chapter 13:

DISORDERS OF THE BLOOD

OBJECTIVES

After studying this chapter you should be able to:

- list the components of the blood;
- describe the structures of blood cells and platelets;
- explain how hemoglobin functions;
- explain how blood clotting occurs;
- give examples of some types of anemias;
- describe the consequences of the genetic defects in sickle cell anemia and the thalassemias;
- explain the diagnoses and treatments of some types of anemias;
- describe some clinical disorders associated with clotting factors.

13.1 INTRODUCTION

Blood is a protein-rich fluid called **plasma** in which erythrocytes and leukocytes, sometimes called red and white blood cells respectively, and platelets are suspended (*Figure 13.1*). The cells constitute about 40–45% of the volume of the blood. The blood is pumped around the body by the heart through the arteries that supply the capillaries and is returned to the heart in the veins (*Chapter 14*). The main functions of the blood are to distribute oxygen, nutrients and hormones and other signaling molecules between tissues and to remove carbon dioxide and other waste products. Plasma contains the proteins of the clotting system and of the immune systems (*Chapters 4* and *5*).

Plasma is blood from which the cells have been removed. It contains a range of plasma proteins in addition to the clotting and immune system proteins mentioned above, nutrients, such as glucose, waste materials, for example urea, and a range of electrolytes in solution. If it is allowed to clot, the clear

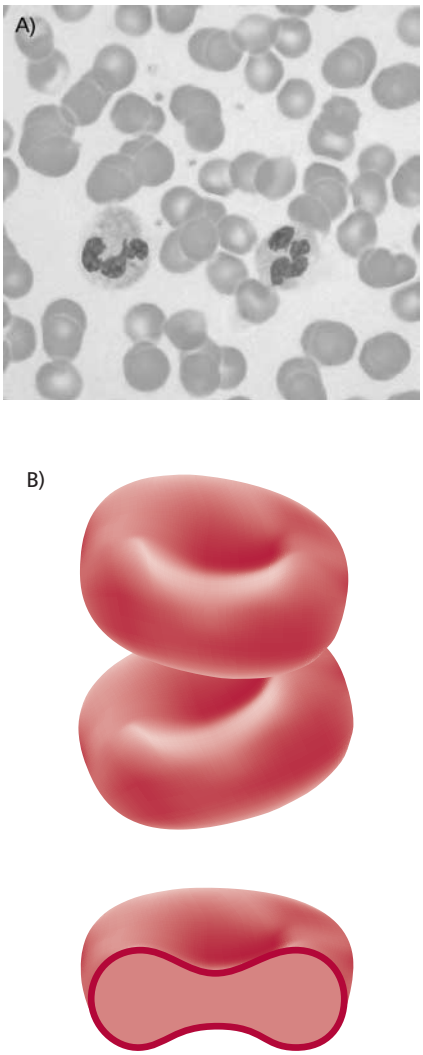


Figure 13.1 (A) Shows the appearance of a normal blood smear when examined with a microscope. The erythrocytes predominate with the occasional leukocyte being visible. (B) The erythrocytes are biconcave in shape and their centers appear lighter in color.

straw-colored liquid remaining after removal of the clot is called **serum**. The composition of the blood and the plasma is given in *Table 13.1*.

Plasma	Concentration
[Total protein] / g dm^{-3}	66
[Fibrinogen] / g dm^{-3}	3.1
[Albumins] / g dm^{-3}	32.6
[Globulins] (excluding fibrinogen)] / g dm^{-3}	30.1
Cells and platelets	**Number**
Erythrocytes male / dm^{-3} female / dm^{-3}	 $4.4–5.9 \times 10^{12}$ $3.8–5.2 \times 10^{12}$
Leukocytes / dm^{-3}	$4–11 \times 10^{9}$
Platelets / dm^{-3}	$2.5–5.0 \times 10^{9}$

*Blood volume of 78 and 66 cm^3 kg^{-1} body weight in males and females respectively

Table 13.1 Composition of the blood*

In a text of this size it is not, of course, possible to discuss each type of blood disorder and attention will focus only on the major types of diseases likely to be normally encountered.

13.2 BLOOD CELLS AND PLATELETS

All of the cells of the blood originate from pluripotent stem cells in the bone marrow (*Figure 13.2*). Chemical signals, such as cytokines (*Chapter 4*), direct primordial stem cells to develop in different ways to produce **erythrocytes**, **leukocytes** of various types, and **megakaryocytes**, which are the precursors of **platelets**.

Normoblasts are erythroid cells that arise from divisions of pluripotent stem cells. Eventually these lose their nuclei giving rise to **reticulocytes,** which contain mRNAs for globins and are still able to synthesize hemoglobin (Hb), and which are the precursors of the erythrocytes. The reticulocytes circulate in the blood for 1–2 days before maturing to erythrocytes, and normally constitute 1–2% of the circulating red cells. Erythrocytes are the most numerous cells in the blood. Adult males and females have erythrocyte counts of about 5.5 and 4.8×10^{12} dm^{-3}, respectively. The number of cells in a given volume of blood can be determined using a hemocytometer (*Figure 13.3*). About 2×10^{11} mature erythrocytes are formed daily. They have no nuclei or other organelles and are biconcave in shape (*Figure 13.1*). Erythrocytes circulate for about 120 days and are then removed from circulation and destroyed by macrophages in the liver and spleen. Hemoglobin is the red protein found in the erythrocytes that carries dioxygen (O_2) and which also plays an important role in buffering, maintaining the pH at 7.4 ± 0.1 (*Chapter 9*). The iron-containing heme is removed from the Hb of defunct erythrocytes and its porphyrin ring is converted to bilirubin, which is excreted in the bile. The iron is conserved and recycled (*Box 13.1*). Iron circulates in the blood attached to a transport

protein called transferrin and stored in the liver and spleen cells in a storage protein called ferritin (*Figure 13.4 (A)* and *(B)*). Excessive breakdown of Hb produces more bilirubin than can be excreted and this accumulates in the tissues causing jaundice (*Chapter 11*).

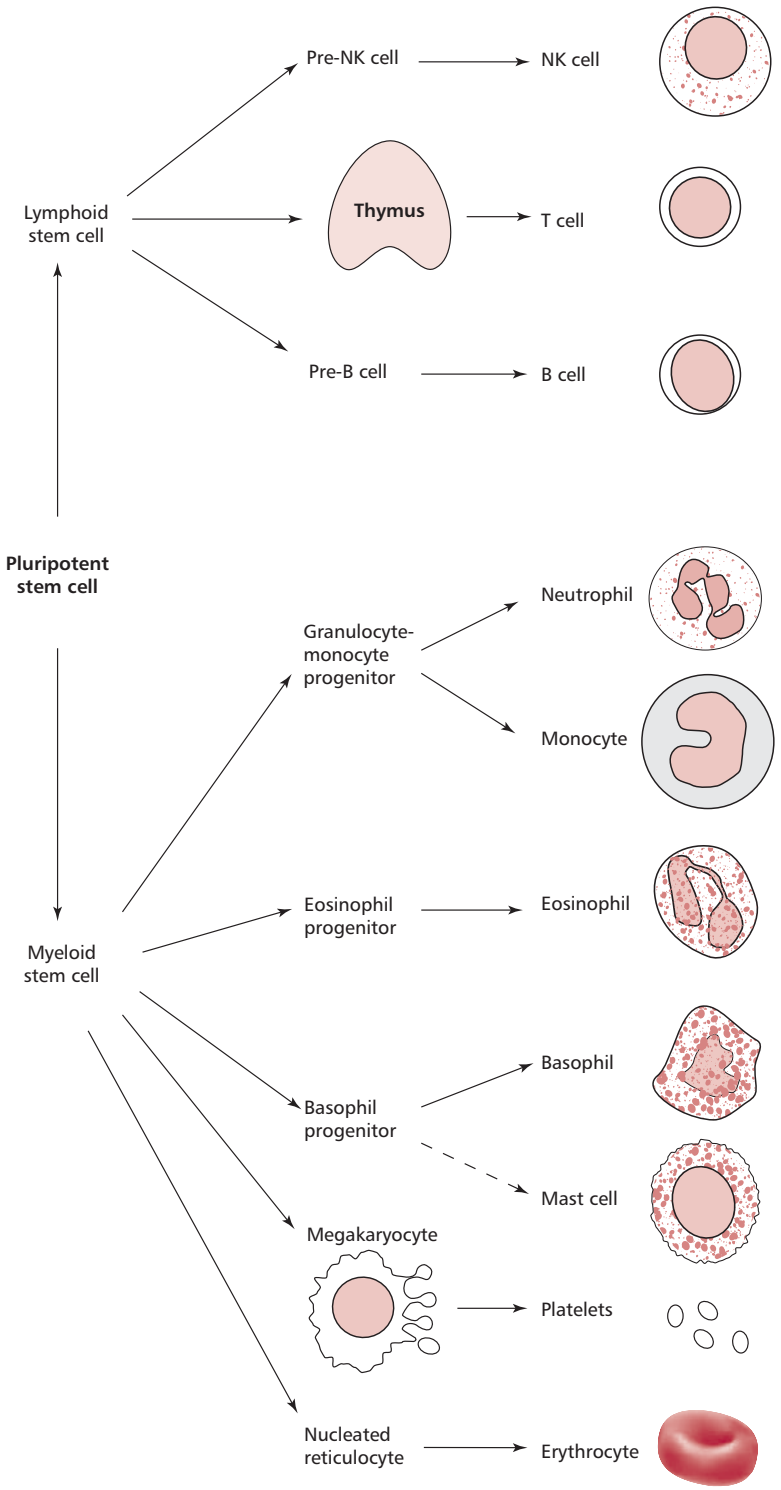


Figure 13.2 **Origin of the cells of the blood. All of the cells of the blood, as well as the platelets, originate in the bone marrow where progenitor stem cells divide and differentiate to produce the different cell types. The process of differentiation is controlled by growth factors.** See *Chapter 4* for more information on lymphocytes (natural killer (NK), T and B cells).

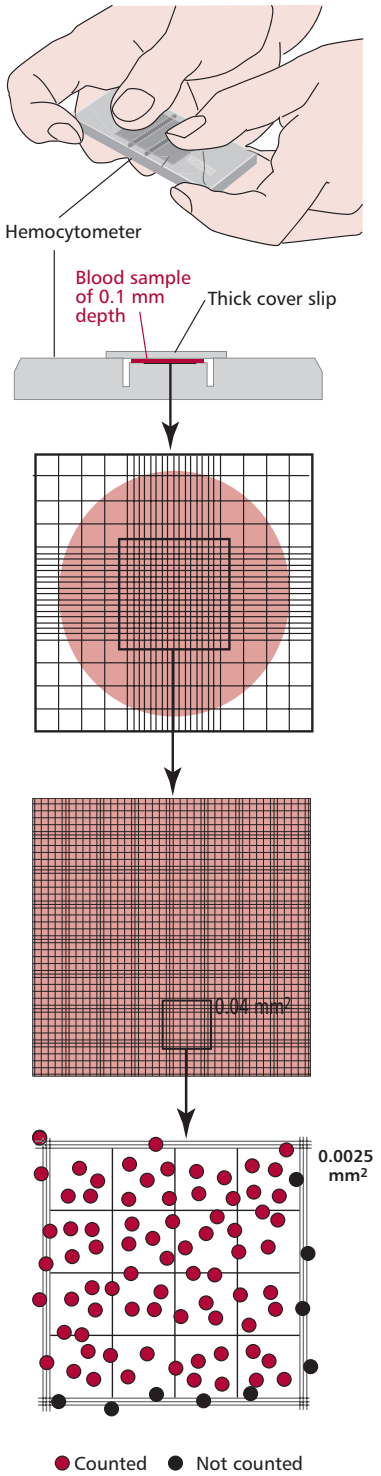


Figure 13.3 **Counting blood cells using a hemocytometer. Blood is diluted and then pipetted into a glass chamber of known volume. The number of cells within the indicated grid is counted under the microscope and this number, multiplied by the dilution, gives the number of cells in the blood sample. Different dilutions need to be done for erythrocytes and leukocytes. In hospital laboratories cells counts are done automatically using instruments called cell counters.**

BOX 13.1 Iron metabolism

Only about 10% of the average daily intake of iron, approximately 20 mg in the UK, is absorbed, mostly by the duodenum and jejunum (*Chapter 11*), although more is absorbed in pregnancy and in iron deficiency anemia (*Section 13.5*). There are obviously increased demands for iron in growth periods and menstruation, when about 0.7 mg iron is lost daily, and in pregnancy (*Chapter 7*). Heme iron from Hb and myoglobin in red meats is better absorbed than nonheme iron. Absorption is controlled by the mucosal cells of the small intestine and the iron is to some extent stored in these cells before being passed to the hepatic portal blood. Stored iron may be lost when these cells are shed. The iron is bound to the iron binding protein transferrin (*Figure 13.4 (A)*) during its transport in the blood. Transferrin can carry two atoms of iron per molecule but is only about one-third saturated on average. The iron is detached from the transferrin in the bone marrow when the protein interacts with specific receptors on erythroblasts and reticulocytes supplying the iron needed for Hb synthesis. Although 60–70% of the body's iron is found in circulation as Hb, with more in the cytochromes and other iron proteins; some is stored in the protein, ferritin (*Figure 13.4 (B)*). This protein is found in most cells, but particularly in those of the liver and spleen.

The body does not have an excretory route for iron, but this is normally a problem only when repeated transfusions are given, such as in the cases of patients with sickle cell anemia and thalassemia. As the transfused erythrocytes are broken down the iron accumulates and may form deposits of hemosiderin in the liver and spleen.

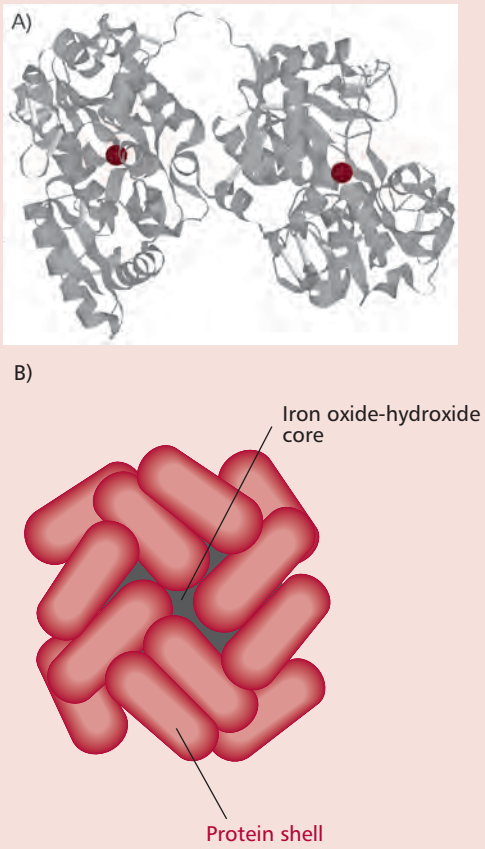


Figure 13.4 (A) Molecular model of transferrin. PDB code 1JNF. (B) Schematic of ferritin that consists of a protein coat of 24 subunits with a central cavity. When completely filled one multisubunit of ferritin can hold more than 2000 iron atoms as hydrated ferric hydroxide.

