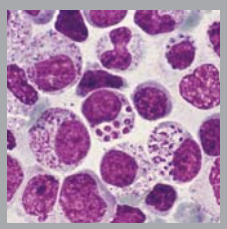

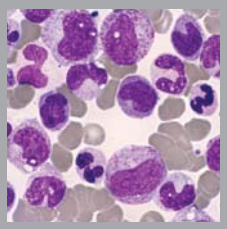

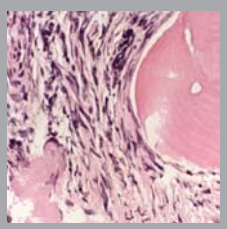

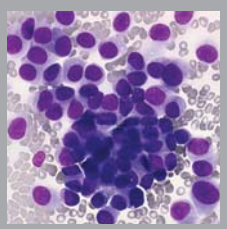

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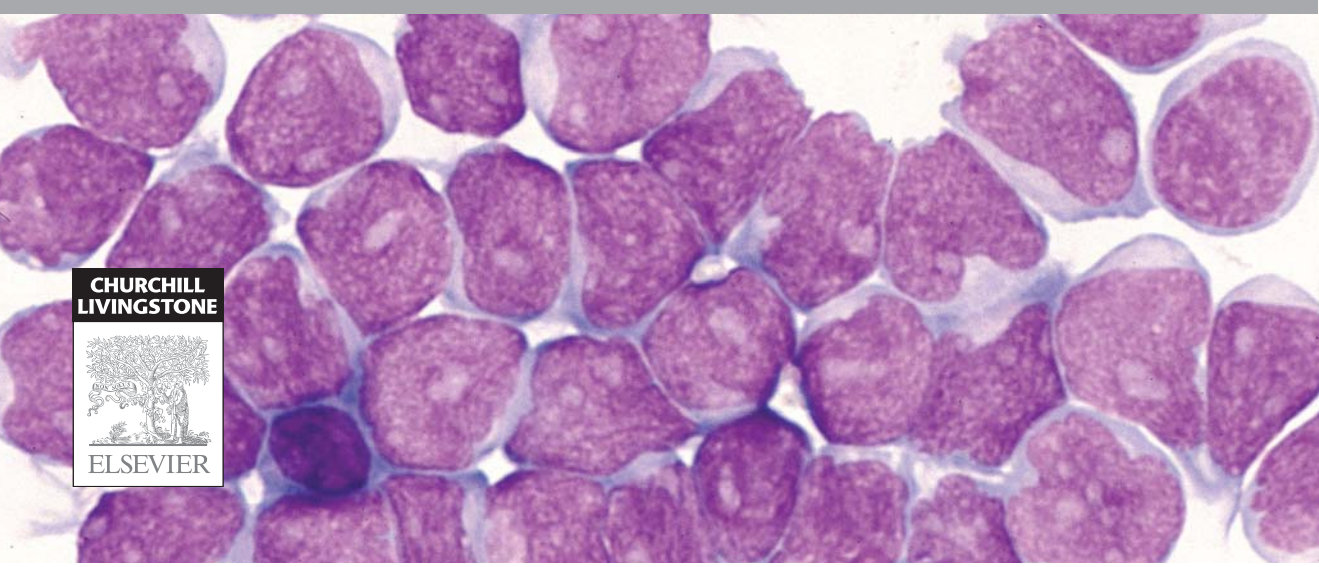

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PREFACE

The third edition of *Microscopic Haematology: A Practical Guide for the Laboratory,* maintains the standard and picture quality achieved in the second edition. The third edition includes descriptions of neoplasms according to the fourth edition of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Additional red cell disorders and white cell neoplasms, including non-Hodgkin lymphoma have been included in this edition. An additional ninety-two images have also been included.

I am indebted to a number of people for their assistance in compiling this book. I would like to thank Professor Robert Lindeman (Director of the Department of Haematology at the Prince of Wales Hospital, Sydney) for allowing me to access all the blood films and bone marrow slides in our laboratory. I am especially indebted to Michael Oakey and Virginia Bentink for their invaluable help and experience in producing a CD-ROM of all 450 images. Thank you to Pauline Dalzell for her expert assistance in updating the cytogenetics of haemopoietic and lymphoid neoplasms. Above all, I wish to thank Narelle Woodland (Senior Lecturer and Coordinator of Haematology at the University of Technology, Sydney) for her advice and continued support during my writing of this third edition.

ABBREVIATIONS

ABL1	Abelson murine leukaemia viral oncogene homolog1
aCML	atypical chronic myeloid leukaemia
add	addition
ADP	adenosine diphosphate
AIDS	acquired immunodeficiency syndrome
AIHA	autoimmune haemolytic anaemia
ALL	acute lymphoblastic leukaemia
AMEGA	amegakaryocytic
AML	acute myeloid leukaemia
AP-AAP	alkaline phosphatase-anti-alkaline phosphatase
APL	acute promyelocytic leukaemia
ATLL	adult T-cell leukaemia/lymphoma
ATPase	adenosine triphosphatase
ATRA	all-trans retinoic acid
AUL	acute undifferentiated leukaemia
BCL2	B-cell CLL/lymphoma 2
BCL6	B-cell CLL/lymphoma 6
BCL10	B-cell CLL/lymphoma 10
BCR	breakpoint cluster region
BL	Burkitt lymphoma
BM	bone marrow
B-PLL	B-cell prolymphocytic leukaemia
BSS	Bernard-Soulier syndrome
CCND1	cyclin D1
cCD	cytoplasmic cluster of differentiation
CD	cluster of differentiation
CDA	congenital dyserythropoietic anaemia
CEL	chronic eosinophilic leukaemia
CHL	classical Hodgkin lymphoma
CLL/SLL	chronic lymphocytic leukaemia/small lymphocytic lymphoma
CMML	chronic myelomonocytic leukaemia
CM	cutaneous mastocytosis
CML	chronic myelogenous leukaemia
CML-AP	chronic myelogenous leukaemia-accelerated phase
CML-BP	chronic myelogenous leukaemia-blast phase
CML-CP	chronic myelogenous leukaemia-chronic phase
CMV	cytomegalovirus
CNL	chronic neutrophilic leukaemia
CNS	central nervous system
CSF	cerebrospinal fluid
CTCL	cutaneous T-cell lymphoma
cyt-μ	cytoplasmic
DAT	direct antiglobulin test
DBA	Diamond-Blackfan anaemia
DC	dyskeratosis congenita

