acute coronary syndromes (p. 808), particularly if aspirin is contraindicated.
- *Glycoprotein IIb/IIIa receptor antagonists* block a receptor on the platelet for fibrinogen and von Willebrand factor (Fig. 8.39). Three classes have been described:
  - (a) murine—human chimeric antibodies (e.g. abciximab)
  - (b) synthetic peptides (e.g. eptifibatide)
  - (c) synthetic non-peptides (e.g. tirofiban).
 They have been used as an adjunct in invasive coronary intervention and as primary medical therapy in coronary heart

disease. Excessive bleeding has been a problem.

- *Epoprostenol* is a prostacyclin which is used to inhibit platelet aggregation during renal dialysis (with or without heparin) and is also used in primary pulmonary hypertension.

The indications for and results of antiplatelet therapy are discussed in the appropriate sections (pp. 806 and 1215).

### Thrombolytic therapy

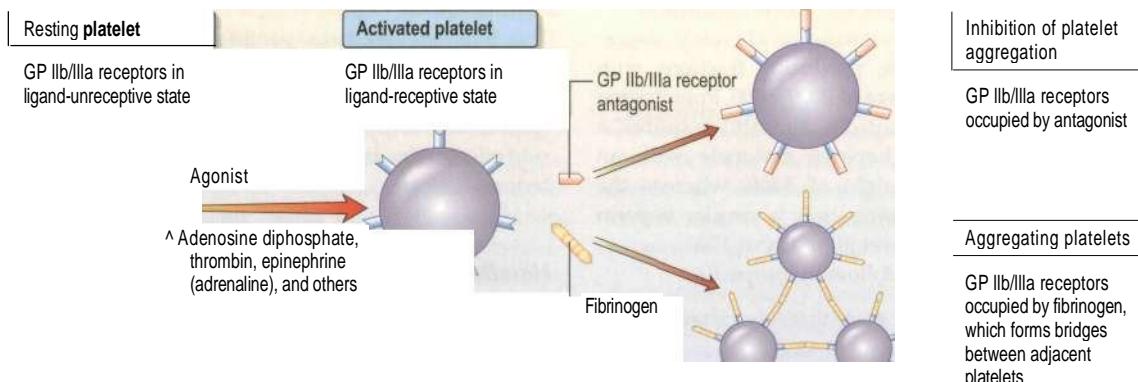
#### *Streptokinase*

Streptokinase is a purified fraction of the filtrate obtained from cultures of haemolytic streptococci. It forms a complex with plasminogen, resulting in a conformational change which activates other plasminogen molecules to form plasmin.

Streptokinase is antigenic and the development of streptococcal antibodies precludes repeated use. Activation of plasminogen is indiscriminate so that both fibrin in clots and free fibrinogen are lysed, leading to low fibrinogen levels and the risk of haemorrhage.

#### *Plasminogen activators (PA)*

Tissue-type plasminogen activators (alteplase (t-PA), tenecteplase (TNK-PA)) are produced by recombinant technology. Reteplase (r-PA) is also a recombinant plasminogen activator. They are not antigenic and do not give allergic reactions. They have a slightly higher risk of intracerebral haemorrhage (see also p. 813).



**Fig. 8.39 The role of glycoprotein IIb/IIIa in platelet aggregation and the inhibition of platelet aggregation by inhibitors of glycoprotein IIb/IIIa receptors.** Modified from Lefkovits J, Plow EF, Topol EJ (1995) *New England Journal of Medicine* 332: 1554, with permission.

### Indications

The use of thrombolytic therapy in myocardial infarction is discussed on page 813. The combination of aspirin with thrombolytic therapy produces better results than thrombolytic therapy alone. The extent of the benefit depends on how quickly treatment is given. They are also used in cerebral infarction (p. 1214) and occasionally in massive pulmonary embolism.

The main risk of thrombolytic therapy is bleeding. Treatment should not be given to patients who have had recent bleeding, uncontrolled hypertension or a haemorrhagic stroke, or surgery or other invasive procedures within the previous 10 days.