There are several types of leukocytes or white cells in the blood, each with its own function. The total white cell count in an adult is between 4 and 11 x 10^9 dm^{-3} but there is considerable variation. White cells were originally classified on the basis of which microscopic stain they took up, whether they had a granular cytoplasm, and whether the nucleus was lobed. The three main types of leukocyte are called **polymorphonucleocytes** (PMN, but sometimes referred to as *polymorphs*), **lymphocytes** and **monocytes** (*Chapter 4*). Polymorphonucleocytes are further subdivided into **neutrophils** (57% of the total white cell population) that contain neutral staining granules, **eosinophils** (3.5%) which contain acid-staining granules, and **basophils** (0.5%) which contain basic staining granules (*Figure 13.2*). Polymorphonucleocytes release chemokines some of which are mediators of inflammation as described in *Chapter 4*. Neutrophils can migrate to areas of infection and phagocytose bacteria. Eosinophils seem to be more concerned with dealing with larger parasites and their number also increases in allergic diseases. Basophils and the similar **mast cells** (*Chapter 4*), which are found mostly in the skin, can release histamine from their granules and this also contributes to some types of allergic responses.

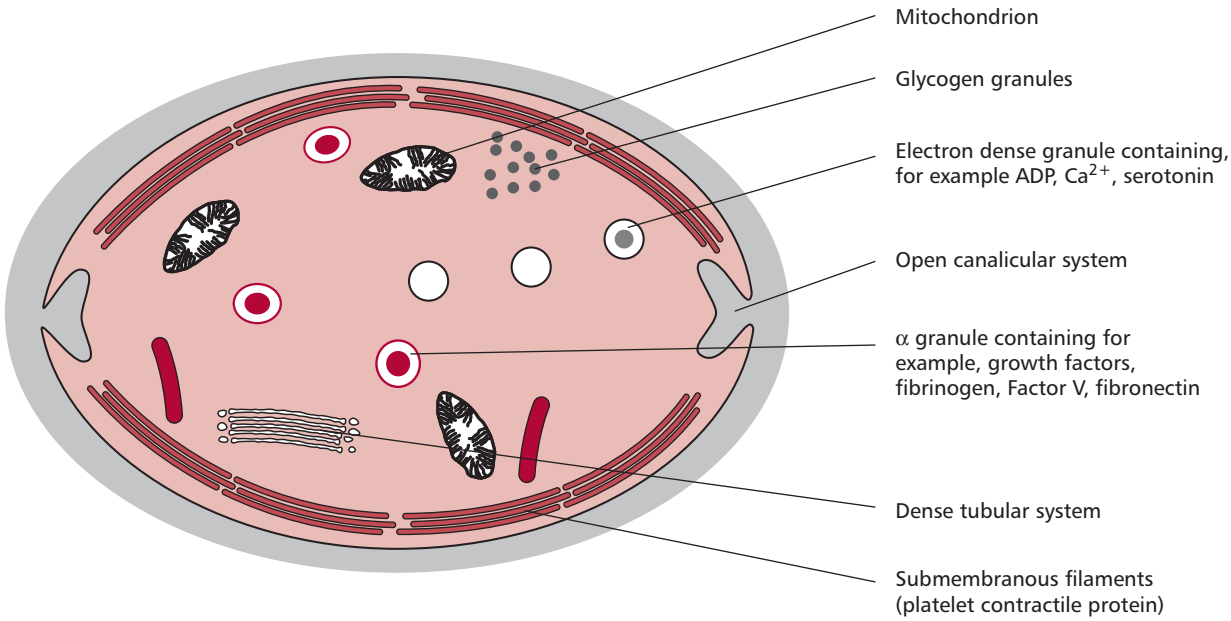


Figure 13.5 Schematic of a blood platelet. The platelet is packed with granules that have a major role in blood clotting.

Lymphocytes are key components of the immune system. Monocytes circulate in the blood for about 72 h and then enter the tissues and transform into macrophages, which play a key role in inflammation and defense (*Chapter 4*).

Platelets are vesicle-like structures about 3 μm in diameter with a volume of 7 fdm^3 (femtodecimeters^3 or femtoliters). They are formed by the fragmentation of large precursor cells called megakaryocytes and, like erythrocytes, are not complete cells although they contain numerous granules, some organelles and a tubular system (*Figure 13.5*). Their lifespan is 10–12 days and they function in blood clotting or hemostasis (*Section 13.4*).

Margin Note 13.1 Platelet concentrates

Platelet concentrates can be prepared by centrifugation and may be stored for up to five days (*Chapter 6*). Such concentrates are used to treat patients suffering from thrombocytopenia (*Section 13.9*) and who have insufficient platelets and to prevent bleeding in patients with bone marrow failure.

13.3 HEMOGLOBINS

Hemoglobin is the red-colored, oxygen-transporting protein in erythrocytes. Its M_r is about 64 000 and it is made up of four subunits, each containing an iron-containing heme group (*Figure 13.6*). Each molecule can carry up to four O_2 molecules. Oxygen is taken up as the blood passes through the lungs and is transported to all parts of the body allowing respiration, the oxidation of fuels, to occur in the mitochondria. The iron in the heme group of Hb remains in the ferrous (Fe(II)) state throughout. Should the iron become oxidized to Fe(III), **methemoglobin** is formed, which is incapable of carrying oxygen. This oxidation happens to a small extent continuously, so that normal blood always contains a few percent of methemoglobin. However, methemoglobin reductase, present in erythrocytes, constantly catalyzes its reduction back to Hb. The rare individuals with a genetic deficiency of this enzyme have severe problems and tend to be cyanosed unless they are treated with a reducing agent.

Margin Note 13.2 Cyanosis – going blue in the face

Cyanosis (from Greek *cyan*, blue) is the bluish complexion resulting from lack of oxygen in the circulating blood. It is most frequently observed under the nails, lips as well as the skin. Cyanosis occurs following an inadequate oxygen intake in the lungs or from many other reasons, for example the stagnation of blood in the circulation during heart failure.

Hemoglobin was one of the first proteins to have its complete structure determined. Indeed, because Hb is so important medically, it is fair to say that more is probably known about Hb than any other protein. It is vital to life because the low solubility of oxygen in water means that insufficient amounts can be carried by blood in solution. The binding of O_2 to Hb in erythrocytes

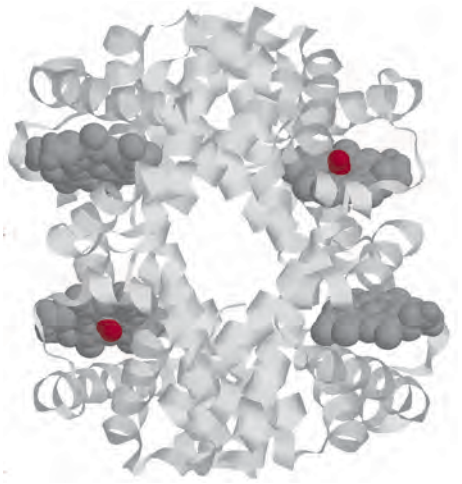
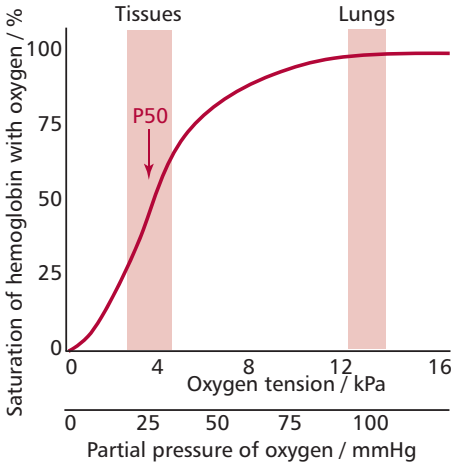


Figure 13.6 Molecular model of adult hemoglobin. PDB code 1GZX. Note how the four subunits interact closely with each other. The bound O_2 are shown in red.



increases the oxygen carrying capacity several thousand-fold. Furthermore, the body has a limited capacity to work anaerobically and so O_2 is vital. Even a small reduction in the amount of Hb in the blood leads to **anemia**, which causes serious clinical problems (*Section 13.5*). Anemia can result from there being too few erythrocytes or from each erythrocyte having too little or a defective Hb and this may be a consequence of a lack of iron. In all cases, medical treatment is usually necessary.

KEY PROPERTIES OF HEMOGLOBIN

Hemoglobin molecules are roughly spherical with the four subunits fitting tightly together. The subunits of Hb are all similar and are in identical pairs. Thus, for example, adult Hb, referred to as HbA, can be described as $\alpha_2\beta_2$. Embryonic and fetal Hbs also occur. The heme groups are hydrophobic and sit inside hydrophobic clefts in the protein. The iron atom within each heme binds one O_2 molecule and so Hb can successively bind four O_2. The strength of this binding, that is the oxygen affinity, can be measured. In the lungs, where there is abundant oxygen, Hb should bind its maximum of four molecules and become saturated. In the tissues, the oxygen must 'unbind' and be released. The strength of binding is critical; too weak and Hb would be ineffective as a carrier, too tight and the tissues would not be supplied with oxygen because the oxyhemoglobin would not release its oxygen.

When an O_2 binds to one subunit, it induces a small change in the shape of the protein making the binding of the next O_2 slightly easier, that is, the strength of binding changes with each successive addition. The consequence is that the graph of oxygen bound against oxygen concentration, the oxygen binding curve (*Figure 13.7*), is S-shaped or sigmoidal. This means that in the lungs the HbA molecule can become nearly 100% saturated with oxygen but in the tissues can release almost all of it.

Figure 13.7 Oxygen binding curve for hemoglobin. Note that it is sigmoidal, indicating that the affinity of O_2 changes as each successive O_2 binds. Thus in the lungs, where the oxygen tension is high, the hemoglobin becomes almost saturated with oxygen. In the tissues, where the oxygen tension is low, the hemoglobin is able to give up almost all of its oxygen.

BOX 13.2 Nomenclature of mutant hemoglobins

Two nomenclature systems are in use, which may be a little confusing. Originally, normal adult Hb was called hemoglobin A (HbA, A for adult, $\alpha_2\beta_2$). Fetal Hb was hemoglobin F (HbF, $\alpha_2\gamma_2$), sickle cell Hb was HbS and so on. However, as more Hb mutations were identified, and hundreds are known, it was realized that the number of mutations known exceeded the number of letters in the alphabet. Subsequently, Hbs began to be named after the geographic location where they were first discovered, for example Hb Dakar, Hb Lepore, Hb Sydney. An additional complication occurs when newly discovered Hbs have the same characteristics as a 'letter' Hb and both nomenclatures may be combined, as in, for instance, HbJ-Capetown. It is appreciated that this nomenclature is not perfect but it is too difficult to change now.

The precise mutation can of course be described in terms of the base change(s) when as is usual the gene sequence is known, but this is a little cumbersome for everyday use. Thus Hb Sydney is caused by a GTG to GGG mutation at position 67 in the gene for β–globin, causing an amino acid residue change from valine to alanine. This results in unstable Hb with poor heme-binding properties, resulting in mild hemolysis. *Table 13.2* gives some examples of mutant Hbs.

Hemoglobin	Codon change	Amino acid changed	Comments
Torino	TTC to GTC	Phe to Val	α-chain (43); decreased O_2 affinity, unstable
Ann Arbor	CTG to CGG	Leu to Arg	α-chain (80); unstable
Bibba	CTG to CCG	Leu to Pro	α-chain (136); dissociates
M-Iwate	CAC to TAC	His to Tyr	α-chain (87); forms met-Hb, benign cyanosis in heterozygotes
Constant Spring	UAA to CAA	STOP to Gln	α-chain mutation of the chain termination codon (142) gives extended α-chain
S	GAG to GTG	Glu to Val	β-chain (6); cells sickle, forms fibrils
C	GAG to AAG	Glu to Lys	β-chain (6); enhances sickling when with HbS
St Louis	CTG to CAG	Leu to Gln	β-chain (28); Fe readily oxidized, polar group in heme pocket, increased O_2 affinity, unstable
Seattle	GCC to GAC	Ala to Asp	β-chain (70); decreased O_2 affinity, unstable
Gun Hill	–	deletion	β-chain (91-95); increased O_2 affinity, unstable
M-Saskatoon	CAT to TAT	His to Tyr	β-chain (63); forms met-Hb, benign cyanosis in heterozygotes

Table 13.2 Some of the many know mutations in hemoglobin genes

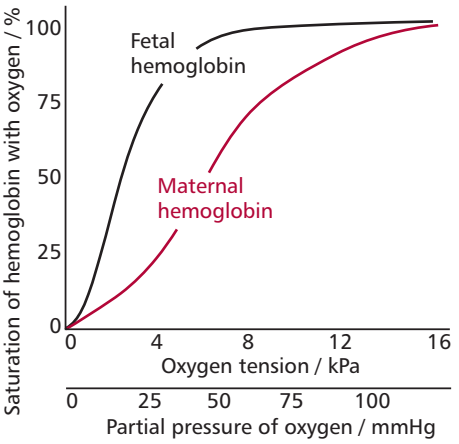


Figure 13.8 Graph showing that fetal hemoglobin has a higher affinity for oxygen than adult, maternal hemoglobin. This allows the fetus to obtain oxygen from the maternal blood.

EMBRYONIC AND FETAL HEMOGLOBINS

The embryonic and the fetal forms of Hb differ slightly from HbA. There are several types of embryonic Hb present early in embryonic life but at about 6 weeks there is a switch to fetal Hb (HbF). Fetal Hb has two α subunits, as in the adult, but two γ subunits, ($\alpha_2\gamma_2$, and there are actually two types of γ subunit). The embryo and fetus obtain their oxygen from the mother's blood in the placenta. Thus their Hbs need to become saturated with oxygen at lower oxygen tensions than maternal HbA so they can obtain it from the mother (*Figure 13.8*). This is possible because embryonic and fetal Hbs have a greater affinity for O_2 than HbA. Adult Hb production starts shortly before birth and by 30 weeks of age it should have replaced all the HbF (*Figure 13.9*).

Margin Note 13.3 Hereditary persistence of fetal hemoglobin

In *hereditary persistence of fetal hemoglobin* (HPFH), the HbF is not replaced. This is presumably due to a failure of the switching mechanism that normally occurs at around the time of birth. Although individuals with HPFH have high concentrations of HbF, the condition does not cause any major hematological abnormalities and does not prevent those affected from having a normal life.

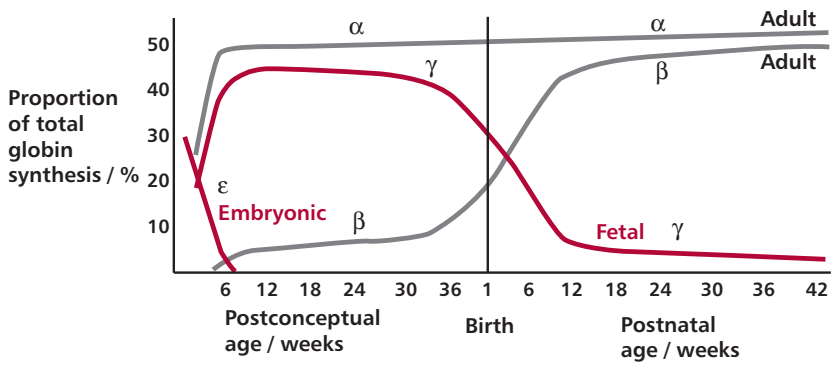


Figure 13.9 The production of human globins during development. There are several types of β-globins in the embryo and the fetus. Any given erythrocyte contains only one type of α- and one type of β-globin.