DEB	diepoxybutane
del	deletion
der	derivative
DIC	disseminated intravascular coagulation
DLBCL	diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
EDTA	ethylenediamine tetraacetic acid
ESR	erythrocyte sedimentation rate
ET	essential thrombocythaemia
ETV6	ETS variant gene
EWS	Ewing sarcoma
FA	Fanconi anaemia
FGFR1	fibroblast growth factor receptor 1
FISH	fluorescence in situ hybridisation
FLI1	interleukin 1 family, member 7 (zeta)
FLT3	FMS-related tyrosine kinase 3
G-CSF	granulocyte colony-stimulating factor
GP	glycoprotein
GPS	gray platelet syndrome
G-6-PD	glucose-6-phosphate dehydrogenase
HbCS	haemoglobin Constant Spring
HbF	fetal haemoglobin
HbH	haemoglobin H
HCL	hairy cell leukaemia
H&E	haematoxylin and eosin
HE	hereditary elliptocytosis
HELLP	haemolysis, elevated liver enzymes and low platelet count
HEMPAS	hereditary erythroblastic multinuclearity with a positive acidified serum test
HES	hypereosinophilic syndrome
HIV	human immunodeficiency virus
HL	Hodgkin lymphoma
HPP	hereditary pyropoikilocytosis
HS	hereditary spherocytosis
HTLV-1	human T-cell leukaemia virus (human T-lymphotrophic virus) type 1
HUS	haemolytic uraemic syndrome
i	isochromosome
IGH	IgG heavy chain Locus
IGK	immunoglobulin kappa
IGL	immunoglobulin lambda
IL3	interleukin 3
IM	infectious mononucleosis
inv	inversion
ISSD	infantile sialic acid storage disease
ITP	idiopathic thrombocytopenic purpura
JAK2	Janus Kinase 2
JMML	juvenile myelomonocytic leukaemia
KIT	V-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
LCH	Langerhans' cell histiocytosis
LDHL	lymphocyte-depleted classical Hodgkin's lymphoma

LGL	large granular lymphocyte
LPL	lymphoplasmacytic lymphoma
LRCHL	lymphocyte-rich classical Hodgkin lymphoma
MALT	mucosa-associated lymphoid tissue
MALT1	mucosa-associated lymphoid tissue lymphoma translocation gene 1
MCCHL	mixed cellularity classical Hodgkin lymphoma
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCL	mast cell leukaemia
MCV	mean cell volume
MDS	myelodysplastic syndrome
MDS/MPD,U	myelodysplastic/myeloproliferative neoplasm, unclassifiable
MDS-U	myelodysplastic syndrome, unclassifiable
MHA	May-Hegglin anomaly
MLL	mixed lineage leukaemia gene
MPAL	mixed phenotype acute leukaemia
MPN,U	myeloproliferative neoplasm, unclassifiable
MPO	myeloperoxidase
MPV	mean platelet volume
MYC	V-MYC avian myelocytomatosis viral oncogene homolog
NaF	sodium fluoride
NAP	neutrophil alkaline phosphatase
N/C ratio	nuclear cytoplasmic ratio
NEC	necrotising enterocolitis
NHL	non-Hodgkin lymphoma
NK	natural killer
NLPHL	nodular lymphocyte predominant Hodgkin lymphoma
NOS	not otherwise specified
NPM1	nucleophosmin/nucleoplasmin family member 1
NRBCs	nucleated red blood cells
NSCHL	nodular sclerosis classical Hodgkin lymphoma
PB	peripheral blood
PAS	periodic acid-Schiff
PBX1	pre-B-cell leukaemia transcription factor 1
PCH	paroxysmal cold haemoglobinuria
PDGFRA	platelet-derived growth factor receptor, alpha
PDGFRB	platelet-derived growth factor receptor, beta
PDW	platelet distribution width
Ph	Philadelphia chromosome
PK	pyruvate kinase
PLL	prolymphocytic leukaemia
PMF	primary myelofibrosis
PNH	paroxysmal nocturnal haemoglobinuria
PV	polycythaemia vera
RA	refractory anaemia
RAEB	refractory anaemia with excess blasts
RAEB-F	refractory anaemia with excess blasts with fibrosis
RARA	retinoic acid receptor alpha gene
RBC	red blood cell
RARS	refractory anaemia with ring sideroblasts
RCC	refractory cytopenia of childhood

RCMD	refractory cytopenia with multilineage dysplasia
RCMD-RS	refractory anaemia with multilineage dysplasia and ring sideroblasts
RCUD	refractory cytopenia with unilineage dysplasia
RDW	red cell distribution width
RN	refractory neutropenia
RNA	ribonucleic acid
RT	refractory thrombocytopenia
RUNX1	runt-related transcription factor 1
SBB	Sudan black B
SDS	Shwachman-Diamond syndrome
SIg	surface immunoglobulin
SM	systemic mastocytosis
SMZL	splenic marginal zone lymphoma
t	translocation
TAM	transient abnormal myelopoiesis
t-AML	therapy-related acute myeloid leukaemia
TAR	thrombocytopenia with absent radii
TCR	T-cell receptor
TdT	terminal deoxynucleotidyl transferase
TEC	transient erythroblastopenia of childhood
T-LGL	T-cell large granular lymphocytic leukaemia
t-MDS	therapy-related myelodysplastic syndrome
t-MDS/MPN	therapy-related myelodysplastic syndrome/myeloproliferative neoplasm
TP53	Tumour protein p53
T-PLL	T-cell prolymphocytic leukaemia
TTP	thrombotic thrombocytopenic purpura
WAS	Wiskott-Aldrich syndrome
WBC	white blood cell
WCC	white cell count
WHO	World Health Organization
ZBTB16	zinc finger-and BTB domain-containing protein 16