### Prevention and treatment of venous thromboembolism

Venous thromboembolism is a common problem after surgery, particularly in high-risk patients such as the elderly, those with malignant disease and those with a history of previous thrombosis (Table 8.29). The incidence is also high in patients confined to bed following trauma, myocardial infarction or other illnesses. The prevention and treatment of venous thrombosis includes the use of anticoagulants.

### Anticoagulants

#### Heparin (standard or unfractionated)

Heparin is not a single substance but a mixture of poly-saccharides. Commercially available unfractionated heparin consists of components with molecular weights varying from 5000 to 35 000 with an average of about 13 000. It was initially extracted from liver (hence its name) but it is now prepared from porcine gastric mucosa.

Heparin has an immediate effect on coagulation by potentiation of the formation of irreversible complexes between antithrombin and activated serine protease coagulation factors (thrombin, Xlla, Xla, Xa, IXa and Vila).

**Table 8.29 Classification of risk of deep vein thrombosis and pulmonary embolism for hospital patients**

<b>Low risk</b> (proximal vein thrombosis 0.4%; fatal pulmonary embolism < 0.2%) Patients < 40 years undergoing major surgery (> 30 minutes) with no other risk factors
Patients undergoing minor surgery (< 30 minutes) with no other risk factors
Patients with minor trauma or illness with no thrombophilia but history of deep vein thrombosis or previous pulmonary embolism
<b>Medium risk</b> (proximal vein thrombosis 2-4%; fatal pulmonary embolism 0.2-0.5%) Major general, urological, gynaecological, cardiothoracic, vascular or neurological surgery in patients > 40 years or with one or more other risk factor(s)
Major acute medical illness such as myocardial infarction, heart failure, chest infection, cancer or inflammatory bowel disease
Major trauma Minor surgery, trauma or illness in patients with previous deep vein thrombosis, pulmonary embolism or thrombophilia
Plastercast immobilization of the leg in patients with minor injury
<b>High risk</b> (proximal vein thrombosis 10-20%; fatal pulmonary embolism 1-5%)
Fracture or major orthopaedic surgery of pelvis, hip or leg
Major pelvic or abdominal surgery for cancer
Major surgery, trauma or illness in patients with previous deep vein thrombosis, pulmonary embolism or thrombophilia
Leg paralysis Critical leg ischaemia or major leg amputation

Verstraete M (1997) Fortnightly review: prophylaxis of venous embolism. *BMJ* 314:124, with permission of the BMJ.

*Low-molecular-weight heparins (LMW heparins)*

These are produced by enzymatic or chemical degradation of standard heparin, producing fractions with molecular weights in the range of 2000-8000. Potentiation of thrombin inhibition (anti-IIa activity) requires a minimum length of the heparin molecule with an approximate molecular weight of 5400, whereas the inhibition of factor Xa requires only a smaller heparin molecule with a molecular weight of about 1700. LMW heparins have the following properties:

- Bioavailability is better than that of unfractionated heparin.
- They have greater activity against factor Xa than against factor IIa, suggesting that they may produce an equivalent anticoagulant effect to standard heparin but have a lower risk of bleeding, although this has not generally been confirmed. In addition, LMW heparins cause less inhibition of platelet function.
- They have a longer half-life than standard heparin and so can be given as a once-daily subcutaneous injection instead of every 8-12 hours.
- They produce little effect on tests of overall coagulation, such as the APTT at doses recommended for prophylaxis. They are not fully neutralized by protamine.

LMW heparins are widely used for antithrombotic prophylaxis, e.g. high-risk surgical patients and for the treatment of established thrombosis (see p. 870).

The main complication of all heparin treatment is bleeding. This is managed by stopping heparin. Very occasionally it is necessary to neutralize unfractionated heparin with protamine. Other complications include osteoporosis with prolonged therapy and thrombo-cytopenia.

*Heparin-induced thrombocytopenia (HIT).* HIT is an uncommon complication of heparin therapy and usually occurs 5-14 days after first heparin exposure. It is due to an immune response directed against heparin/platelet factor 4 complexes. All forms of heparin have been implicated but the problem occurs less often with LMW heparins. A separate and unimportant immediate thrombocytopenia has also been described.