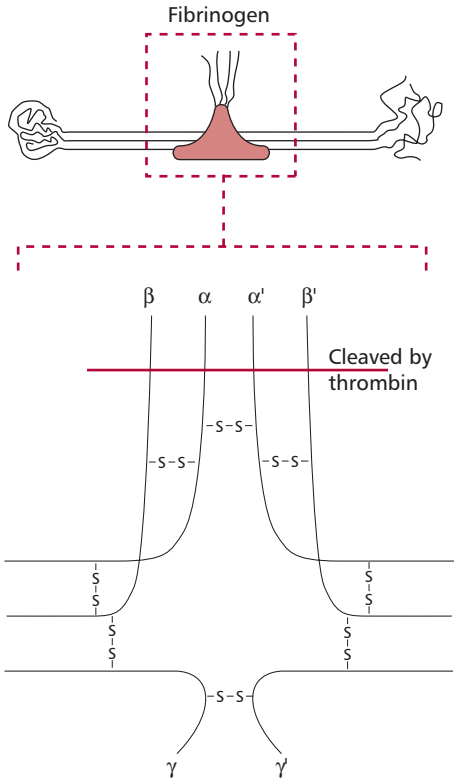


Figure 13.10 The structure of fibrinogen. Fibrinogen has a M_r about 340 000 and consists of two units, each containing three polypeptides (α, β, γ) joined together at their *N*-terminal ends by a number of disulfide bonds. Short lengths of the amino terminal regions of the two α and two β polypeptides project outwards and cleavage of these by thrombin, as indicated, allows the resulting fibrin molecules to aggregate to form a 'soft clot'. This is subsequently strengthened by the cross-linking action of Factor XIIIa which is a transglutaminase.

13.4 HEMOSTASIS AND BLOOD CLOTTING

The circulatory system is self-sealing. Hemostasis rapidly stops all but the most catastrophic bleeding in normal individuals. If the lining of a blood vessel is damaged, eventually a platelet plug is formed that prevents further blood loss. Blood clotting is then initiated leading to the deposition of fibrin and the formation of a clot to seal the wound. Wound healing can then begin.

Blood clotting occurs in one of two pathways, the so-called intrinsic and extrinsic pathways. These pathways each have a number of unique reactions but, in the end, both pathways activate the final clotting stage, which is the formation of fibrin. The clotting pathways involve a group of plasma proteins that act in sequence, each activating the next in line. The end result is the conversion of soluble fibrinogen to the insoluble fibrin, which polymerizes to form a clot at the site of the damage and critically not elsewhere (*Figure 13.10*).

About 20 plasma proteins or plasma clotting factors are produced by the liver and circulate in the blood as inactive proteins. They were called factors long before anything was known of their chemical nature. They are identified by Roman numerals although some of them also have common names (*Table 13.3*). However, their numbering is unfortunately not in a logical order with

Factor number	Name	Active form/ function	Associated diseases
I	fibrinogen	fibrin subunit	afibrinogenemia (uncommon)
II	prothrombin	serine protease	defective function (extremely rare)
III*	tissue factor	receptor	–
IV	Ca^{2+}	–	
V	labile factor	cofactor	–
VI	initially wrongly identified, now known to be Va		
VII	proconvertin	serine protease	deficiency known (but rare)
VIII	antihemophilia	cofactor	hemophilia A (classical hemophilia,
	factor		see main text)
IX	Christmas factor	serine protease	Christmas disease (hemophilia B)
X	Stuart-Prower factor	serine protease	deficiency known (but extremely rare)
XI	plasma thromboplastin antecedent	serine protease	rare, primarily in Ashkenazi Jews
XII	Hageman factor	serine protease	–
XIII	fibrin stabilizing factor	transglutaminase	rare, poor wound healing, scarring
	prekallikrein	precursor of kallikrein, a serine endopeptidase	–
	von Willebrand factor (vWF)	–	vWB disease (up to 125 in 10^6)
	high M_r kininogen	–	–

*factor III is used as a synonym for thromboplastin, a mixture of tissue factor and phospholipid.

Table 13.3 Clotting factors

reference to the clotting process. Their activated forms are distinguished by a lower case 'a'; thus the activation of Factor VIII produces Factor VIIIa. Many of them become proteases when activated but, as each factor catalytically activates the next one in sequence, a small amount of the first factor produces a very large reaction at the end of the pathway. Thus the cascade of clotting reactions is an amplification of an initial small stimulus and leads to the production of large amounts of fibrin forming a clot.

It is convenient to divide clotting factors into four groups based on what they do in the clotting process. The first group consists of **zymogens,** which become active proteases when subjected to specific proteolytic cleavage. The second group contains **cofactor proteins** that bind to zymogens and their protease products, increasing the specificity and speed of the activation. The third group of factors contains protease inhibitors, which inactivate the proteases after their roles in the clotting process are complete. The fourth group is a miscellaneous group. It includes fibrinogen, which is cleaved to form fibrin, Factor XIII/XIIIa, a transglutaminase that catalyzes the formation of covalent cross-links between fibrin molecules, which stabilizes the clot, and von Willebrand factor which can anchor platelets to the endothelium of blood vessels and which also carries Factor VIII in the plasma.

THE INTRINSIC PATHWAY

The intrinsic pathway is a sequence of reactions that are catalyzed by enzymes, which become activated when tissue is injured (*Figure 13.11*). This pathway can take several minutes for completion. Damage to the wall of a blood vessel or to the endothelium that lines it (*Chapter 14*) results in the exposure of collagen to which platelets stick and become activated. The platelets degranulate, releasing a range of highly active substances, including serotonin (5-hydroxytryptamine or 5-HT) and ADP, as well as certain growth factors, such as platelet derived growth factor (PDGF), which has a role in subsequent wound healing. Serotonin (*Figure 7.5*) is a powerful vasoconstrictor and causes the blood vessel to constrict, temporarily limiting the blood supply to the damaged area until clotting takes place. This is a temporary effect, and a clot must form soon. The period before blood flow stops is called the **bleeding time** and is normally two to six min. **Platelet aggregation** occurs as the released ADP activates other platelets and these in turn activate others to extend pseudopodia and become sticky and clump with those already adhering to the damaged area. This forms a plug that prevents further blood loss. Fibrin is then deposited in the loose platelet plug, which traps more platelets as well as blood cells, strengthening the clot. The **clotting time** is usually six to 12 min.

A complex cascade of reactions is required to produce fibrin. These are initiated by the binding of Factor XII (Hageman factor) to tissue collagen and anionic surfaces that somehow changes its conformation to Factor VIIa allowing it to convert prekallikrein to kallikrein. Kallikreins are serine endopeptidases that are widely distributed in mammalian tissues and body fluids. Plasma kallikrein, also known as kininogenin or Fletcher Factor, cleaves bonds in kininogen to produce a varied group of polypeptides, which include angiotensin, bradykinin, substance P and secretin. Kallikrein also activates the bound Factor XII to form Factor XIIa, in a reaction that also requires high M_r kininogen as a cofactor. Factor XIIa and high M_r kininogen activate Factor XI (plasma thromboplastin antecedent) to Factor XIa. Factor XIa also binds to the exposed surfaces of the tissue where injury has occurred. Here it catalyzes the conversion of Factor IX (Christmas factor) to Factor IXa in a reaction that requires Ca^{2+}. The final reaction, unique to the intrinsic pathway, involves the conversion of Factor X (Stuart factor) to Factor Xa, the final product, and is catalyzed by Factor IXa. This reaction also requires Factor VIII, the antihemolytic factor found on the surface of aggregated platelets and Ca^{2+}.

THE EXTRINSIC PATHWAY

The extrinsic pathway is so called because it requires the nonplasma protein, thromboplastin to initiate the cascade (*Figure 13.11*). Thromboplastin is an integral membrane protein found in many tissues but especially the walls of blood vessels, brain, lung and placenta. The initial reaction is the conversion of Factor VII (proconvertin) to Factor VIIa in a reaction that requires Ca^{2+} and phospholipids released from the injured tissue or from the surface of aggregated platelets. Damaged tissues also release thromboplastin which, in combination with Factor VIIa and Factor IXa from the intrinsic pathway, directly activates Factor X to Xa. The final result of this part of the pathway is the production of Factor Xa. Thus the final product of the extrinsic pathway, like the intrinsic, is the production of Factor Xa. Despite this, deficiencies in either pathway result in prolonged bleeding times.

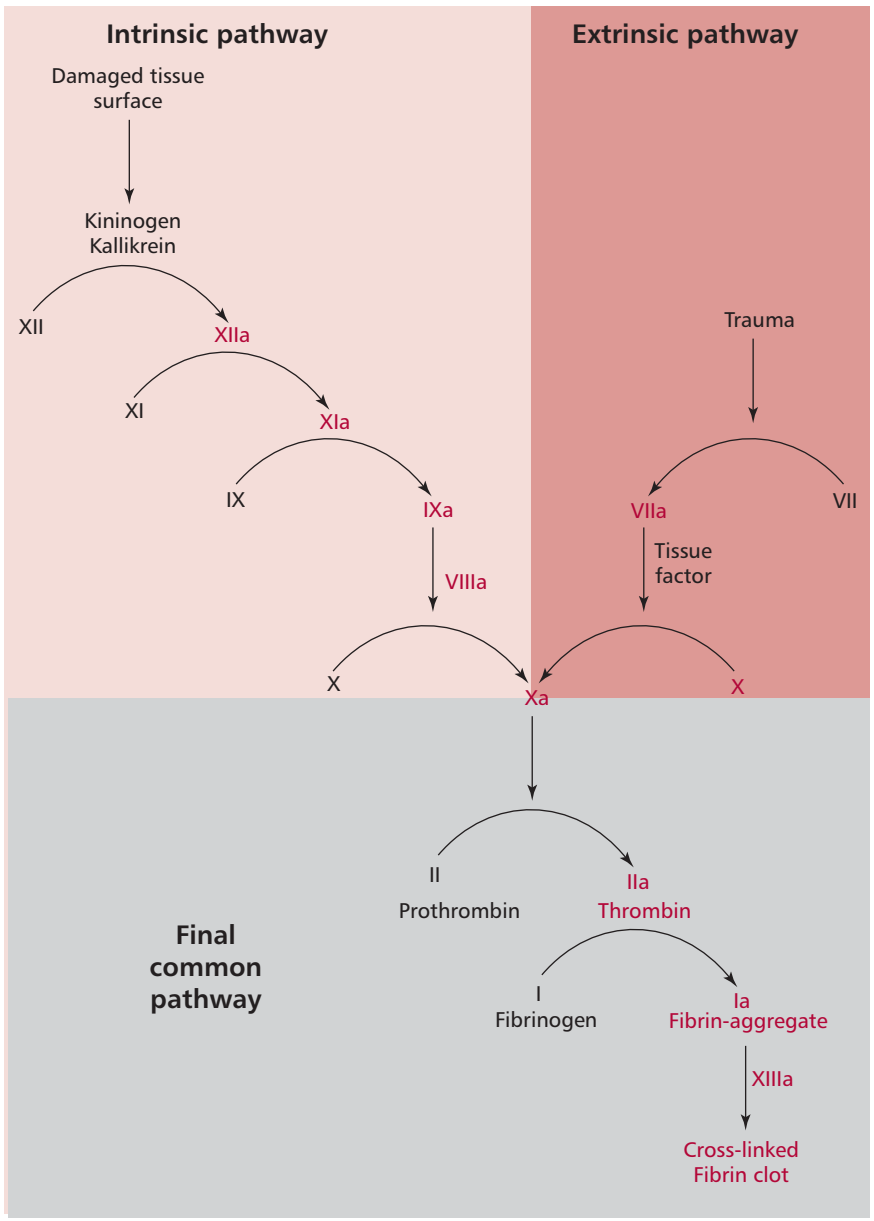


Figure 13.11 A simplified form of the blood clotting cascade consisting of the intrinsic, extrinsic and final common pathways. See text for details.

COMMON FINAL PATHWAY

Factor Xa from the intrinsic and extrinsic pathways catalyzes the hydrolysis of Factor II, or prothrombin to thrombin (*Figure 13.11*). There is an intermediate stage as prothrombin is first converted into prethrombin, which is slowly converted to thrombin. Factor Xa by itself is a relatively slow prothrombin activator, but its activity is enhanced about 20 000-fold by Ca^{2+}, Factor V (proaccelerin) and negatively charged phospholipids, for example phosphatidylserine, from damaged cell membranes. These types of phospholipids occur almost exclusively on the cytosolic sides of cell membranes, which, of course, are not usually in contact with the blood. Hence clotting reactions take place on the surface of the platelets so that the clotting action is confined to the sites of injuries.

Thrombin is a serine protease that catalyzes the hydrolysis of fibrinogen to fibrin, the final reaction of the clotting cascade. Fibrinogen forms 2–3% of the plasma proteins. It consists of three pairs of polypeptides and two pairs of oligosaccharides (*Figure 13.12*).

Thrombin cleaves four peptide bonds in the fibrinogen molecule, releasing two A and two B fibrin peptides from the amino terminal ends of the fibrinogen polypeptides changing the net charge from positive to negative. In the presence of Factor XIIIa and Ca^{2+}, the fibrin monomers polymerize producing a stable clot. The clots formed are strengthened by cross-links formed by the transaminase, Factor XIIIa, which is also activated by thrombin. Within the clot, the platelets contract, reducing the clot to less than half its original size and one that is tougher and more elastic. The process also draws the edges of the wound together.

PREVENTION OF CLOTTING

It is clearly important that clots do not form in the absence of injury. There are several systems to prevent this occurring. The protein factors constantly circulate in the blood but, of course, require specific activation before clotting can take place; in addition, the liver removes activated factors. However, this is a slow preventative measure. A more rapid mechanism is effected by antithrombin III, which is a natural clotting inhibitor that binds to all of the serine proteases of the clotting cascade, especially thrombin, and inhibits their proteolytic activities. Heparin (*Figure 13.13*), a sulfated polysaccharide found in the circulation, activates antithrombin III. In addition, the plasma protein, α_2-macroglobulin also inhibits the clotting cascade. Patients with antithrombin III deficiency have an increased risk of thrombosis and resistance to the action of heparin.

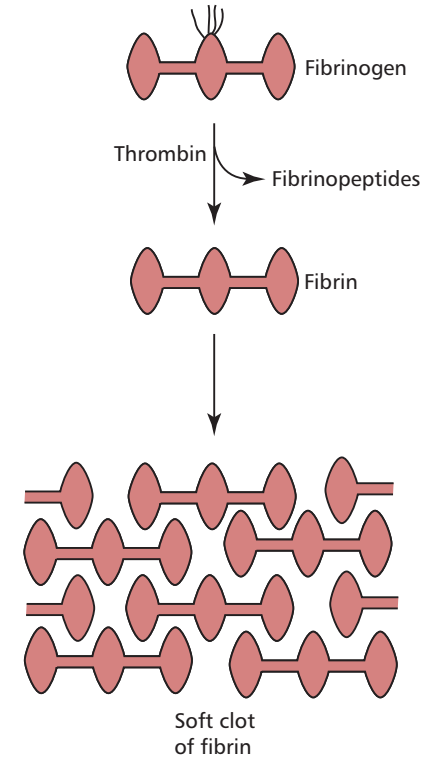


Figure 13.12 Schematic showing the conversion of fibrinogen to fibrin. The cleavage of small peptides from fibrinogen catalyzed by thrombin forms fibrin monomers that aggregate to give a soft clot (see *Figure 13.10*).

Margin Note 13.4 Fibrinogen

Fibrinogen is an **acute phase protein** whose concentration is substantially increased in certain clinical situations such as acute inflammation caused by surgery, infections and myocardial infarction.