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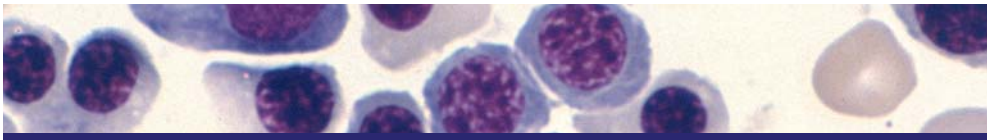


PART A

ERYTHROCYTES

A1 Erythropoiesis

NORMOBLASTIC ERYTHROPOIESIS

Erythropoiesis is divided into a number of stages. The earliest recognisable red cell precursor in the bone marrow is known as the proerythroblast; this gives rise to the basophilic erythroblast, the polychromatic erythroblast, the orthochromatic erythroblast, the polychromatic red cell (reticulocyte) and the mature red cell.

Normal erythropoiesis is characterised by the following progressive changes:

(a) Reduction in cell size

(b) Maturing of the cytoplasm: as the cytoplasm gradually acquires haemoglobin it changes from a basophilic to an eosinophilic colour. This change is accompanied by a gradual loss of RNA

(c) Maturing of the nucleus: the chromatin strands gradually become condensed and pyknotic; nucleoli are lost and the nucleus is finally extruded at the orthochromatic stage while the cell is still within the bone marrow. The resulting polychromatic red cell or reticulocyte still contains some RNA, which, after a period of 1–2 days, completely disappears and a fully haemoglobinised mature red cell or erythrocyte results.

These cell characteristics are seen in fixed preparations stained with a Romanowsky stain.

Proerythroblast

The proerythroblast varies from 12 to 20 μm in diameter and has a large nucleus that occupies most of the cell. The chromatin strands are fine, giving an even reticular appearance. Nucleoli are present. The cytoplasm is intensely basophilic—much more so than is seen in blast cells of the white cell series. Refer to Fig A1-1.

Basophilic erythroblast

The basophilic erythroblast varies from 10 to 16 μm in diameter. The nucleus is still relatively large and the chromatin strands are thick, giving a coarse appearance; there are no nucleoli present. The cytoplasm is still very basophilic. Refer to Fig A1-2.

Polychromatic erythroblast

The polychromatic erythroblast varies from 8 to 14 μm in diameter. The nucleus is smaller and the chromatin strands more dense, tending to form clumps giving a characteristic cartwheel-shaped appearance. The cytoplasm is no longer basophilic but polychromatic or mauve coloured as it has begun to acquire haemoglobin. Refer to Fig A1-3.

Orthochromatic erythroblast

The orthochromatic erythroblast varies from 8 to 10 μm in diameter. The nucleus is small, with a coarse, pyknotic chromatin pattern. The cytoplasm is pale pink with a polychromatic hue signifying that it has acquired more haemoglobin. As the cell matures, the nucleus becomes smaller and is finally extruded whilst still within the bone marrow. Refer to Fig A1-4.

Polychromatic red cell

The polychromatic red cell is a young erythrocyte that is slightly larger than the mature red cell. It is polychromatic in colour since it still contains some RNA remnants, which can be demonstrated by the use of a supravital stain such as new methylene blue or brilliant cresyl blue, in which case the cell is termed a reticulocyte. Once this cell has lost all its RNA, it develops into a mature fully haemoglobinised red cell or erythrocyte. Refer to Fig A1-5.

Mature red cell

The mature red cell (erythrocyte) is a biconcave disc approximately 7 μm in diameter with an area of central pallor occupying less than one-third of its diameter. Red cells exhibit an eosinophilic reaction when stained with any of the Romanowsky stains. The average life span of a red cell is 120 days. Refer to Fig A1-6.

MEGALOBLASTIC ERYTHROPOIESIS

Megaloblasts are abnormal erythroblasts produced in the bone marrow of patients deficient in vitamin B_{12} and/or folic acid. Vitamin B_{12} and folic acid are vital for DNA synthesis and thus for the normal maturation and growth of red cells.

Megaloblastic changes occur in all stages of red cell maturation. Megaloblastic erythropoiesis is classified according to the normoblastic series: promegaloblast, basophilic megaloblast, polychromatic megaloblast, orthochromatic megaloblast and mature macrocyte.

Megaloblasts differ from normoblastic erythroblasts in the following respects:

(a) They are larger at every stage of their development.

(b) Nuclear maturation is abnormal, since vitamin B_{12} and folic acid are vital for DNA synthesis. Deficiency or absence of either leads to abnormal maturation of the nucleus and asynchronous development; the nucleus lags behind the cytoplasm at every stage in the maturation process. This is most evident in the polychromatic megaloblast, where the cytoplasm is polychromatic and the chromatin strands of the nucleus are still very fine and open—unlike the polychromatic erythroblast, where they are dense and form clumps.

(c) Mitoses are common and sometimes abnormal in appearance.

Refer to Figs A1-7 to A1-12.

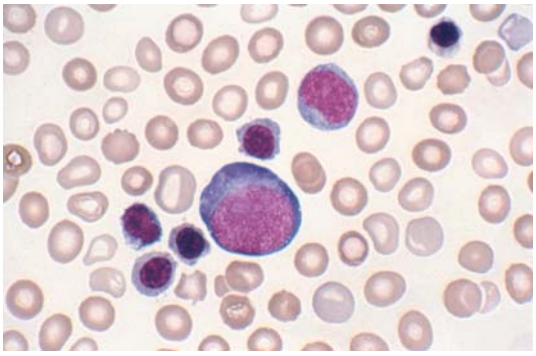


Figure A1-1
Proerythroblast and polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