HIT is paradoxically associated with severe thrombosis and when diagnosed all forms of heparin must be discontinued, including heparin flush. Unfortunately the diagnosis can be difficult to make because patients on heparin are often very sick and may be thrombocytopenic for many other reasons. Laboratory tests based on bioassay or

immunoassay are available but are neither sensitive nor specific and management decisions often have to be made before results are available.

It is usually necessary to continue some form of anti-coagulation in patients with HIT and the choice lies between the heparinoid danaparoid and the antithrombin hirudin. The introduction of warfarin should be covered by one of these agents, as warfarin alone may be ineffective or even exacerbate thrombosis as protein C levels fall.

*Fondaparinux*

This is a new synthetic pentasaccharide which inhibits activated factor X, similar to the LMW heparins. It will now be necessary to establish comparative efficacy (prevention or treatment of thrombosis) against risk of side-effects (bleeding) before the position of fondaparinux becomes clear.

*Hirudin*

A recombinant form of hirudin, lepirudin, is available. Lepirudin is used for anticoagulation in patients with HIT. Hirudins act directly on thrombin and can be monitored by the use of the APTT. They are excreted by the kidney and must be used with caution in renal failure.

*Oral anticoagulants*

These act by interfering with vitamin K metabolism. There are two types of oral anticoagulants, the coumarins and indandiones. The coumarin warfarin is most commonly used because it has a low incidence of side-effects other than bleeding.

The dosage is controlled by PT tests. Thromboplastin reagents for PT testing are derived from a variety of sources and give different PT results for the same plasma. It is standard practice to compare each thromboplastin with an international reference preparation so that it can be assigned an international sensitivity index (ISI). The international normalized ratio (INR) is the ratio of the patient's PT to a normal control when using the international reference preparation. Therapeutic ranges using the INR for oral anticoagulation in various conditions are shown in Box 8.2.

Each laboratory can use a chart adapted to the ISI of their thromboplastin to convert the patient's PT to the INR. Suitably selected control plasmas can also be used to achieve the same objective. The use of this system means that PT tests on a given plasma sample using different thromboplastins result in the same INR and that anticoagulant control is comparable in different hospitals across the world.

**Box 8.2 Indications for oral anticoagulation and target INR (British Society for Haematology 1998)**

**Target INR**

- |     |   |
|-----|---|
| 2.5 | Pulmonary embolism, proximal and calf deep vein thrombosis, recurrence of venous thromboembolism when no longer on warfarin therapy, symptomatic inherited thrombophilia, atrial fibrillation, cardioversion, mural thrombus, cardiomyopathy. |
| 3.5 | Recurrence of venous thromboembolism while on warfarin therapy, antiphospholipid syndrome, mechanical prosthetic heart valve, coronary artery graft thrombosis  |

*Contraindications* to the use of oral anticoagulants are seldom absolute and include:

- severe uncontrolled hypertension
- non-thromboembolic strokes
- peptic ulceration (unless cured by *Helicobacter pylori* eradication)
- severe liver and renal disease
- pre-existing haemostatic defects
- non-compliance.

Oral anticoagulants should be avoided in pregnancy because they are teratogenic in the first trimester and may be associated with fetal haemorrhage later in pregnancy. When anticoagulation is considered essential in pregnancy, specialist advice should be sought. Self-administered subcutaneous heparin should be used as an alternative, although this may not be as effective for women with prosthetic cardiac valves.

Many drugs interact with warfarin (see Ch. 16). More frequent PT testing should accompany changes in medication, which should occur with the full knowledge of the anticoagulant clinic.