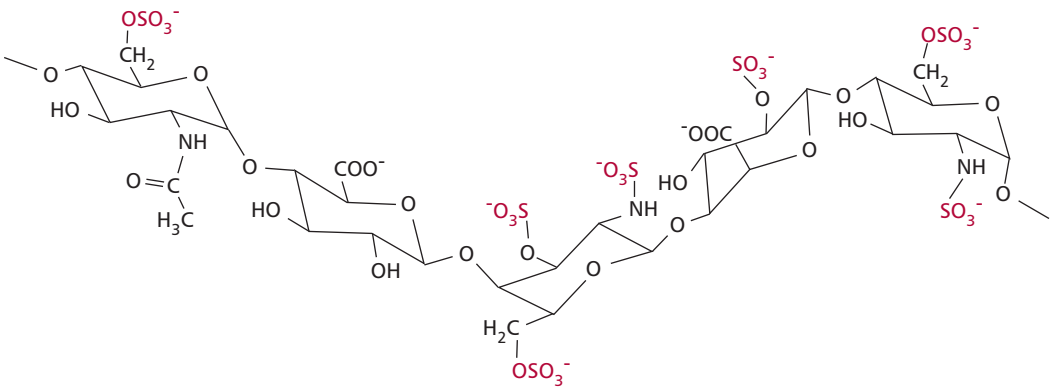


Figure 13.13 A pentasaccharide portion of the anticoagulant polysaccharide, heparin. The M_r of a complete molecule is approximately 17 000.

DISSOLUTION OF CLOTS

Clots on the skin surface eventually scab and are largely removed by abrasion. However, internal clots are eventually destroyed by a process called **fibrinolysis**. Again, a cascade of reactions is involved. Fibrin activates plasminogen activator, a protein that as its name implies, converts plasminogen to plasmin. Plasmin is a hydrolytic enzyme that catalyzes the digestion of fibrin and dissolves the clot. Plasminogen activator has a high affinity for fibrin clots and it and a tissue-type plasminogen activator (t-PA), bind to the clot and activate plasminogen.

In some instances, such as prolonged bleeding, shock and some types of cancer, plasminogen activator can be activated in the absence of fibrin. In these cases, plasminogen activators, such as streptokinase or t-PA are given to patients to dissolve blood clots to try to reduce the damage caused by myocardial infarction (*Chapter 14*).

13.5 ANEMIAS

Anemia develops when the amount of Hb in the blood falls below the reference levels for an individual's age and sex (*Table 13.4*) and there is insufficient iron for Hb synthesis. Anemia may be caused by major blood loss, or as a consequence of defects with Hb, the hemoglobinopathies, and by deficiencies of, for example, iron or some vitamins (*Section 13.6*). The characteristic signs of anemia are pallor, tachycardia, a fast heart rate, cardiac failure (*Chapter 14*) and epithelial changes including brittle nails, spoon-shaped nails, atrophy of the tongue papillae, angular stomatitis and brittle hair. Other signs specific to the type of anemia may also be present. However, anemic patients may be asymptomatic, even when the anemia is quite severe, or may present with various nonspecific symptoms, such as fatigue, headache, breathlessness, angina on effort or palpitations (*Chapter 14*). Rapid onset of anemia tends to cause more symptoms than slow onset, and the elderly tolerate anemia less well than the young when the normal compensation of increased cardiac output is impaired. The body responds to anemia with a variety of physiological responses. For example, the heart responds with an increased stroke volume and tachycardia to increase its output (*Chapter 14*). The oxygen binding curve of Hb can be modified by the production of 2,3 bisphosphoglycerate (BPG), which increases the release of O_2 from oxyhemoglobin to the tissues. In iron deficiency anemia, the concentration of BPG can increase by 40–75%.

Margin Note 13.5 Hematological indices

The measured erythrocyte values for Hb concentration, packed cell volume (PCV) and erythrocyte count (RBC) allows four hematological indices to be calculated. These are the packed cell volume (PCV), mean cell volume (MCV), mean cell Hb (MCH) and mean cell Hb concentration (MCHC). The PCV can be measured using a hematocrit (*Figure 13.14*) or an automated cell counter (*Figure 13.15*). It can also be derived as the product of the MCV and RBC. The MCV is obtained by dividing the PCV by the RBC. The MCH can be calculated by dividing the Hb concentration of whole blood by RBC. The MCHC can be obtained by dividing the Hb concentration by the PCV. The normal values for these indices are given in *Table 13.4*.

	Males	Females
[Hemoglobin] / g dm^{-3}	135–175	115–155
PCV* (hematocrit) / %	40–52	36–48
MCV* / fdm^3	80–95	80–95
MCH* / pg	27–34	27–34
MCHC * / g dm^{-3}	200–350	200–350
[Serum iron] / µmol dm^{-3}	10–30	10–30
Total iron-binding capacity / µmol dm^{-3}	40–75	40–75

*PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell Hb; MCHC, mean cell Hb concentration (*Margin Note 13.5*).

Table 13.4 Normal blood values

The reduced amount of Hb in anemia is usually accompanied by a reduction in the erythrocyte count and the packed cell volume that are usually determined in hospital laboratories using an automated blood analysis system (*Figure 13.15*). These types of systems can determine all hematological indices, for example the Hb concentration, packed cell volume (PCV) and the erythrocyte count (RBC), for numerous samples rapidly and efficiently. The mean cell volume (MCV), mean cell Hb (MCH) and mean cell Hb concentration (MCHC) can be derived from these measured values (*Margin Note 13.5*). The investigation of anemia should also consider the reticulocyte, white cell and platelet counts and any abnormal morphology as seen in a blood and/or marrow when examined with a microscope.

The three major types of anemias are the normocytic, microcytic and macrocytic, which are classified largely in terms of erythrocyte indices, especially their MCVs. Normocytic anemias, that is with normal sized erythrocytes, are associated with acute blood loss and a variety of disease states. However, the microcytic and macrocytic anemias are associated with observable changes to the sizes of erythrocytes in the blood sample and distinctive changes to the bone marrow appearance (*Table 13.5*). Thus the classification of anemias starts from routine hematological investigations. Their diagnosis involves taking a medical history and a clinical investigation of the patient, especially, of course, blood and marrow examinations to determine any changes in erythrocyte size.

Erythrocyte appearance	Diameter / μm; MCV / fdm^3	Causes
Microcytic and hypochromic (small cells, pale due to reduced Hb content)	< 7; < 80	iron deficiency, thalassemias, sideroblastic anemia, chronic disease
Normocytic and normochromic (normal size and color)	7; 76 to 96	acute blood loss, infection, collagen disease, malignancy, endocrine disease, chronic disease
Macrocytic (large cells; oval or round in shape)	> 9; 96	deficiencies of vitamins B_{12} or folate (oval), alcoholism (round), liver disease (round)

Table 13.5 A classification of anemias

MICROCYTIC ANEMIAS

The major causes of microcytic anemias are iron deficiency, thalassemias (*Section 13.6*), sideroblastic anemia and the anemia of chronic disease. Iron is difficult to absorb because of problems connected with the low solubility of its salts, its oxidation state, and interaction with other components of the diet. Loss of iron also occurs in hemorrhage and menstruation (*Box 13.1*).

Iron deficiency anemia, the commonest cause of anemia worldwide, shows a number of characteristic features. The erythrocytes (*Figure 13.16*) are microcytic, with an MCV of less than 80 fdm^3, and hypochromic, the MCH being less than 27 pg. There is variation in, cell sizes, anisocytosis and poikilocytosis, that is abnormal shapes, and a reduced reticulocyte count. There are also changes in the bone marrow, for example erythroid hypoplasia and decreased iron deposits. The serum iron decreases while the serum iron binding capacity increases compared with their normal concentration

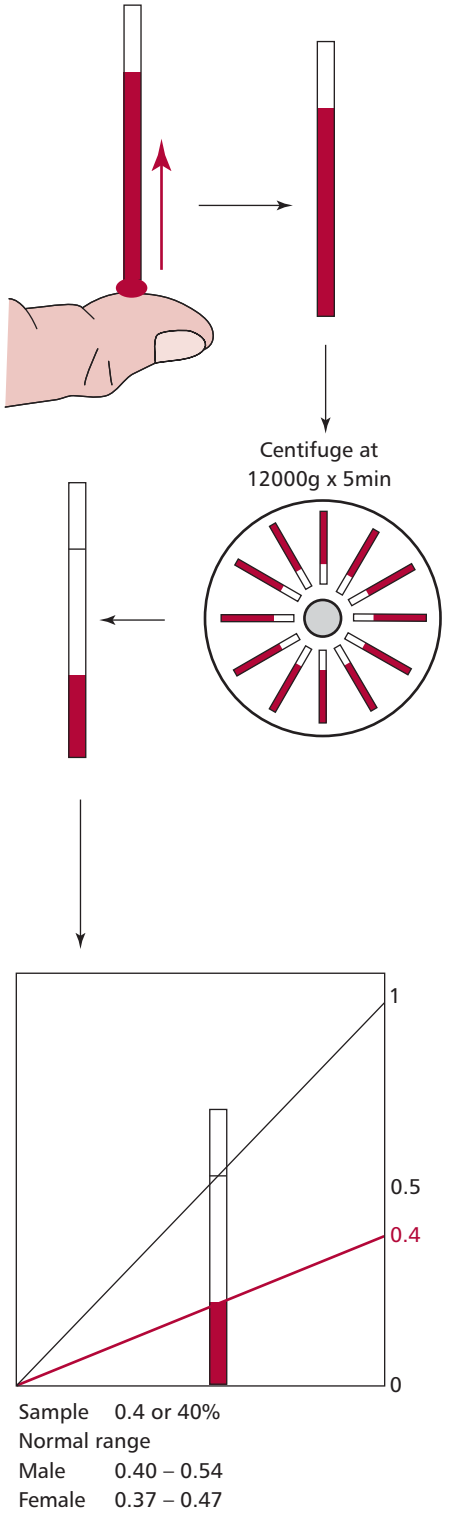


Figure 13.14 Determining PCV with a hematocrit following the collection of blood in a capillary from a thumb prick.

Figure 13.15 An automated blood analysis system that can determine all hematological indices automatically. Courtesy of Department of Clinical Biochemistry, Manchester Royal Infirmary, UK.

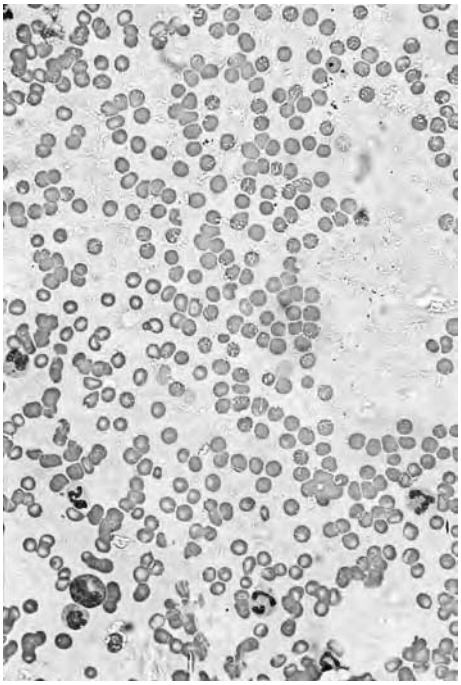


Figure 13.16 A photomicrograph of a peripheral blood smear from a patient suffering from severe iron deficiency. The cells are microcytic and hypochromic.

(*Table 13.4*). The transferrin saturation, that is the proportion of serum iron to its total iron binding capacity, falls below 19%, compared with a more usual 30% or so. The concentration of serum ferritin resulting from cellular degradation is regarded as the most reliable measurement of anemia. Patients may also show an impaired ability to maintain body temperature, depressed muscle function and abnormal thyroid hormone metabolism.

The underlying cause of the iron deficiency should be identified by the appropriate tests and taking a careful history of the patient. To counteract the deficiency, 600 mg of ferrous sulfate is given orally each day. If there are side effects, such as nausea, diarrhea or constipation, then ferrous gluconate may be substituted. Failure to respond to the treatment may be due to lack of patient compliance, continuing hemorrhage, severe malabsorption, or another cause for the anemia. It may be necessary to give iron parentally if absorption is defective, as for example in patients with ulcerative colitis or Crohn's disease (*Chapter 11*).

MACROCYTIC ANEMIA

Macrocytic anemias are characterized by the presence of anemia and erythrocytes of variable shapes but with diameters in excess of 9 μm and MCVs characteristically greater than 96 fdm^3 (*Table 13.4*). The condition may be caused by certain liver diseases, including alcoholism, that produces large rounded cells or by megaloblastic anemia, which is associated with enlarged oval cells. The latter is also indicated by the presence of the erythrocyte precursors, erythroblasts (megaloblasts) in blood. The increased proportion of immature forms of all cell lines reflects the premature death of cells in the process of development (*Figure 13.17*). The cells are large, although there is a substantial variation in size, and they have large, immature nuclei. The basis of the problem is the inability to synthesize deoxythymidine monophosphate from methylated deoxyuridine monophosphate. The methyl group is supplied by the folate coenzyme, methylene tetrahydrofolate polyglutamate and deficiency of folate reduces its supply. A deficiency of vitamin B_{12} (*Figure 13.18(A)*) also reduces its supply by slowing the demethylation of methyl tetrahydrofolate. Thus deficiencies of vitamins B_{12} or folate (*Chapter 10*) or other defects, for example genetic ones, that affect DNA biosynthesis in the bone marrow, produce an asynchrony between nuclear and cytoplasmic development and a delayed maturation of blood cells and result in megaloblastic anemia.

Vitamin B_{12}, also called cobalamin, is found in animal products and is produced by certain microorganisms but not by plants. It is liberated from protein complexes by gastric enzymes and binds to a glycoprotein called **intrinsic factor** (*Chapters 10* and *11*). This is secreted by the gastric parietal cells along with H^+ and carries vitamin B_{12} to specific receptors on the mucosal surface of the ileum. Although the vitamin enters the ileal enterocytes, the intrinsic factor remains in the lumen of the gut. Transport in the blood is by another protein, transcobalamin. Atrophy of the gastric mucosa and consequent failure to produce intrinsic factor leads to the malabsorption of the vitamin, whose deficiency results in pernicious anemia. Cytotoxic IgG antibodies directed against gastric parietal cells and/or against intrinsic factor are found in the serum in about 90% of individuals with pernicious anemia. In a majority of these individuals the antibodies are also present in the gastric juice and either prevent the binding of vitamin B_{12} to intrinsic factor or inhibit the absorption of the vitamin B_{12}: intrinsic factor complex.

The onset of the disease is insidious with progressively increasing symptoms of anemia. Patients show **achlorhydria**, a low or absence of gastric acid secretion, and lack of secreted intrinsic factor. There may be jaundice because of excessive breakdown of Hb and because erythropoiesis in the bone marrow is deficient. The serum bilirubin may be increased and the serum vitamin B_{12} concentration is usually considerably below its physiological value of approximately 160 ng dm^{-3}. However, the polyneuropathogical symptoms make it important that treatment is not delayed as they can become irreversible; patients present with symmetrical paraesthesia in the fingers and toes, an early loss of vibration sense and ataxia. Paraplegia may be the result. Pernicious anemia is predominantly a disease of the elderly with one in 8000 of the over 60 population being affected in the UK. It also seems to be associated with certain autoimmune diseases, such as thyroid and Addison's diseases (*Chapter 7*).

The causes of folate (*Figure 13.18 (B)*) deficiency are nutritional, for example a poor intake of green vegetables, such as broccoli and spinach and offal, alcohol excess, cancer, or excessive utilization in pregnancy and lactation and the use of antifolate drugs, such as methotrexate, phenytoin and pyrimethamine. The clinical manifestations of folate deficiency are megaloblastic anemia with a serum folate concentration that is lower than the reference value of 4 to 18 μg dm^{-3}.