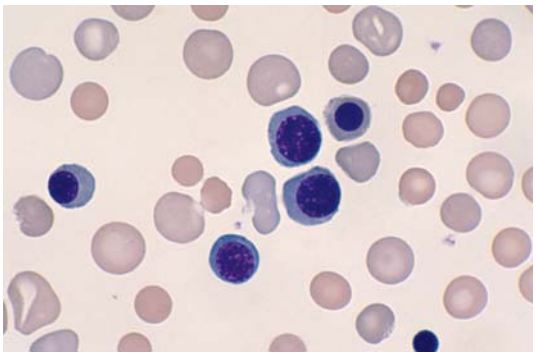


Figure A1-2
Basophilic and polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

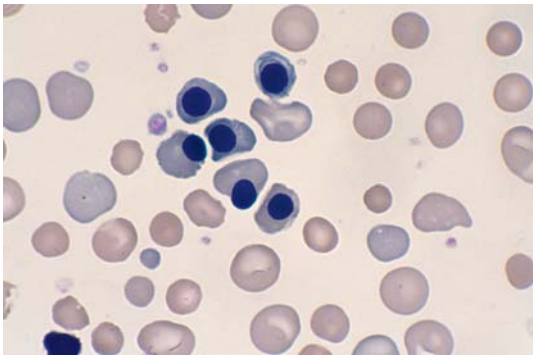


Figure A1-3
Polychromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

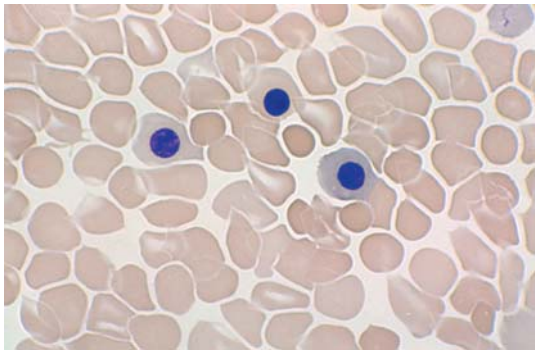


Figure A1-4
Polychromatic and orthochromatic erythroblasts in the peripheral blood of a newborn infant with haemolytic disease of the newborn. (x 1000)

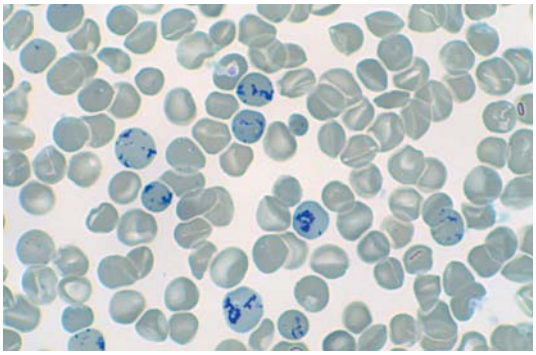


Figure A1-5
Reticulocytes in the peripheral blood stained with new methylene blue stain. (x 1000)

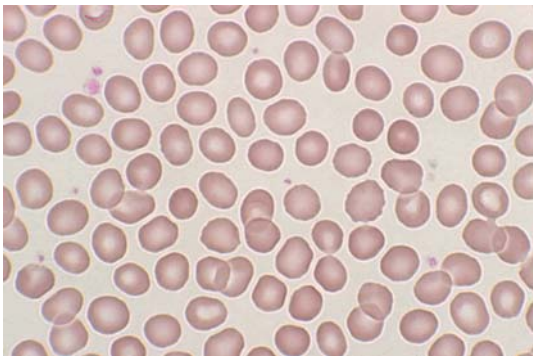


Figure A1-6
Mature red cells in the peripheral blood. (x 1000)

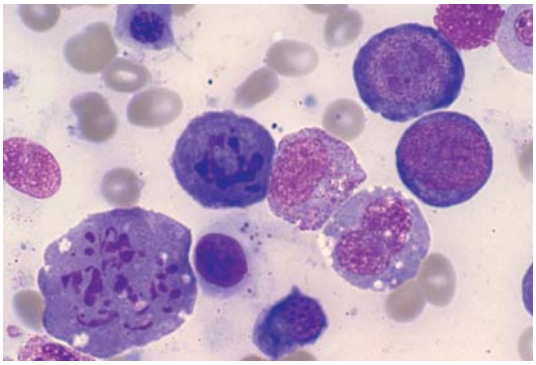


Figure A1-7
Abnormal mitoses in the bone marrow in megaloblastic anaemia. (x 1000)

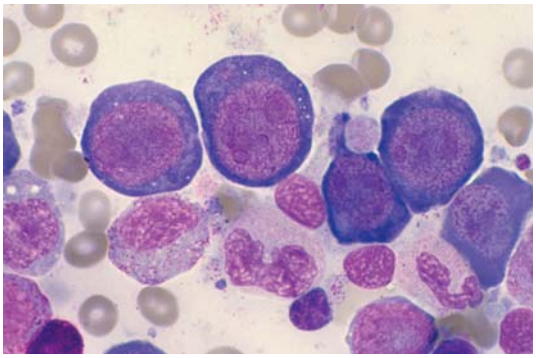


Figure A1-8
Promegaloblast, basophilic megaloblasts and myeloid precursors in a bone marrow aspirate from a patient with megaloblastic anaemia. (x 1000)

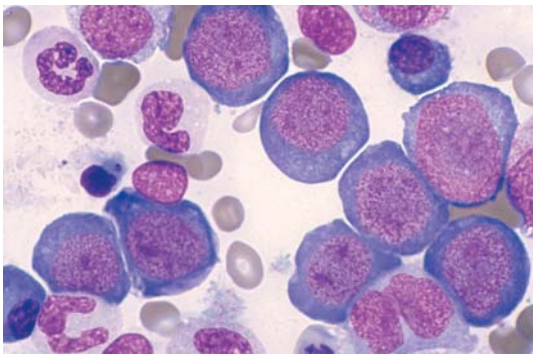


Figure A1-9
Basophilic megaloblasts in the bone marrow in megaloblastic anaemia. (x 1000)