An increased anticoagulant effect due to warfarin (Emergency box 8.1) is usually produced by one of the following mechanisms:

- drugs causing a reduction in the metabolism of warfarin, including tricyclic antidepressants, cimetidine, sulphonamides, phenothiazines and amiodarone
- drugs such as clofibrate and quinidine which increase the sensitivity of hepatic receptors to warfarin

#### **Emergency Box 8.1**

#### **Management of bleeding and excessive oral anticoagulation (modified from British Society for Haematology 1998)**

INR > 3.0 < 6.0 (target INR 2.5)	(1) reduce warfarin dose or stop
INR > 4.0 < 6.0 (target INR 3.5)	(2) restart warfarin when INR < 5.0
INR > 6.0 < 8.0 no bleeding or minor bleeding	(1) stop warfarin (2) restart when INR < 5.0
INR > 8.0, no bleeding or minor bleeding	(1) stop warfarin (2) restart warfarin when INR < 5.0 (3) if other risk factors for bleeding give 0.5-2.5 mg of vitamin K (oral)
Major bleeding	(1) stop warfarin (2) give prothrombin complex concentrate 50 units/kg or FFP 15 mL/kg (3) give 5 mg of vitamin K (oral or i.v.)

If unexpected bleeding occurs investigate the possibility of a local anatomical cause.

- drugs interfering with vitamin K absorption (such as broad-spectrum antibiotics and colestyramine) which also potentiate the action of warfarin
- displacement of warfarin from its binding site on serum albumin by drugs such as sulphonamides (this is not usually responsible for clinically important interactions)
- drugs that inhibit platelet function (such as aspirin) which increase the risk of bleeding
- alcohol excess, cardiac failure, liver or renal disease, hyperthyroidism and febrile illnesses which result in potentiation of the effect of warfarin.

*A decreased anticoagulant effect due to warfarin.* This is usually produced by drugs that increase the clearance of warfarin by induction of hepatic enzymes that metabolize warfarin, such as rifampicin and barbiturates.

#### *Ximelagatran*

This oral direct thrombin inhibitor is a potential alternative to warfarin and has already been evaluated in patients with venous thromboembolism, atrial fibrillation and myocardial infarction. It has a rapid onset of action and can be administered in a fixed dose without the need for monitoring. Drug interactions are also less than for warfarin but elimination is primarily renal and abnormalities of liver function have been

described during its use.

#### **Prophylaxis to prevent venous thromboembolism**

Prophylactic measures to prevent venous thrombosis during surgery are aimed at procedures for preventing stasis, such as early mobilization, elevation of the legs, compression stockings, and possibly calf-muscle stimulation and passive calf-muscle exercises during surgery, and methods for preventing hypercoagulability, usually using heparin.

*Low-risk patients* (Table 8.29) require no specific measures other than early mobilization.

*Moderate-risk patients* should receive a standard dose of LMW heparin e.g. enoxaparin 20 mg (2000 i.u.) subcutaneously daily until the patient is ambulatory.

In *high-risk patients*, such as patients undergoing total hip replacement, LMW heparin once daily, such as enoxaparin 40 mg (4000 i.u.), has been shown to be more effective than standard low-dose heparin in preventing thrombosis. There is evidence to suggest that it is most effective when administered for a total of 1 month post-operatively rather than merely during the admission for surgery.

LMW heparin has replaced standard low-dose heparin for all surgical prophylaxis and is increasingly used for medical prophylaxis.

#### **Treatment of established venous thromboembolism**

The aim of anticoagulant treatment is to prevent further thrombosis and pulmonary embolization while resolution

## Haematological disease

of venous thrombi occurs by natural fibrinolytic activity. Anticoagulation is started with heparin as it produces an immediate anticoagulant effect. There is no evidence that it is necessary to use heparin for any longer than it takes for simultaneously administered warfarin to produce an anticoagulant effect (INR 2.5) usually about 3<sup>rd</sup> days.

LMW heparin (e.g. tinzaparin 175 units/kg daily) is equally effective and as safe as unfractionated heparin in the immediate treatment of deep vein thrombosis and pulmonary embolism. This creates the opportunity for treatment of venous thromboembolism without admission to hospital, in compliant patients without coexisting risk factors for haemorrhage.

Anticoagulation (with warfarin approximately 3-9 mg daily for 6 weeks) is sufficient for patients after their first thrombosis provided there are no persisting risk factors. There is much interest in the use of longer-term anticoagulation in patients with previous thrombosis. It has

been suggested that a lower INR might be safer and equally effective but the current view is that the target INR should be 2.0 to 3.0 where oral anticoagulation is used. Secondary prophylaxis should be offered to patients with unprovoked venous thromboembolism, for a period of 6-12 months. Very long-term treatment should be reserved for those with repeated episodes or continuing risk factors. Outpatient anticoagulation is best supervised in anticoagulant clinics. Patients are issued with national booklets for recording INR results and anticoagulant doses.