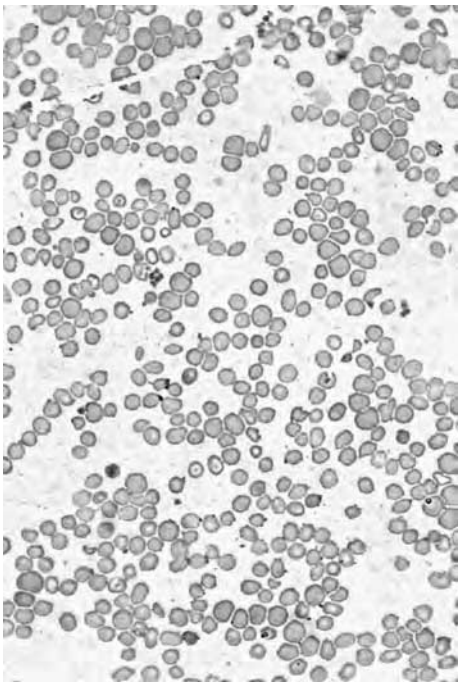


Figure 13.17 A photomicrograph of a marrow smear from a patient with megaloblastic anemia. The MCV is over 95 fdm^3 and the macrocytes are typically oval in shape. The bone marrow is usually hypercellular and the normoblasts (erythroblasts) are large and show failure of nuclear maturation with an open, fine, primitive (stippled) chromatin pattern. Giant and abnormally shaped cells called myelocytes are present.

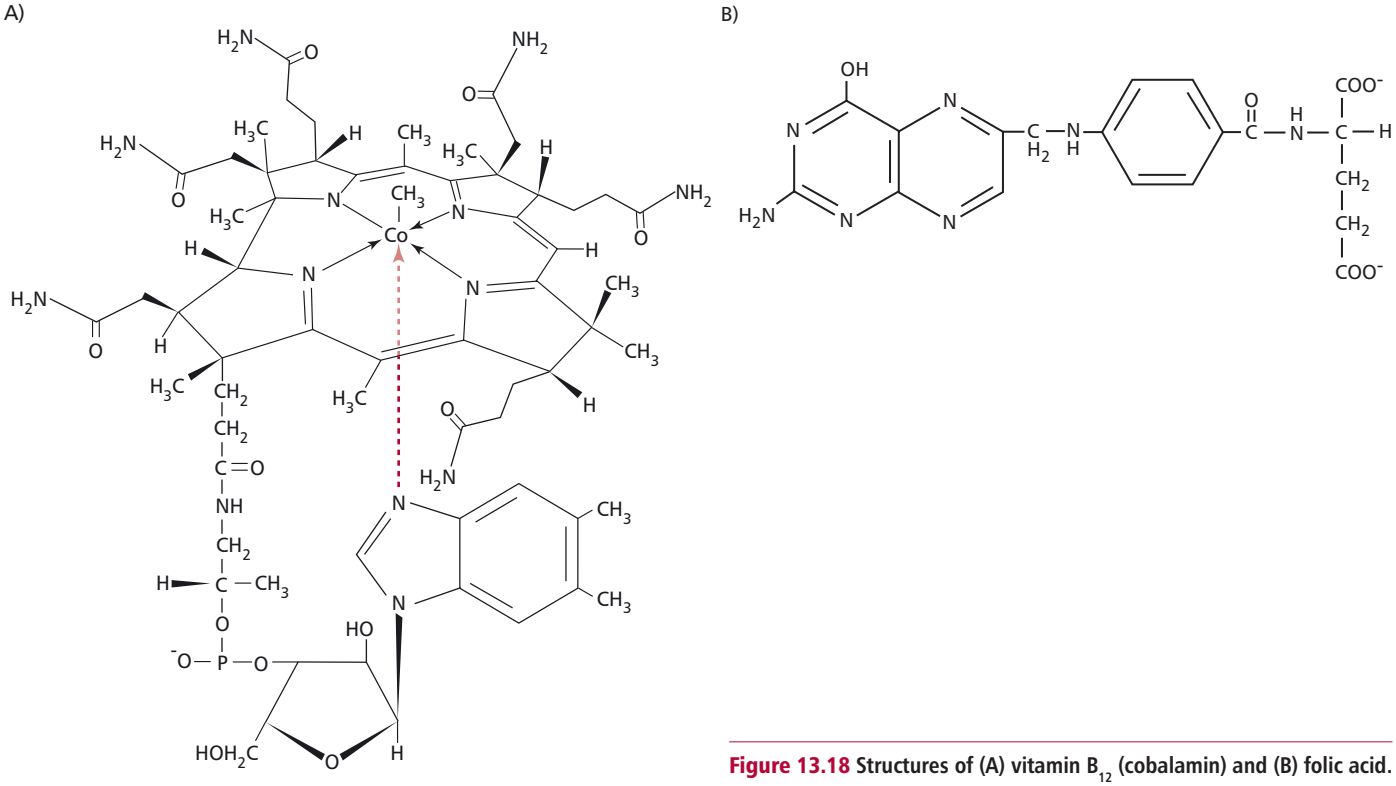


Figure 13.18 Structures of (A) vitamin B_{12} (cobalamin) and (B) folic acid.

The deoxyuridine suppression test for megaloblastic anemia is performed by adding tritiated thymidine (^{3}H-thymidine) to a sample of bone marrow. Bone marrow samples may be obtained by aspiration or by trephine. Aspiration, using a specialized needle, is usually carried out at the iliac crest with a local anesthetic. In normal marrow less than 5% of the ^{3}H-thymidine is usually taken up, but in megaloblastic marrow up to 50% of it may be used. The microscopic picture of the bone marrow can be investigated by using the aspirate to make a smear on a microscopy slide. If a larger sample of bone marrow is required, the posterior iliac crest is used but a longer and wider needle is used to obtain a 'core' of bone. This core is fixed and decalcified over several days and then stained for microscopy.

It is important to distinguish pernicious anemia from other causes of megaloblastic anemia, such as folate deficiency, because this will affect treatment. However, this is usually clear from the blood concentrations of these two vitamins. Also, the ability to absorb vitamin B_{12} can be measured using the Schilling test in which patients are given vitamin B_{12} radioactively-labeled with ^{58}Co. The urine is collected over a period of 24 h and the amount of radioactivity measured.

Vitamin B_{12} deficiency is treated by intramuscular injection of 1 mg of the pure vitamin, to a total of 6 mg over a period of three weeks. Oral administration is obviously unsuccessful in pernicious anemia because of the lack of intrinsic factor. A maintenance dose of 1 mg every three months is then given for the rest of the patient's life. Clinical improvement may occur within a few days (provided that the neuropathy has not been long-standing) and a reticulocytosis is observed a few days later. Folate deficiency can be corrected by giving 5 mg of folic acid daily, and this usually produces a rapid hematological response. Prophylactic folate is recommended for all women during pregnancy and especially for women who have had a previous child with a neural tube defect.

13.6 HEMOGLOBINOPATHIES

Hemoglobinopathies are clinical conditions that result from mutations that change the sequences of bases in DNA of the genes for globins (*Chapter 15*). If the bases in the DNA are changed even by a single one, then a modified protein may be produced (or no protein at all). The consequences can be negligible, severe or fatal. Mutations are inherited and, if the disease is not fatal, then the disease symptoms will be inherited too. The severity of the disease may depend on whether one or both copies of the gene in question carry the mutation, in other words, whether the individual is homozygous or a heterozygote. The mutations involved in hemoglobinopathies include point mutations, the largest group, that substitute one amino acid residue for another, insertions or deletion of one or more residues, drastic changes caused by frameshift mutations (*Margin Note 13.6*) and alterations in the lengths of the polypeptide chains by mutations that produce or destroy stop codons.

In normal adult humans, there are two α- and one β-globin genes, coding for polypeptides of 141 and 146 amino acid residues respectively, which go to form HbA, $\alpha_2\beta_2$. In a diploid cell there are actually four α and two β genes. Each of these genes has two introns (*Margin Note 13.7*). The α genes are located on chromosome 16 and the β genes on chromosome 11. If there is a mutation, it may have been inherited from one or both parents giving a heterozygous or homozygous condition respectively. A mutation in an α gene tends to have less serious consequences than one in a β gene because there may still be nonmutated copies of the α gene present. Nevertheless, even small changes in the structure of the Hb protein can sometimes result in disastrous clinical effects. Over 750 Hb mutations are known. They usually only affect one type of subunit because there are separate genes for the α- and the β-globins (*Table 13.2*).

Originally, many of the different mutant Hbs were identified by their mobilities in electrophoresis (*Figure 13.19*) and peptide mapping (*Box 13.3*) but now, of course, the DNA can be analyzed directly. A major technical advance has been the ability to make DNA probes that are specific for α- or β-chains. This means it is possible to identify which mRNAs are being produced and identify any mutations present. Thus the different *clinical* variants can be understood at the *molecular* level. For example, in so-called 'hemoglobin H disease' it has been shown that there is only one of the four possible α-globin genes present and functioning, so that only 25% of the normal amount of α-chain mRNA is produced. The mutation causing this situation is a deletion not a point mutation.

The majority of mutations are harmless and therefore do not produce a hemoglobinopathy because they do not cause disease. For example, mutations

Margin Note 13.6 Frameshift mutations

In the genetic code, sequences of three bases or **codons** code for each amino acid residue. A change in one base may cause the incorporation of a different (or 'wrong') residue, such as occurs in HbS. However, if one or more bases is lost or added then the reading frame of the code is shifted. Instead of a single amino acid being changed, a totally new sequence may be produced, which may result in the production of a different protein or often no protein at all, depending upon where in the sequence the frameshift occurs.

Margin Note 13.7 Introns and exons

The majority of genes in eukaryotic cells are not continuous but are arranged in sections along the DNA of the chromosome. The coding sequences are called **exons**, and the noncoding portions in between are called intervening sequences or introns. In order to make the messenger RNA that can be translated to produce a polypeptide, an RNA transcript of the DNA is made, that is one with introns and exons transcribed, and then the intron coded sections are cut out and the exposed ends joined together in a process called splicing. This is a normal part of the processes that produce a mRNA that can be translated to produce a polypeptide in eukaryotic cells.

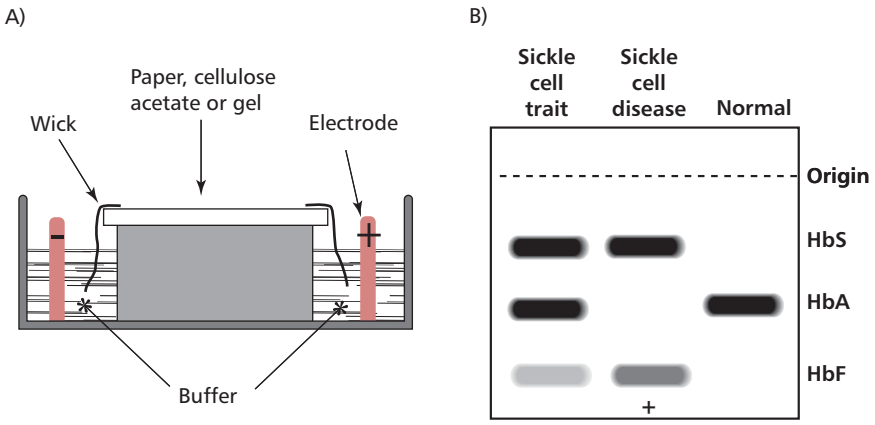


Figure 13.19 Electrophoresis to identify mutant hemoglobins that have different charges from normal adult hemoglobin. A hemolysate of erythrocytes is subjected to electrophoresis, for example sickle cell hemoglobin (HbS) moves more slowly towards the positive electrode because a glutamic acid residue in the β-chain (negatively charged) is replaced by a valine residue (zero charge) so that the whole molecule of HbS has two fewer negative charges than HbA.

Margin Note 13.8 Heinz bodies

Heinz bodies or Heinz-Ehrlich bodies were first reported in 1890 by the German physician Heinz (1865–1924) as inclusions in the erythrocytes of some patients with hemolytic anemia. They are known to be aggregates of denatured, precipitated Hb, which associate with the erythrocyte membrane (*Figure 13.20*). This causes the erythrocyte to become misshaped and leads to anemia. The bodies are best seen when the blood is stained with crystal violet. Heinz bodies are associated with certain type of hereditary hemolytic anemia, for example hemolytic anemia of infancy. Oxidative damage to Hb by a number of toxic chemicals, including nitrobenzene, diphenylamine, naphthalene and hydroxylamine and a number of its derivatives can also result in Heinz body formation. They also have iatrogenic causes and may result from sensitivity to some drugs, for example primaquine especially in glucose 6-phosphate dehydrogenase deficiency (*Section 13.7*) and sulfonamides and can appear after a splenectomy.

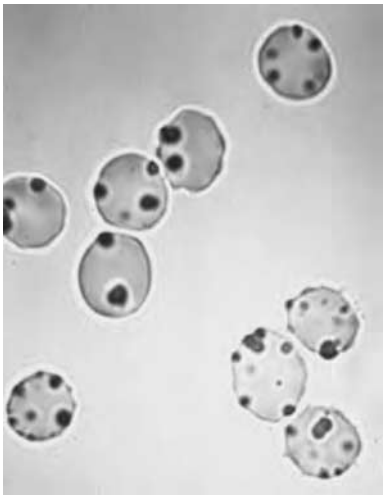


Figure 13.20 Heinz bodies in erythrocytes are formed from denatured, precipitated hemoglobin. Courtesy of Dr Ian Quirt, Department of Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, Canada.

distant from the heme binding cleft, or from the regions of subunit contact may have little effect on the properties of the Hb. However, mutations may change the shape of the globin subunit(s), the binding of the heme groups or even prevent globin synthesis, all with severe clinical consequences. To function properly, the four subunits in the Hb molecule must fit together tightly but still produce a molecule that is flexible. The regions of contact have been conserved in evolution and are essential for normal functions, such as the cooperative binding of O_2 (*Figure 13.7*). Thus mutations can upset the delicate balance of interactions between the amino acid side chains with several consequences. The molecule may dissociate upon deoxygenation and, in some cases, the monomers may precipitate in the erythrocytes reducing O_2 affinity. Microscopically, the denatured and precipitated Hb can be seen as Heinz bodies (*Margin Note 13.8* and *Figure 13.20*). A deletion of one or more amino acid residues or substitution mutations can produce this effect, as in Hb Leiden and Hb Philly respectively. There may also be cell membrane damage, with intravascular hemolysis, anemia, reticulocytosis and splenomegaly as consequences. In other cases, a small change in the regions that bind the heme groups may make the pockets slightly less hydrophobic so that it does not bind appropriately, and again, the denatured Hb can precipitate to form Heinz bodies. Thus only two of the four subunits may have heme groups. In other cases, the change in the pocket allows the iron to become oxidized to the Fe^{3+}(III) state (methemoglobin), which will not bind O_2. The resulting condition is referred to as methemoglobinemia and patients become cyanosed because they lack oxygen (*Margin Note 13.2*).

This chapter will concentrate on two hemoglobinopathies, **sickle cell anemia**, which arises from a point mutation, and a group of diseases called the **thalassemias** that seem to originate from point mutations or very small deletions.

SICKLE CELL ANEMIA

Sickle cell anemia was first described in a black patient in the USA in 1904, a time when little was known of the structures of proteins. The patient presented with severe pain and a microscopic examination of a blood sample showed sickle shaped erythrocytes (*Figure 13.21*). The gene for sickle cell Hb (HbS) differs from that for HbA by a single point mutation in the β-globin gene at the codon responsible for the amino acid residue at position 6. This substitutes a thymine for an adenine base. Given that one β-globin gene is inherited from each parent, the condition may be homozygous (Hb^SHb^S) or heterozygous (Hb^AHb^S).