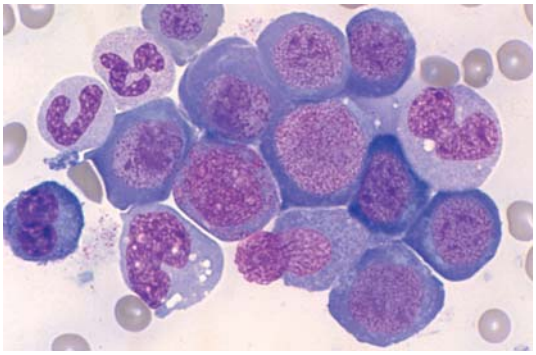


Figure A1-10
Basophilic and polychromatic megaloblasts in the bone marrow in megaloblastic anaemia. (x 1000)

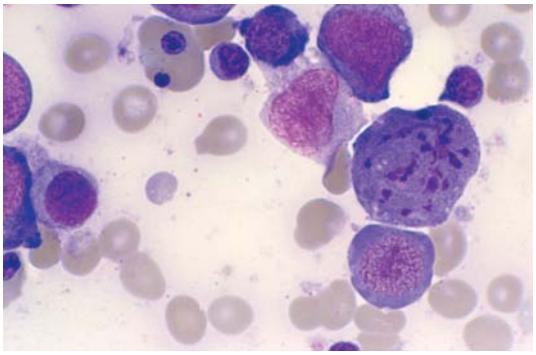


Figure A1-11
Polychromatic and orthochromatic megaloblast in the bone marrow in megaloblastic anaemia. (x 1000)

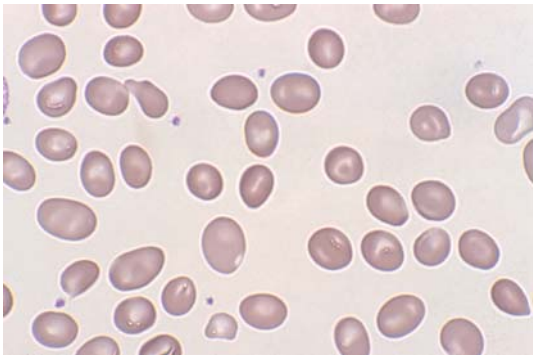


Figure A1-12
Megaloblastic mature red cells in the peripheral blood in megaloblastic anaemia. (x 1000)

A2 Deficiency anaemias

IRON DEFICIENCY ANAEMIA

Iron deficiency anaemia occurs when the iron content of the body is less than normal. It is characterised by decreased or absent iron stores, low serum iron concentration, high transferrin with low saturation, low haemoglobin concentration, low haematocrit and low red cell number. The red cells in iron deficiency anaemia are microcytic and hypochromic. Specific red cell parameters such as the mean cell volume (MCV) and mean cell haemoglobin (MCH) are reduced, while the red cell distribution width (RDW) is increased.

A major cause of iron deficiency anaemia is blood loss. It may also result from an inadequate diet and rarely from malabsorption. Pregnancy and growth are associated with greater requirements for iron; thus the risk of development of iron deficiency is high at these times. Microcytic hypochromic red cells are characterised by an MCV less than 80 fL and an MCH less than 27 pg. Red cell size may be assessed by comparing the red cell with a small lymphocyte.

The classical features found on the blood film in iron deficiency include anisocytosis, microcytes, hypochromasia, elliptocytes, and pencil cells and fragmented cells. Thrombocytosis is often present. When iron-deficiency anaemia is treated, a dimorphic blood film will result, that is, one in which there are two distinct populations of red cells: microcytic and hypochromic as well as normocytic and normochromic.

Severe cases of iron deficiency anaemia may also be detected in the bone marrow by the presence of smaller than normal erythroblasts with ragged and incompletely haemoglobinised cytoplasm. Iron stores may be assessed from the bone marrow by performing a Perl's Prussian blue stain. Haemosiderin, which is present in the marrow fragments, will stain a turquoise colour in the presence of iron; decreased or absent haemosiderin is characteristic of iron deficiency. Refer to Figs A2-1 to A2-4.

MEGALOBLASTIC ANAEMIA

Megaloblastic anaemia is due to a lack of vitamin B_{12} and/or folic acid. Vitamin B_{12} deficiency is usually due to malabsorption. One form of malabsorption is pernicious anaemia, an autoimmune disease in which there is a lack of intrinsic factor production by the gastric parietal cells. Less commonly, vitamin B_{12} deficiency results from dietary insufficiency. Folic acid deficiency results from an inadequate diet, particularly of leafy green vegetables and fruit, the additional requirements of pregnancy and, less frequently, from impaired absorption.

Classical features of megaloblastic anaemia are seen in both the peripheral blood and bone marrow. Nuclear cytoplasmic asynchrony is a characteristic feature leading to macrocytic red cells with an MCV ranging from 100 to 150 fL. The red cells are oval in shape and may contain basophilic stippling and Howell-Jolly bodies. Teardrop poikilocytes are often present. The neutrophils are hypersegmented and giant metamyelocytes can be seen in the bone marrow. Megaloblastic anaemia due to inadequate diet often coexists with a microcytic hypochromic anaemia due to the presence of iron deficiency. In such cases, hypochromic microcytes will also be present and the blood picture is described as a 'mixed' deficiency. Refer to Figs A2-5 to A2-9.

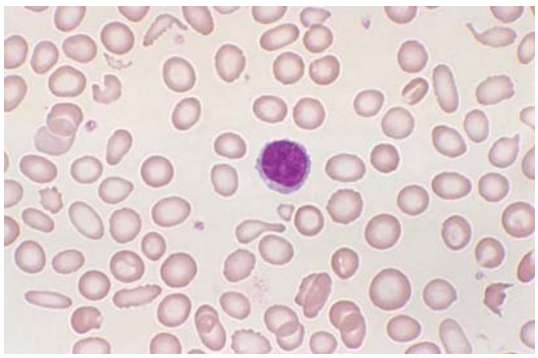


Figure A2-1
Iron deficiency anaemia: peripheral blood film showing hypochromic microcytes, elliptocytes and fragmented cells. (x 1000)