The role of thrombolytic therapy in the treatment of venous thrombosis is not established. It is sometimes used in patients with massive pulmonary embolism and in patients with extensive deep venous thrombi.

Thrombolytic therapy should be followed by anti-coagulation with heparin for a few days and then by oral anticoagulants for a few months to prevent rethrombosis.

### FURTHER READING

- British Society for Haematology guidelines documents: (1998) Guidelines on oral anticoagulation, 3rd edition. *British Journal of Haematology* **101**: 374-387  
(2000) Guidelines on the investigation and management of the antiphospholipid syndrome. *British Journal of Haematology* **109**: 704-715  
(2001) Investigation and management of heritable thrombophilia. *British Journal of Haematology* **114**: 512-528  
(2003) Guideline on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias. *British Journal of Haematology* **120**: 556-573  
(2003) Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. *British Journal of Haematology* **120**: 574-596  
(2004) The diagnosis for deep vein thrombosis in symptomatic outpatients and the potential for clinical assessment and D-dimer assays to reduce the need for diagnostic imaging. *British Journal of Haematology* **124**: 15-25.

### CHAPTER BIBLIOGRAPHY

- Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U (2001) *Williams Hematology*, 6th edn. New York: McGraw-Hill. Hoffbrand AV, Pettit J (2000). *Atlas of Clinical Haematology*, 3rd edn, Edinburgh: Churchill Livingstone.

- Online. Available:  
<http://www.bcsghguidelines.com>;  
<http://www.bcsh.org.uk>;  
<http://www.transfusionguidelines-org.uk>. Buller, HR, Prins MH (2003) Secondary prophylaxis with warfarin for venous thromboembolism. *New England Journal of Medicine* **349**: 702-704. Dahlback B (2000) Blood coagulation. *Lancet* **355**: 1627-1632.  
George JN (2000) Platelets. *Lancet* **355**: 1531-1539. Gerhardt A et al. (2000) Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *New England Journal of Medicine* **342**: 374-380. Lowe GDO, Sandercock PAG, Rosendaal FR (2003) Prevention of venous thromboembolism after major orthopaedic surgery: is fondaparinux an advance? *Lancet* **362**: 504-505. Shapiro SS (2003) Treating thrombosis in the 21st century. *New England Journal of Medicine* **349**: 1762-1764. Topol EJ, Byzova TV, Plow EF (1999) Platelet GP IIb-IIIa blockers. *Lancet* **353**: 227-231.

- Hoffbrand AV, Pettit J, Moss P (2001) *Essential Haematology*, 4th edn. Oxford: Blackwell Science.  
Murphy MF, Pamphilon D (2001) *Practical Transfusion Medicine*. Oxford: Blackwell Science. Provan D, Gibben J (2000) *Molecular Haematology*. Oxford: Blackwell Science. Weatherall D, Clegg J (2001) *The Thalassaemia Syndromes*, 4th edn. Oxford: Blackwell Science.

## **SIGNIFICANT WEBSITES**

<http://www.bloodline.net>

*General website on haematology* <http://www.transfusion.org>

*Journal of the American Association of Blood Banks* <http://www.shotuk.org>

*Serious Hazards of Transfusion (SHOT) scheme, covering*

*UK and Ireland NHS and private hospitals, affiliated to the*

*Royal College of Pathologists (based Manchester Blood*

*Transfusion Centre)* <http://www.blood.co.uk>

*IK National Blood Service*

<http://www.doh.gov.uk/bbt2>

*UK CMO's Better Blood Transfusion Conference* <http://www.bcsghguidelines.com>

*British Society for Haematology guidelines* <http://www.wfh.org>

*World Federation of Hemophilia* <http://www.hemophilia.org>

*US National Hemophilia Foundation* <http://www.med.unc.edu/isth/>

*International Society on Thrombosis and Haemostasis*

*(ISTH)*