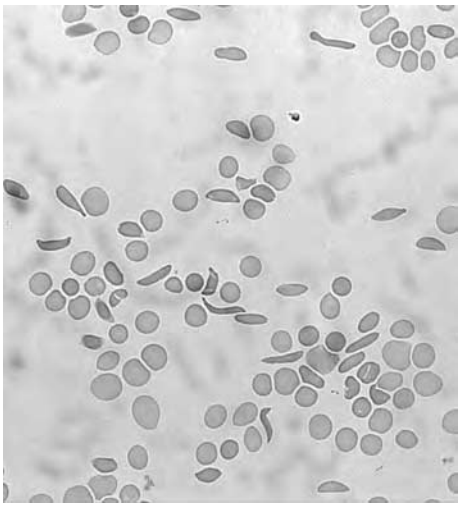


Figure 13.21 A photomicrograph of a blood smear from a patient with sickle cell anemia. Note the sickled erythrocytes.

BOX 13.3 Sickle cell anemia: a molecular disease

The identification of the precise mutation in sickle cell disease was a significant step in the understanding of molecular diseases. The use of peptide mapping in two dimensions on large sheets of chromatography paper enabled the differences between the HbA and HbS to be identified. The change from a glutamate to a valine residue means the β-globin molecule has lost one negative charge and become more hydrophobic leading to sickle cell anemia, as described in the main text:

Hemoglobin A …Pro-**Glu**…

Hemoglobin S …Pro-**Val**…

PEPTIDE MAPPING

The sequence of amino acids in a protein may be determined by hydrolyzing the polypeptide into small fragments, for example by digestion with trypsin, and then separating the fragments and determining the sequence of each. It is relatively easy to determine the amino acid sequence of short peptides. The sequences of these then have to be aligned to give the sequence for the complete polypeptide.

The traditional way to separate many short peptides was two-dimensional separation on a large sheet of filter paper. The separation was usually by electrophoresis in one dimension, followed by chromatography in the second to produce a peptide map, sometimes called a fingerprint. The colorless peptides were located on the paper by staining them with ninhydrin. When a point mutation has occurred, it is often possible to observe that just one peptide has changed its position provided that the mutation has produced a change in charge, as is the case with HbS (*Figure 13.22*), or that the substitute amino acid residue is substantially different in M_r to the original. The stained 'spots' can then be cut out and the peptide eluted from the paper and its sequence determined. This method is no longer used. Typically HPLC is now used to separate and purify the small peptides and the use of mass spectrometry can give the sequence quickly.

The identification of the precise defect in the mutant Hb in sickle cell patients as an adenine to thymine mutation in codon 6 of the β-globin gene was a major step in the understanding of genetic diseases. The condition was the first one to be referred to as a *molecular disease*.

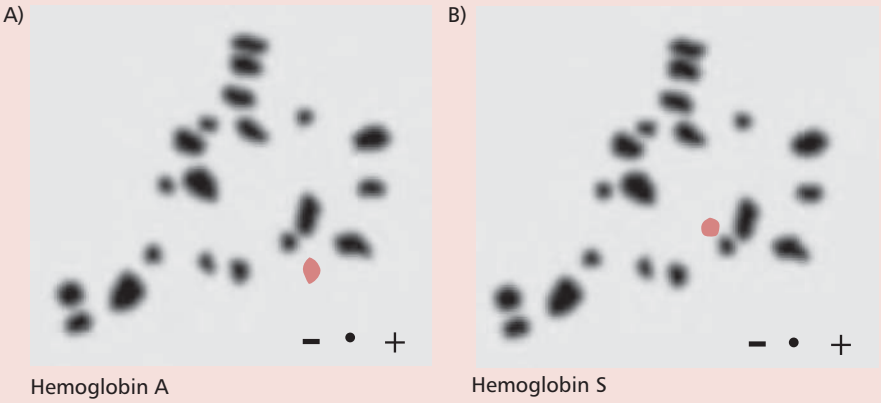


Figure 13.22 Peptide mapping to show the difference, highlighted in red, between (A) normal and (B) sickle cell hemoglobins. See text for details.

The mutation means that an acidic, hydrophilic glutamate residue is replaced by a hydrophobic valine. The presence of the valine residue means that the Hb molecule is a little more hydrophobic or 'sticky' in two places on its surface because there are two β-chains present. The sticky patches are more exposed in the deoxygenated state when the conformation of HbS changes as the molecule releases its oxygen in the tissues. The HbS molecules therefore aggregate forming stiff fibrils that cause the sickling of the erythrocytes although, even after years of study, it is still not completely understood how these changes occur. The deformed erythrocytes are less flexible than normal ones and cannot squeeze through the capillaries in the tissues and block them. This leads to hemostasis, anoxia and severe pain and, because the sickling occurs as a result of changes occurring in deoxyhemoglobin, the effects are exacerbated and more cells become sickle-shaped. The life of a typical erythrocyte is reduced from 120 to about 10–12 days in sickle cell patients: the abnormal cells are destroyed in the spleen and consequently anemia ensues.

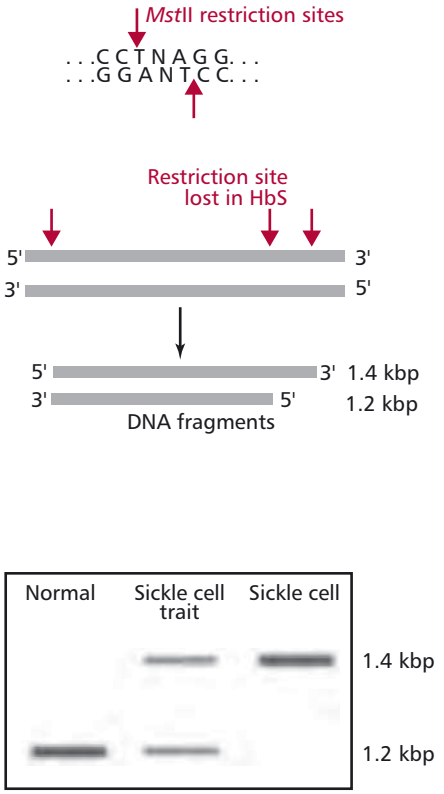


Figure 13.23 The mutation in sickle cell anemia changes one nucleotide base in the gene for the β-globin producing a new site, which the restriction enzyme, *Mst*II cannot attack. Consequently, different sized fragments of DNA are produced from normal compared with sickle cell hemoglobin genes. Gel electrophoresis separates the fragments, which may be detected with a probe for the β-globin gene to reveal the fragments of different sizes. This technique can also be applied to the prenatal diagnosis of sickle cell disease.

At low concentrations, HbS shows a normal oxygen binding curve, but at high concentrations, as would occur in the erythrocytes of a homozygote for sickle cell anemia, the oxygen affinity is decreased. Again, the reasons for this are not fully understood, although the resulting shift in the oxygen dissociation curve to the right means a greater proportion of the oxygen is released and ameliorates the effects of the anemia. Indeed, the amino acid substitution that causes the condition does not affect the structure of the oxygen binding site or the ability of the molecule to bind and carry oxygen.

Patients who are homozygous for sickle cell anemia present with crises of intense pain that can occur anywhere in the body caused by blockage of capillaries. Crises tend to occur when the circulation is slow or when there is hypoxia; about 15 s of low oxygen tension are required to produce sickling so when the circulation is reasonably rapid there is insufficient time for this to happen. Clinical complications of sickle cell disease are highly variable, and the clinical consequences may include megaloblastic erythropoiesis, aplastic crises, stroke, bone pain crises, proneness to infection, especially by *Pneumococcus*, *Salmonella* and *Haemophilus* due to hyposplenism, and acute chest syndrome. Acute chest syndrome is a common form of crisis in children with sickle cell disease and is sometimes fatal. It occurs in about 40% of all people with sickle cell disease. It is characterized by severe chest pain and difficulty in breathing. It is probably caused either by a chest infection or by blocked pulmonary capillaries resulting from a blood clot. In developed countries the mortality in sickle cell disease is relatively low but this is not the case in developing countries. In general, there seems to be an approximate 10% mortality in the first few years of life but, again, this depends on the treatment available, namely whether the infant has 'Western-style' medical care. The probability of surviving to 29 years is about 84% but there are few data on longevity. Infections seem to be the commonest cause of death at all ages.

A diagnosis of sickle cell anemia may now be carried out on the DNA (*Figure 13.23*) of the embryo obtained by chorionic villus sampling or amniocentesis. The parents may then make an informed decision whether or not to continue with the pregnancy. Treatment includes analgesia for the pain during crises and antibiotics and vaccination against the likely life-threatening infections. The pain is often so acute as to require morphine. Sometimes inhalation of nitric oxide can help by producing a vasodilation but this treatment is only dealing with the symptoms. Blood transfusions are also possible but they can lead to iron overload, as well as other complications, as the transfused erythrocytes are removed from the circulation. Chelating agents such as desferrioxamine may be used. In was observed that the severity of sickle cell disease in some populations was reduced by the presence of high concentrations of HbF. Fetal Hb is almost as good as HbA in transporting oxygen and, of course, does not sickle. Hydroxyurea and butyrate are used as therapeutic agents to try to induce higher levels of HbF in sickle cell patients. Hydroxyurea is thought to kill selectively precursor cells in the bone marrow whilst sparing the erythroblasts that produce HbF. However, this compound is an antineoplastic agent and its long-term effects are unknown. Butyrate seems to activate transcription of the γ-globin gene so that HbF is produced in the adult. Both agents have met with reasonable success in treating sickle cell patients and, in some cases, may be used synergistically to increase HbF up to 20% with a marked clinical improvement.

The mutation that causes HbS production is not the only one that leads to sickling of erythrocytes but the many other variants are rather rare. The second commonest of these in black Americans occurs in HbC, in which a lysine residue replaces glutamate at position 6 in the β chain. Hemoglobin C is rather insoluble and crystals of it can sometimes be seen in peripheral blood smears. Heterozygotes for HbC are asymptomatic but homozygotes have a mild hemolytic anemia.

BOX 13.4 Sickle cell trait and malaria

Heterozygotes, those with genes for HbA and HbS, have sickle cell trait. Individuals typically have about 30% HbS and life expectancy is about the same as for normal persons. The condition causes relatively few problems except at high altitudes and when flying in nonpressurized aircraft. Sickle cell disease, the homozygous condition, is present in approximately 8% of American blacks and may be as high as 45% in some African populations. It is thought to cause 60 000–80 000 deaths in African children annually. It may be asked why such a deleterious gene should have persisted. The answer is probably that the possession of a single HbS gene, that is sickle cell trait, increases resistance to malaria caused by *Plasmodium* spp (*Chapter 2*). Malaria is typically endemic in areas where the sickle cell trait reaches high levels (*Figure 13.24*). The same may also be true of thalassemia (*see below*). The reason for resistance seems to be that as *Plasmodium* parasites grow in the erythrocytes, they lower the intracellular pH and generate hydrogen peroxide. The lower pH promotes sickling of the erythrocytes and the hydrogen peroxide damages cell membranes, which become more permeable to K^+. The resulting intracellular decrease in K^+ kills the parasites.

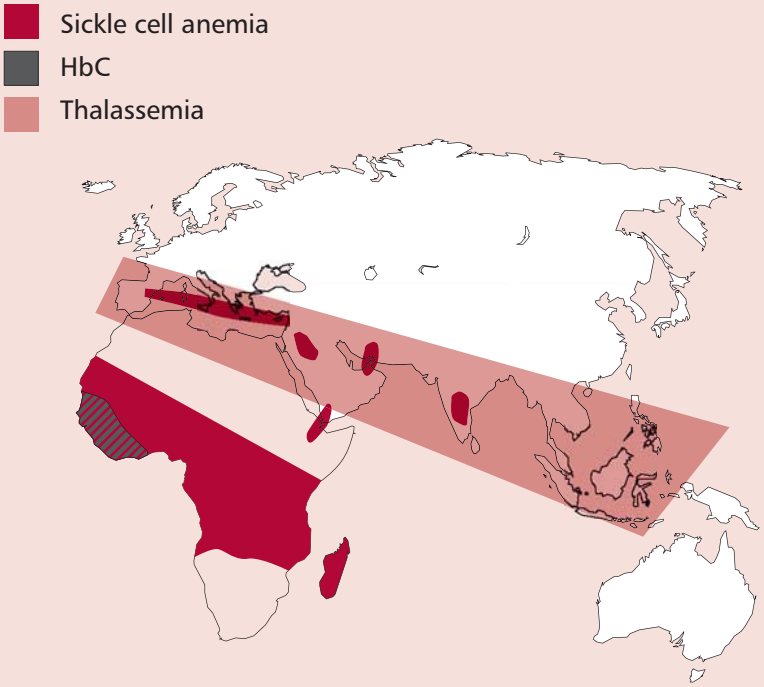


Figure 13.24 Areas of the world where sickle cell disease and thalassemia are common.

As a result of the coincident distribution of the genes for HbS and HbC, heterozygotes for both Hbs are not uncommon producing HbSC disease. This is milder than true sickle cell disease but patients can show practically all the same complications. Furthermore, it is symptomatic in the heterozygous state. There can also be co-inheritance of the sickle cell and thalassemia genes, which generates a wide spectrum of clinical symptoms whose severity depends on the type of thalassemia mutation (*see below*).

THE THALASSEMIAS

Thalassemia, from 'thalassa', which is Greek for 'the sea', are a group of hemoglobinopathies originally discovered in people living near the

Mediterranean sea. However, the thalassemias are also relatively common in southeastern Asia, the Philippines, China and worldwide and is perhaps the most common group of hereditary diseases. Cooley first accurately described them clinically in 1925 and the disease used to be called Cooley's anemia, a term now reserved for β-thalassemia.

In the late 1930s, thalassemia was shown to be an inherited disorder. However, it was not until protein analytical techniques improved in the 1960s that the disease was shown to be the result of an imbalance in the amounts of α- and β-globins synthesized. The severe anemias associated with some thalassemias stimulate the production of erythrocyte precursors. As a result, bone marrow is able to expand to all areas of the skeleton leading to skeletal deformities. If this occurs within the spine and compresses the spinal cord it can cause intense pain. Some forms of the disease are fatal causing death *in utero*, while others require copious blood transfusions for the anemia. α-Thalassemia, for example, is caused by a complete or partial failure to produce α-globin. It is fatal in its severe form when α-chains are not produced. In β-thalassemia, a partial or complete failure to produce β-globin means that patients require lifelong blood transfusions.

There are a number of forms of the disease because there is more than one gene for the globin polypeptides and a mutation may not affect all of them. Consequently there may not be complete absence of a given globin chain and the disease will be less severe. Thalassemias are classified according to which globin chains are reduced in amount, are mutated or are absent. The more copies of the gene are missing or inactive, the greater the severity of the disease. The anemia is caused by an ineffective erythropoiesis and from precipitation of excess free globin within the erythrocytes. The cells have a shortened life span and the spleen removes the abnormal erythrocytes leading to splenomegaly.

α-Thalassemias can vary from a condition in which only one α-globin chain is missing, producing a 'silent' mutation that is practically a symptom-free, carrier state, to other forms where two, three or all four genes are absent or inactive (*Table 13.6*). The presence of a single functional α-globin gene is usually sufficient to preclude serious morbidity. A decreased synthesis of α-globin leads to the formation of two abnormal Hb tetramers. Hemoglobin Barts is found in umbilical cord blood and arises because of a lack of α-chains but normal production of the fetal γ-chains; thus Hb Barts is γ_4. If the infant survives, β-chain synthesis begins and β_4 (HbH) tetramers form. Unfortunately, neither γ_4 nor β_4 can take up cooperatively O_2 but bind it so tightly it cannot be delivered to the tissues. Hemoglobin H precipitates in older erythrocytes

Syndrome	Genotype	Number of α-genes	Clinical severity	Hemoglobin
Hydrops fetalis	--/--	0	lethal *in utero*	mostly γ_4, little β_4
HbH disease	--/-α	1	severe microcytosis	about 25% HbA, mostly β_4
α-thalassemia trait	--/αα or -α/α-	2	asymptomatic	variable
Silent carrier	-α/αα	3	none	functional

Table 13.6 Characteristics of α-thalassemias

and under oxidant stress, such as when the patient is treated for malaria with primaquine. A total absence of a α-globin gene results in the lethal *in utero* condition, hydrops fetalis.