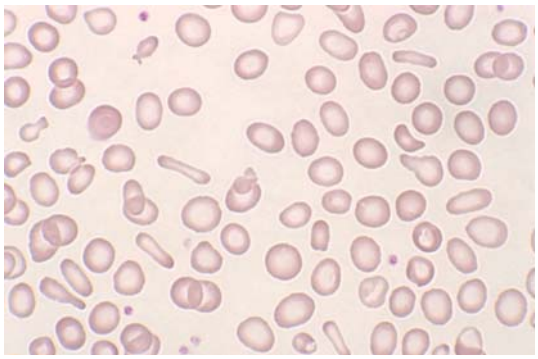


Figure A2-2
Iron deficiency anaemia: peripheral blood film showing hypochromic microcytes, elliptocytes, pencil cells and fragmented cells. (x 1000)

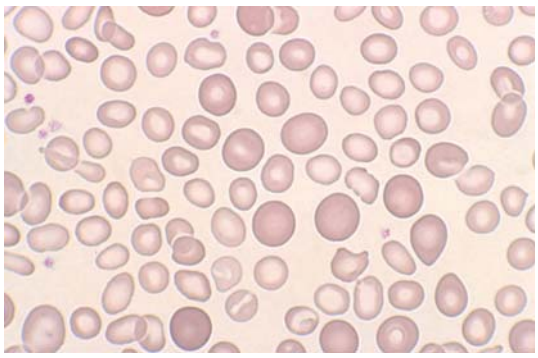


Figure A2-3
Response to iron therapy in a child: dimorphic blood picture showing two populations of red cells: hypochromic microcytic and normochromic normocytic. (x 1000)

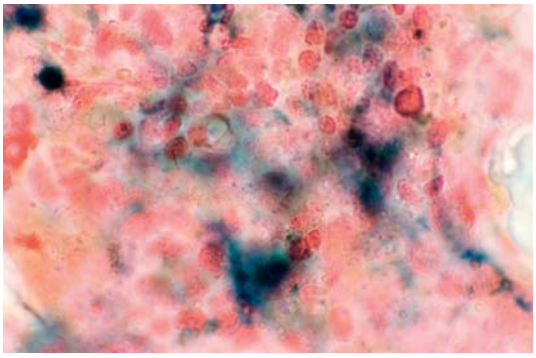


Figure A2-4
Perl's Prussian blue stain showing haemosiderin in a fragment of bone marrow. (x 1000)

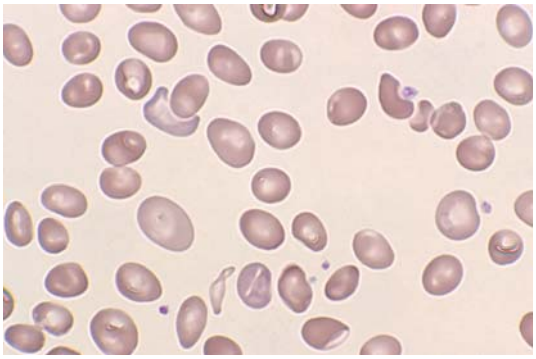


Figure A2-5
Megaloblastic anaemia: peripheral blood showing many oval macrocytes. (x 1000)

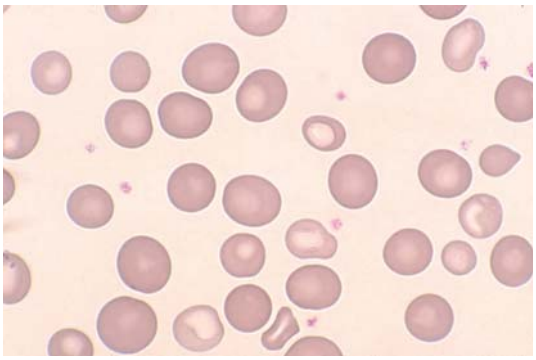


Figure A2-6
Round macrocytes in the peripheral blood in alcoholic liver disease with low serum and red cell folate levels. (x 1000)

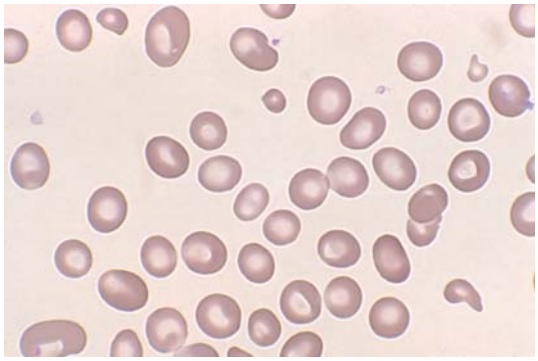


Figure A2-7
'Mixed deficiency' in the peripheral blood film from a 4-month-old child with vitamin B_{12}, folic acid and iron deficiency. This child was being breastfed by a vegan mother. (x 1000)

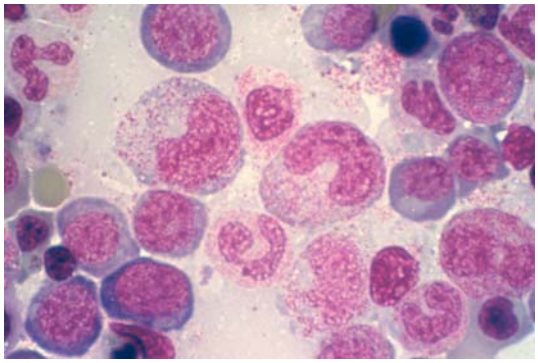


Figure A2-8
Megaloblastic anaemia: bone marrow showing two giant metamyelocytes. (x 1000)

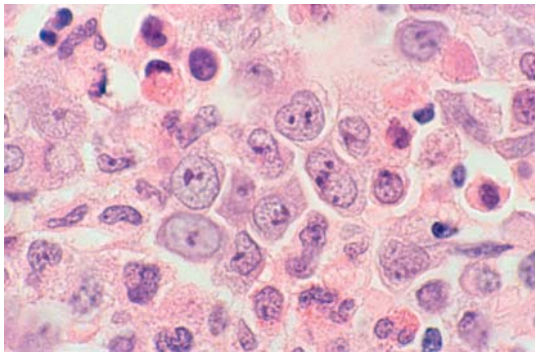
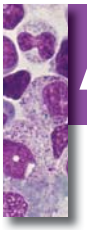


Figure A2-9
Megaloblastic anaemia: bone marrow trephine infiltrated with megaloblasts. (H&E) (x 1000)



A3 Haemolytic anaemias

AUTOIMMUNE HAEMOLYTIC ANAEMIA

Autoimmune haemolytic anaemia (AIHA) is due to antibodies produced by the body's immune system against its own red cells. These antibodies are either warm or cold and in some instances may have a wide thermal amplitude extending from warm to cold.