Some mutations that produce thalassemia are referred to as nondeletion mutations. The mutation occurs in the STOP codon for the α-chain, consequently translation continues beyond the normal end point to the next STOP codon, extending the polypeptide by 31 residues. An example of this produces Hb Constant Spring, the incidence of which is fairly common in southeastern Asian populations where the usual STOP codon UAA is mutated to CAA. It appears that the extended mRNA for the α-chain is unstable and leads to a reduced rate of Hb synthesis. If this mutation is present together with a lack of one of the α-globin genes, then HbH disease results. However, in heterozygotes only about 1% of the α-globin produced is the Constant Spring mutant type.

β-Thalassemias result from mutations in the β-globin genes. In some ways, it would be more appropriate to call them *'thalassemias* of the β-globin gene family' because the genes for δ-, and γ-globins and the single β-globin are all grouped together on chromosome 11. In β-thalassemia there is reduced synthesis of β-globin with or without a reduced synthesis of δ- or γ-globins. β-Thalassemia is not a single disease. The molecular defects in this and related disorders are highly heterogeneous, with about 200 mutations having been identified to date. These include many single nucleotide substitutions that affect the expression of β-globin genes. Examples include nonsense and frameshift mutations in the exons, point mutations in the intron–exon splice junctions and mutations in the 5′ region and the 3′-polyadenylation sites. The latter are extended sequences of adenine nucleotides at the 3′ end of mRNA molecules that stabilize the mRNA molecules and help in their transport from the nucleus to the cytoplasm. A number of heterozygous states are also known but these usually only give rise to mild clinical symptoms. In some forms of β-thalassemia, abnormal δ-β fusion polypeptides may be formed. For example, in Hb Lepore there is a fusion gene caused by nonhomologous crossing over between the δ-gene on one chromosome and the β-gene on the other. Thus normal β- and δ-genes are absent.

β-Globin chains are not required until after birth (*Figure 13.9*) and β-thalassemia infants are usually born normally at term. Clinical problems begin two to six months later, when γ-globin synthesis, and therefore the amount of HbF, has declined. Consequently β-thalassemia is a crippling disease of childhood, characterized by the precipitation of excess α-globin chains, destruction of erythrocytes in the bone marrow and circulation, and deficiency of functional Hb tetramers. In β-thalassemia major, formerly Cooley's anemia, β-globin synthesis is strongly depressed or absent causing massive erythroid proliferation and skeletal deformities.

Thalassemias can be diagnosed from their general clinical symptoms and the anemia, including the precipitation of excess free globin chains. Hypochromic erythrocytes with a clear center and a darker rim containing the Hb are visible in blood smears as are poikilocytes, which are abnormally shaped cells produced by the spleen when it removes target cells. There is also splenomegaly. Electrophoresis of hemolyzed erythrocytes will provide information about the relative proportions of A, A_2 and F globins. In HbH disease, the Hb (tetramer of β-globin) is detected as a rapidly moving band at pH 8.4.

The treatment for thalassemias, as with sickle cell disease, is to give repeated blood transfusions combined with chelation therapy to remove the iron with, for example, desferrioxamine. The latter is necessary because the body has no real excretion route for iron, and an iron overload may be fatal due to deposition cardiomyopathy by the second decade of life. Transfusions may also put the patient at risk from hepatitis and AIDS, especially in developing

countries. Other treatments, such as giving hydroxyurea to augment HbF synthesis, are also used (*see above*) and local irradiation may bring immediate relief when expansion of bone marrow has happened.

In general, β-thalassemias are more severe, cause more patient suffering and are more expensive to treat because more medical intervention is required, than is the case with α-thalassemias.

13.7 GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD)

Glucose 6-phosphate dehydrogenase is the first enzyme of the pentose phosphate pathway (PPP), sometimes called the hexose monophosphate shunt, of glucose metabolism. It catalyzes the reaction:

$$\text{Glucose 6-phosphate} + \text{NADP}^+ \rightarrow \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

The PPP offers an alternative route from glycolysis and the TCA cycle for the complete oxidation of glucose. Erythrocytes can carry out glycolysis and generate ATP but have no mitochondria and so cannot use the TCA cycle. Their pentose phosphate pathway is also necessary to produce the NADPH, a form of reducing power essential for a number of metabolic activities. The gene for glucose 6-phosphate dehydrogenase is on the X chromosome and G6PD is therefore a sex-linked condition affecting males (*Chapter 15*). It is, however, carried by females who have half the normal level of the enzyme. Female carriers, like sickle cell patients, are more resistant to the malarial parasite, presumably because the host cells provide a less suitable habitat for the malarial parasite and/or because the cells lyse before the parasite can mature. However, the presence of G6PD in males, who only have one X chromosome, or in homozygous females, has little antimalarial effect for reasons that are not clear.

Genetic deficiency of glucose 6-phosphate dehydrogenase (G6PD) is common in Africa, the Middle East, South East Asia and the Mediterranean region. It is estimated that about 400 million people are affected making it the most common inherited disease. Many hundreds of different mutations are known but the commonest is the African or A type present in about 11% of blacks. The degree of deficiency is mild with enzyme activity being about 10% of usual levels and erythrocytes can manufacture sufficient NADPH under normal circumstances.

Several hundred different mutant variants of the enzyme are known that are unstable or have abnormal kinetics resulting in a reduced enzyme activity. Erythrocytes are most severely affected in G6PD because they have a long life in circulation and cannot carry out protein synthesis to replace the defective enzyme. However, most patients can make enough NADPH under normal conditions and the defect may only become apparent when the person takes a drug, such as the antimalarial, primaquine (*Table 13.7*), that greatly increases the demand for NADPH. Many different drugs besides antimalarials, that require NADPH for their detoxification, can bring on a crisis. In individuals with a severe form of the disease, oxidative stress may lead to severe hemolytic anemia with a loss of 30–50% of the erythrocytes. Heinz bodies (*Margin Note 13.8* and *Figure 13.20*) may be present. The urine may turn black because of the high concentrations of Hb and its degradation products, and a high urine flow must be maintained to prevent renal damage.

The requirement for NADPH relates to the need for glutathione (GSH) a sulfur containing tripeptide that was met in *Chapter 12*, also *18*. Glutathione contains a thiol (–SH) group that is readily oxidized (*Figure 13.25*). A major function of glutathione in erythrocytes is to eliminate hydrogen peroxide, H_2O_2, which is

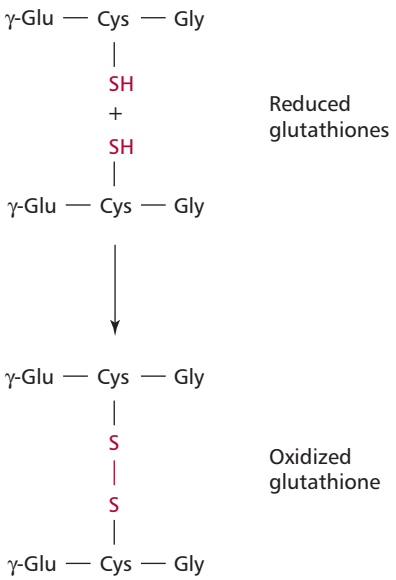


Figure 13.25 Structure of glutathione (GSH), a γ-linked tripeptide of glutamate, cysteine and glycine residues. Oxidation of the SH groups between two GSH molecules produces one molecule of the oxidized form (GSSG). See also *Figures 12.6* and *18.4*.

Group	Examples
Antimalarials	pamaquine, primaquine, pentaquine, atabrine, quinine*
Analgesics	aspirin (high dose), phenacetin
Antibacterials	chloramphenicol*, nitrofurantoin, furazolidine
Sulfonamides	sulfacetamide, sulfanilamide, sulfapyridine
Sulfones	dapsone, thiazolesulfone, diphenylsulfone
Arsenicals	neoarsphenamine
Chemicals	methylene blue, naphthalene, phenylhydrazine, toluidine blue, nitrite, ascorbic acid (large doses), nalidixic acid

*only in the Mediterranean type.

Table 13.7 Examples of drugs and chemicals that can cause acute hemolytic anemia in individuals with G6PD deficiency

a toxic product of a number of reactions. Hydrogen peroxide can react with unsaturated fatty acids in the erythrocyte membrane forming hydroperoxides, damaging the membrane (*Chapter 12*) and leading to premature cell lysis. The peroxides are eliminated by the action of glutathione peroxidase, which requires glutathione as a reducing agent:

$$2GSH + R\text{-}O\text{-}O\text{-}H \xrightarrow{\text{Glutathione peroxidase}} GSSG + ROH + H_2O$$

reduced glutathione — peroxide — oxidized glutathione

There is only a limited amount of glutathione in the cell and the reduced form must be regenerated. This takes place in an NADPH-requiring reaction, catalyzed by glutathione reductase:

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+$$

Consequently a steady supply of NADPH is required for erythrocyte integrity.

Glucose 6-phosphate dehydrogenase activity is highest in the immature erythrocytes or reticulocytes and declines as the mature erythrocytes age. In a hemolytic crisis, the older erythrocytes are destroyed first, leaving behind reticulocytes and so giving a higher reticulocyte count. Consequently, measuring the G6PDH activity in the erythrocytes following a crisis can lead to spuriously high values. If the patient survives the crisis, with or without transfusions, recovery will usually occur quite rapidly as the reticulocyte count increases even if the individual continues to take the antimalarial, because the reticulocytes can synthesize glucose 6-phosphate dehydrogenase.

FAVISM

Favism is a hemolytic crisis brought on by the consumption of fava or broad beans (*Vicia fava*) by some, but not all, individuals with G6PD. Infants are especially susceptible. It is not understood why only some patients are susceptible but it has been suggested that another mutation must also be present for favism to be shown. Broad beans contain small quantities of toxic glycosides, which, like the antimalarials, increase the demand for NADPH.

Margin Note 13.9 Favism

Broad beans were the only edible bean known in Europe until new species were brought from the New World. However, the philosopher Pythagoras forbade his followers to eat broad beans on the grounds that the beans contained the souls of the dead.

They are a popular item in the diet in the Mediterranean, an area where G6PD is endemic. Favism is frequently fatal unless a large volume of blood is transfused promptly.

13.8 CLINICAL ASPECTS OF CLOTTING

Clinical problems occur if the blood fails to clot as, for example, in hemophilia (*Box 13.5*), or if it clots too easily or inappropriately, as in a coronary thrombosis (*Chapter 14*). Thus analytical methods to clinically investigate the clotting abilities of blood samples are necessary. These blood samples require the addition of Ca^{2+} chelating agents, such as oxalate, citrate, EDTA or heparin to prevent them clotting and centrifugation to remove blood cells. The addition of excess Ca^{2+} or the removal of the chelating agents to the remaining plasma allows it to clot. Thus, the clotting of blood from patients can be studied in the pathology laboratory and any defects identified to help with the diagnosis and monitoring of treatment. For example, the prothrombin time, a measure of the activity of the extrinsic pathway, can be estimated. Thromboplastin is added to blood from the patient into a tube containing citrate to chelate the Ca^{2+} and the time taken for a fibrin clot to form noted. The thrombin time is a measure of the activity of thrombin. It is determined by adding thrombin to plasma and waiting for the clot to form. It is also possible to measure the activity of the intrinsic pathway by estimating the partial thromboplastin time. Calcium ions and phospholipids are added to plasma which is then exposed to a surface to activate Factor XII and the time taken for a clot to form is noted.

VITAMIN K AND ANTICOAGULANTS

Like most of the other blood clotting proteins, prothrombin is synthesized in the liver in a process that requires vitamin K, one of the fat-soluble vitamins (*Chapter 10*). A lack of vitamin K leads to the production of abnormal prothrombin that is activated by Factor X at only about 1% of the normal rate. Normal prothrombin contains 10 γ-carboxyglutamate residues. Vitamin K (*Figure 13.26 (A)*) is a cofactor required for the enzymic conversion of the relevant glutamates in the protein to the γ-carboxyglutamate residues (*Figure 13.26 (B)*). Dicoumarol and warfarin (*Figure 13.27 (A)* and *(B)*) are competitive

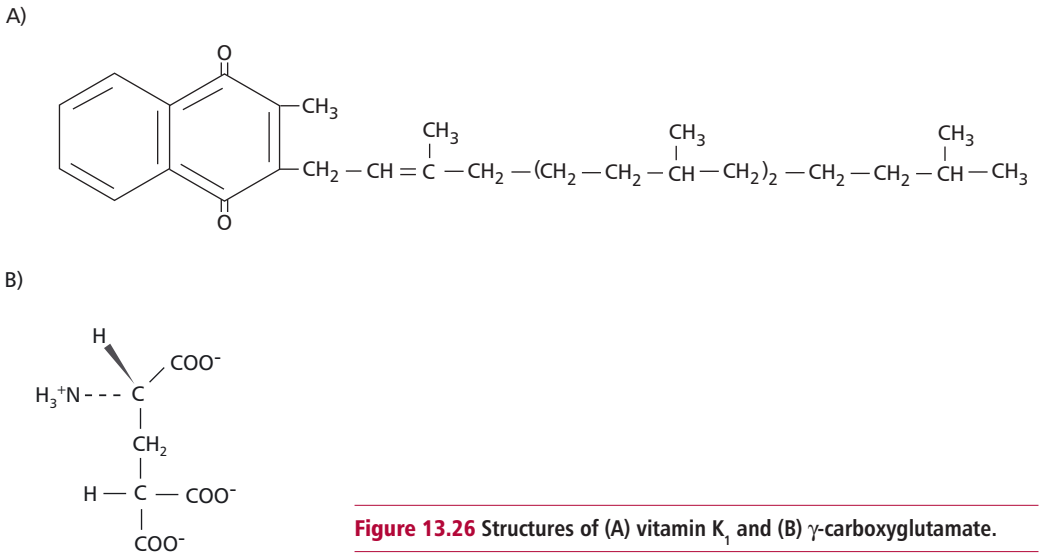


Figure 13.26 Structures of (A) vitamin K_1 and (B) γ-carboxyglutamate.

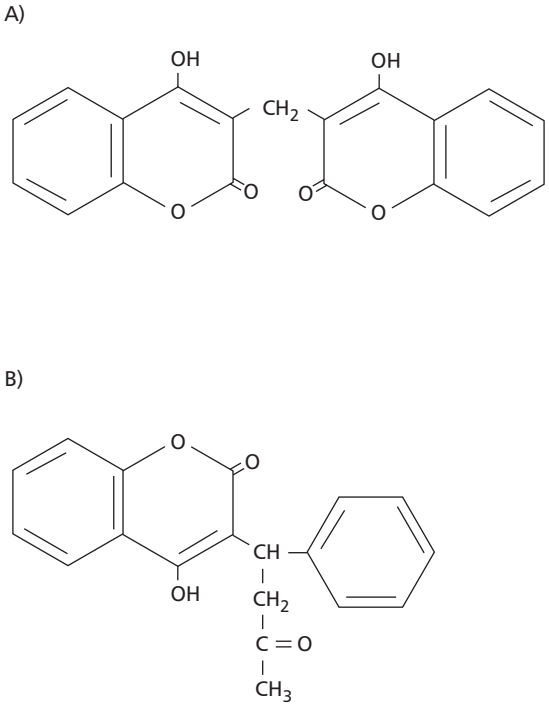


Figure 13.27 Structures of the clotting inhibitors (A) dicoumarol and (B) warfarin.

inhibitors of this process. Dicoumarol was first discovered in spoiled sweet clover because it causes fatal hemorrhages in cattle, and warfarin was originally developed as rat poison. Agents such as warfarin are used for anticoagulant therapy. They are slow to produce effects but they have long plasma half-lives and so can be active for days (*Chapter 14*).