Warm-antibody AIHA is the most common type; the antibodies produced are of the IgG class, which have maximal activity at 37°C. Cold-antibody AIHA results from the production of antibodies of the IgM class, which act at temperatures below 37°C.

Examination of the blood film from a case of AIHA reveals the presence of spherocytes, polychromasia, and nucleated red cells. In cold AIHA, auto-agglutination will also be present.

The diagnosis of AIHA is established by performing a direct antiglobulin test (DAT) on the patient's red cells. A positive result indicates the presence of antibody or complement on the red cell surface, thus confirming the diagnosis of AIHA. The DAT can also be used to differentiate AIHA from hereditary spherocytosis (HS): both disorders have a similar blood picture but HS is characterised by a negative DAT. Refer to Figs A3-1 to A3-3.

PAROXYSMAL COLD HAEMOGLOBINURIA (PCH)

Paroxysmal cold haemoglobinuria is an autoimmune haemolytic anaemia described by Julius Donath and Karl Landsteiner in 1904. It occurs in children under 5 years of age. The blood picture resembles that of an AIHA with spherocytes, reticulocytes and nucleated red cells. It is positive for the Donath-Landsteiner antibody which is a polyclonal IgG that binds to various red cell antigens such as I, i, P and p on the red cell surface. The P antigen is its primary target. The polyclonal IgG anti-P autoantibody binds to red blood cell surface antigens in the cold. When the blood returns to the warmer central circulation, the red cells are lysed with complement, giving rise to intravascular haemolysis. The anaemia is DAT (C3d) positive. The blood film sometimes shows monocytic and granulocytic erythrophagocytosis. Refer to Figs A3-4 to A3-6.

NON-IMMUNE HAEMOLYTIC ANAEMIA

Clostridial sepsis

Septicaemias induced by *Clostridium welchii* and *C. perfringens* give rise to severe rapidly progressive intravascular haemolytic anaemia characterised by the presence of microspherocytes. It is thought that these bacteria produce a toxin containing a proteolytic agent capable of destroying spectrin. This toxin is responsible for red cell membrane destruction often involving the entire red cell mass. Refer to Figs A3-7 and A3-8.

Paroxysmal nocturnal haemoglobinuria (PNH)

PNH is a haemopoietic stem cell disorder present in red cells, white cells and platelets. This red cell abnormality predisposes the red cells to intravascular complement-mediated lysis. Lack of red cell membrane proteins, in particular the 'decay accelerating factor'

(DAF CD55), the 'membrane inhibitor of reactive lysis' protein (MIRL CD59) and the 'homologous restriction factor' (HRF), leads to severe clinical haemolysis. These proteins negatively regulate the haemolytic action of complement on red cells. Patients with PNH present with a severe anaemia with Hb levels ranging from less than 5.0 g/L to normal. They may also present with pancytopenia as well as aplastic anaemia. There is a macrocytosis due to the presence of increased reticulocytes and in some cases a microcytic hypochromic anaemia due to iron deficiency. Refer to Fig A3-9.

Haemolytic anaemia due to lead poisoning

The ingestion of lead interferes with haem synthesis. It does so by inhibiting several of the enzymes directly involved with haem synthesis. Pyrimidine 5′-nucleotidase is one such enzyme. In its absence, pyrimidine nucleotides accumulate in the red cells, preventing iron from being incorporated into haem at a normal rate. This leads to a shortened red cell life span resulting in a mild haemolytic anaemia. The blood film shows characteristic fine to coarse basophilic stippling in the red cells as seen with any of the Romanowsky stains. The anticoagulant ethylenediamine tetraacetic acid (EDTA) can mask lead-induced stippling if blood films are not made fresh and fixed immediately.

Refer to Fig A3-10.

MICROANGIOPATHIC HAEMOLYTIC ANAEMIA

The term 'microangiopathic' means small vessel disease; hence microangiopathic haemolytic anaemia results from physical damage to red cells as they pass through very small orifices or damaged and sclerosed vessels.

The blood film shows increased numbers of red cell fragments that have characteristically sharp projections. These fragments are referred to as schistocytes, red cells produced by a microangiopathic process. They are fractured or ripped as they pass across strands of fibrin in damaged vessels or as they pass across a damaged or prosthetic heart valve. Thrombocytopenia is a classical finding in some types of microangiopathic haemolytic anaemia.

A variety of disorders are associated with a microangiopathic blood picture, namely haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), human immunodeficiency virus (HIV) infection, disseminated intravascular coagulation (DIC), valvular heart disease, HELLP or preeclampsia of pregnancy, necrotising enterocolitis (NEC), malignancy and acute renal failure. Microangiopathic haemolytic anaemia may also result from the use of the immunosuppressive agent cyclosporin. Refer to Figs A3-11 to A3-20.

Haemolytic uraemic syndrome (HUS)

HUS occurs most commonly in infancy and early childhood and is initiated by infection with *Escherichia coli* strain 0157. This bacterium produces a verocytotoxin that is attracted to the vascular endothelium, especially the endothelium lining the glomeruli of the kidney. This toxin induces severe glomerulonephritis that in turn leads to a microangiopathic blood picture.

The blood film of HUS shows schistocytes and a marked thrombocytopenia. Refer to Fig A3-11.

Thrombotic thrombocytopenic purpura (TTP)

TTP is a microangiopathic haemolytic anaemia seen mostly in adults. It is characterised by a pentad of clinical features, namely fever, thrombocytopenia, anaemia, neurological symptoms and renal disease; schistocytes are seen on the blood film.