HEMOPHILIA

The bleeding disease, hemophilia, is caused by a genetic lack of clotting factors. In these disorders, the bleeding time is normal but the clotting (or coagulation) time prolonged. The symptoms are a tendency for hemorrhages, that is blood loss, either spontaneous or from even small injuries. Some patients with hemophilia A may have a normal prothrombin time because their concentration of tissue factor is high. The commonest of the hemophilias is hemophilia A, in which Factor VIII is deficient. In north America, about 80% of the cases are hemophilia A. This deficiency is sex-linked to males, with a frequency of about one in 5000 to 10 000. The plasma levels of Factor VIII in patients with severe hemophilia A are less than 5% of normal. Hemophilia B, the second commonest form of hemophilia, is due to a lack of Factor IX. Factor IX is also called Christmas Factor because it was first found to be missing in a patient named Stephen Christmas.

In the past, hemophilias were treated by blood transfusion. However, repeated transfusion brings the possibility of infection with HIV or hepatitis, as well as the possibility of immune reactions and iron overload. Initial treatments involved purifying the factor from human plasma obtained from pooled blood. Modern treatment involves injections of Factor VIII prepared by recombinant DNA technology. This eliminates any chance of infection but is expensive. The same treatments are applicable to hemophilia B.

BOX 13.5 Hemophilia and Queen Victoria

Hemophilia, the inability of blood to clot properly, results from deficiency in one of the clotting factor proteins of the clotting cascade. If untreated it is accompanied by internal bleeding into the joints and muscles as well as there being a risk of uncontrolled bleeding in the case of injury or surgical operation. Treatment involves injections of the purified clotting factor that is absent. The commonest form of the disease, hemophilia A, is caused by a recessive gene carried on the X chromosome leading to a deficiency of Factor VIII. Factor VIII has a half-life of 12 h, so it needs to be administered twice daily to maintain the required therapeutic concentrations in the plasma, such as after an injury or before an operation.

The incidence of type A hemophilia varies from one in 5000 to 10 000 of the male population. In males, the presence of the defective allele on their sole X chromosome means that they will show the disease, because the Y chromosome does not carry an allele of the gene. Females can have the recessive gene on one X chromosome and a normal gene on their other X chromosome and in this case their blood will clot normally. Such females are carriers. Given hemophilia affects only about 0.02%, or less, of males in the population, so the frequency of X chromosomes carrying the allele for hemophilia is about 0.0002 among human males. Therefore among human females the occurrence cannot exceed $(0.0002)^2$; so female hemophiliacs are very uncommon. Thus hemophilia affects mainly the males and is normally transmitted by a female carrier who shows no symptoms. The following genotypes are seen in:

females	X^HX^H (normal)	X^HX^h (carrier)	X^hX^h (hemophiliac)
males	X^HY (normal)	X^hY (hemophiliac)	

The human gene for Factor VIII was cloned in 1984. It is an enormous gene of 186 kilobases forming about 0.1% of the DNA in the X chromosome. It is subjected to various genetic defects, including deletions, point mutations and insertions. Spontaneous mutations in the Factor VIII gene are fairly common.

Hemophilia is associated with members of the royal families of Europe. Queen Victoria (*Figure 13.28*) appears to have received a mutant allele from one of her parents and, as shown in *Figure 13.29*, this was passed on. Prince Albert could not have been responsible because male-to-male inheritance is impossible. One of Victoria's sons, Leopold, Duke of Albany, died of hemophilia at the age of 31 and at least two of Victoria's daughters were carriers. Through various intermarriages the disease spread from throne to throne across Europe, including the son of the last Tsar of Russia.

Figure 13.28 Queen Victoria.

13.9 THROMBOCYTOPENIA AND THROMBOCYTOSIS

In thrombocytopenia there is reduced platelet production. The clotting time may be only slightly prolonged but the clot formed is soft and does not retract. The condition can result from many diseases where bone marrow does not produce enough platelets, or where they become entrapped in an enlarged spleen and destroyed, or is caused by some drugs. The symptoms of thrombocytopenia include bleeding in the skin, pinpoint bruises, bleeding gums and it can be life threatening. Suspected thrombocytopenia can be investigated by platelet counts, assessing the size of the spleen and by bone marrow biopsy. Treatments would be cessation of drugs, if that is the cause, and by transfusions of platelets.

In thrombocytosis, in contrast, there is a high platelet count and an increased chance of thrombosis. The condition can be primary, myeloproliferative disease, or secondary, for example following splenectomy or some other operations, bleeding following extreme exercise or by inflammatory diseases. Clinically patients present with bleeding disorders. The usual treatments are to give antiplatelet agents, such as aspirin or dipyridamole.

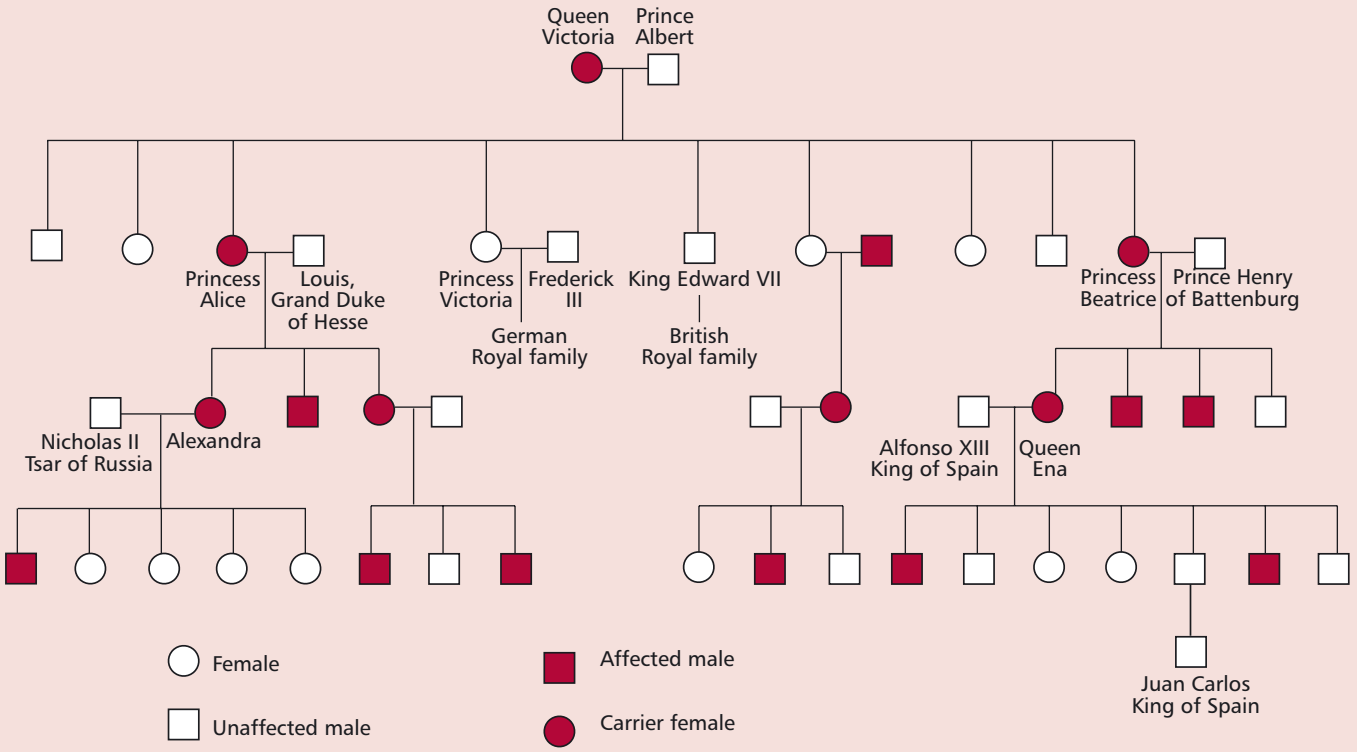


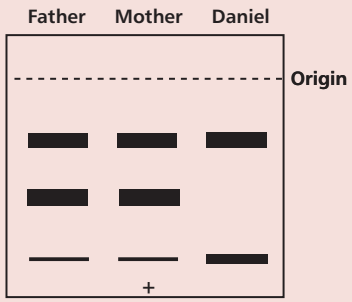
Figure 13.29 Family tree to show the inheritance of the hemophila A gene in descendants of Queen Victoria.

CASE STUDY 13.1

Daniel, a four-year-old child of Jamaican origin, was brought into the hospital because of frequent severe headaches and abdominal pain. He had also been tired and sleepy for several months. His sclerae were yellow and abdomen distended. Palpation revealed an enlarged spleen. His blood samples give the following data (references ranges in parentheses):

Erythrocyte count	2.2×10^{12} dm^{-3}	(4.4 – 5.9 × 10^{12} dm^{-3} (men))
Hemoglobin	47 g dm^{-3}	(140–180 g dm^{-3})
Serum bilirubin (unconjugated)	+++	(±)

A fresh smear of blood showed a few crescent-shaped cells and so an electrophoresis strip was run of hemolyzed erythrocytes from Daniel and both his parents, with the following result:



CASE STUDY 13.1 *continued*

Questions

(a) What is the most likely diagnosis?

(b) Outline the sort of treatment that would be appropriate.

(c) What is the chance of the parents' next child having this disease?

CASE STUDY 13.2

A 57-year-old man, Bill, lost weight and complained of weakness, shortness of breath, sore tongue and had difficulties with swallowing. On referral it was found that his skin was yellowish and his tongue was shiny and smooth. He said that he had gradually lost his appetite and recently only taken liquid foods in order to avoid abdominal pain. He also mentioned numbness and tingling of the hands. His temperature was a little above normal at 39°C. Blood index measurements give the following data (references ranges in parentheses):

Erythrocyte count	3.7×10^{12} dm^{-3}	(4.4 to 5.9×10^{12})
Total hemoglobin	82 g dm^{-3}	(135 g dm^{-3})
PCV	37%	(40–52%)

A blood smear showed large erythrocytes of unusual shape. His bone marrow sample contained basophilic megaloblasts. Gastric secretion was small in volume, 0.3 dm^3 in 24 h compared with the normal 2.5 dm^3 and contained little hydrochloric acid.

Questions

(a) Calculate the MCV, MCH and MCHC.

(b) What is the most likely diagnosis and its most probable cause?

(c) Suggest plausible reasons for the gastric problems.

(d) How should this patient be treated?

CASE STUDY 13.3

Alan has sickle cell anemia and has frequent sickling crises. His brother, Michael, is known to have sickle cell trait. However, their sister Liza seems to be normal and does not show any symptoms. However, she is about to marry and wants to start a family. She is therefore concerned to know her sickle cell status. A Hb electrophoresis on her blood was carried out and showed that she had 53% HbA, 46% HbS and 1% HbF .

Questions

(a) What molecular biology test could be carried out to confirm Liza's Hb distribution?

(b) If Liza's proposed marriage partner was heterozygous for sickle cell anemia, what are the chances that their first child would have sickle cell anemia?

13.10 SUMMARY

The blood system has many components, erythrocytes, leukocytes and platelets suspended in the complex protein rich plasma. Hemoglobin transports oxygen from the lungs to the tissues. Anemia results when the Hb level decreases and this is usually accompanied with a reduction in the erythrocyte count. Anemias may be associated with normal sized erythrocytes or ones that are microcytic or macrocytic, depending upon the cause.

Many hundreds of mutations are known that affect Hb giving rise to hemoglobinopathies. These range from relatively mild conditions to ones that are severe and life-threatening. In sickle cell anemia, the molecules of deoxyhemoglobin aggregate to form fibrils, which distort the erythrocytes, making them less flexible when passing through the capillaries. This leads to a lack of oxygen in the tissues, severe pain and hemolysis. It is due to a point mutation in the gene for β-globin. Thalassemias are a group of relatively common hemoglobinopathies, caused by the defective synthesis of the α- or β-globins. Thalassemias, again, vary from the relatively mild to very severe, depending upon the precise defect.

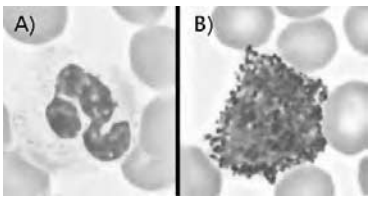
The commonest blood disease is glucose 6-phosphate dehydrogenase deficiency. Typically this is asymptomatic until the patient takes a particular drug that produces an excessive demand for glutathione in the erythrocytes and this leads to a severe hemolytic anemia.

Many defects of the blood clotting system are known. The commonest is hemophilia A, which is caused by lack of Factor VIII, one of the protein factors active in the clotting mechanism.

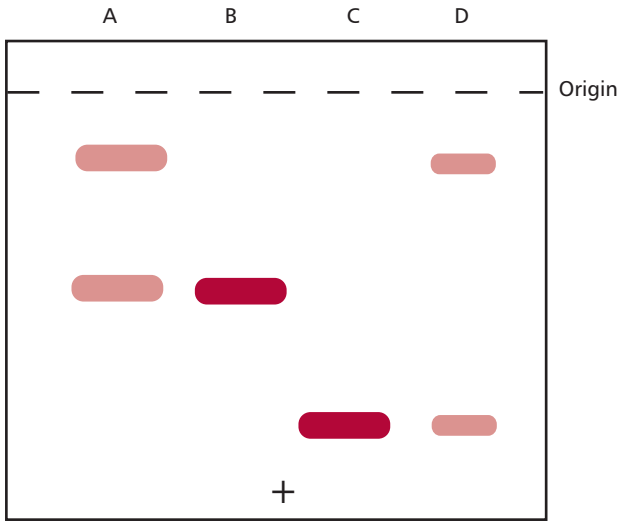
QUESTIONS

1. Which of the following statements about iron-deficiency anemia is/are true?
   (a) The erythrocytes are small and hypochromic.
   (b) The serum iron-binding capacity falls.
   (c) There is angular stomatitis and brittle hair.
   (d) Deposits of hemosiderin will be found in the liver and spleen.
   (e) The erythrocytes are microcytic in appearance.

2. Identify the blood cells, A and B, in the accompanying figure.



3. The accompanying figure shows an electrophoresis of Hb from four individuals. In sickle cell disease the change at position 6 in the β-globin chain is from Glu to Val, and in HbC from Glu to Lys. Individual C is normal, with HbA present. What can be said about individuals A, B and D?



4. Explain why hereditary persistence of fetal hemoglobin (HbF) does not have serious consequences.

5. Indicate in the following table whether the listed hematological indices are not affected (NA), decreased (D) or increased (I) in iron deficiency and pernicious anemias.

Index	iron deficiency anemia	pernicious anemia
PCV		
MCV		
MCH		
MCHC		

6. Elsa went to her doctor complaining of pain in her lower spine. The doctor noticed some deformity of her skull and facial bones. A CT scan of the bones of her spine showed an area of spinal cord compression in the upper lumbar region. Blood tests indicated severe anemia and thalassemia was diagnosed. Explain these findings and comment on possible treatment.

7. Explain the role of platelets in blood clotting.

8. Simon, a nine-month-old male infant, was brought to the Accident & Emergency Department with a painful, expanding mass in his left thigh. His mother has noticed this a few hours after he had fallen down on a hard floor, and the child was in considerable pain. An X-ray revealed that there were no fractures, but the soft swelling was shown to be a hematoma caused by bleeding into the tissues. On questioning, Simon's mother said that soon after he began to crawl Simon's knees became painful and swollen. The pediatrician suspected a coagulation disorder. Tests showed that Simon had only about 5% of the normal level of plasma Factor VIII. What is the most likely diagnosis and treatment?

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