TTP has been reported in patients with the acquired immunodeficiency syndrome (AIDS)-related complex. Refer to Fig A3-12.

Disseminated intravascular coagulation (DIC)

DIC occurs when small blood vessels become blocked by platelet and fibrin thrombi, thus altering the patency of the vessel and inducing intravascular haemolysis. The blood film shows schistocytes and thrombocytopenia. Refer to Fig A3-14.

Valvular heart disease

Microangiopathic haemolytic anaemia occurs in valvular heart disease and also in some patients who have had prosthetic heart valves inserted. The high shear forces produced by the abnormal blood flow seen in such disorders produce a blood film characterised by the presence of schistocytes, classical of a microangiopathic process. The platelet count is invariably normal. Refer to Fig A3-15.

Malignancy

A microangiopathic haemolytic anaemia may be associated with metastatic carcinoma, especially mucin-secreting adenocarcinoma of the breast and stomach. Metastases occurring in the microvascular system, especially the lung, give rise to a microangiopathic blood picture with thrombocytopenia. Refer to Fig A3-16.

Cyclosporin

The immunosuppressive agent cyclosporin is nephrotoxic and hence may give rise to a microangiopathic blood picture with thrombocytopenia. Refer to Fig A3-18.

HELLP syndrome

The HELLP syndrome (haemolysis, elevated liver enzymes and low platelet count) is a multisystem syndrome occurring in severe preeclampsia and eclampsia. It affects both primiparous and multiparous women in the third trimester of pregnancy. HELLP is characterised by a microangiopathic haemolytic anaemia, hepatic dysfunction, renal failure and in severe cases, DIC.

Delivery of the fetus is the initial treatment; however, the disease remains active after delivery and appears to achieve peak intensity during the 24–48 hour post delivery period. Refer to Fig A3-20.

OXIDANT-DRUG-INDUCED HAEMOLYTIC ANAEMIA

The use of oxidant drugs can be easily recognised from the blood film, provided that the patient has not had a splenectomy. Two frequently used oxidant drugs, dapsone and sulfasalazine (Salazopyrin), may give rise to a Heinz-body-positive haemolytic anaemia resulting in a blood picture characterised by the presence of bite and blister cells. Heinz bodies are precipitates of denatured haemoglobin and are the manifestation of the oxidative challenge that the red cell has suffered. They are rapidly removed or pitted out by the spleen, giving rise to bite cells. Should the red cell membrane of the bite cell rejoin, a blister cell will result. The red cells of premature and term neonates are more susceptible to oxidants. Prolonged exposure to naphthalene may give rise to a haemolytic anaemia in infants despite normal levels of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD). Refer to Figs A3-21 to A3-24.

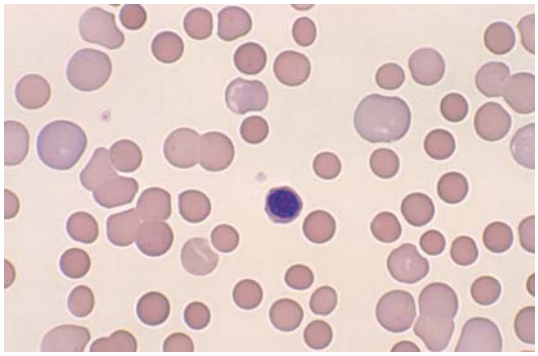


Figure A3-1
Autoimmune haemolytic anaemia (warm antibody): peripheral blood film showing spherocytes, reticulocytes (polychromasia) and a nucleated red cell. (x 1000)

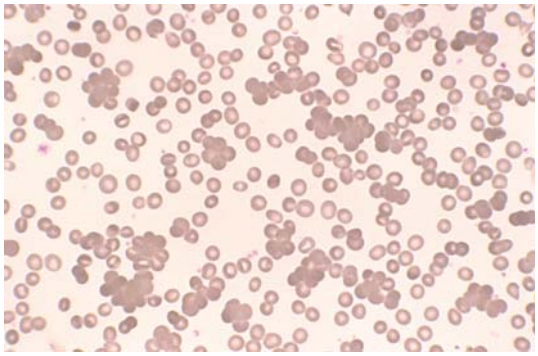


Figure A3-2
Autoimmune haemolytic anaemia (cold antibody): peripheral blood film showing marked auto-agglutination. Blood samples with cold agglutination will have a falsely raised mean cell volume (MCV) unless the sample is prewarmed to 37°C prior to processing through the blood cell analyser. (x 400)

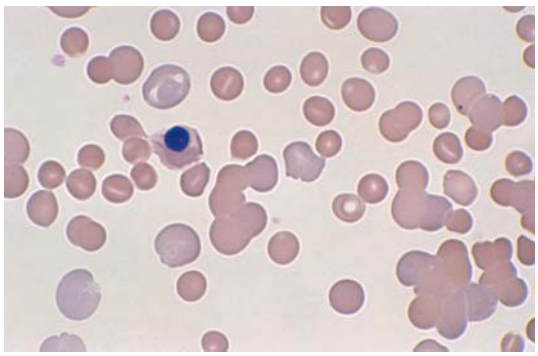


Figure A3-3
Peripheral blood film showing the presence of a wide-thermal-amplitude auto-antibody giving rise to features of both a warm and cold autoimmune haemolytic anaemia. (x 1000)

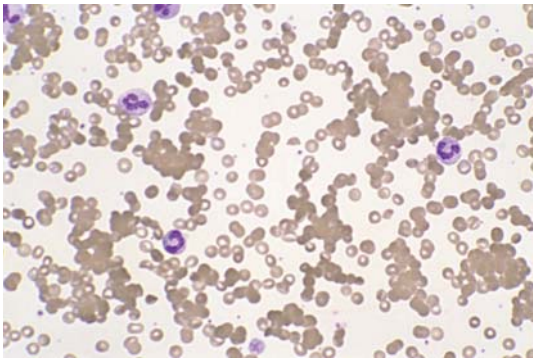


Figure A3-4
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing auto-agglutination, spherocytes and reticulocytes. (x 400)

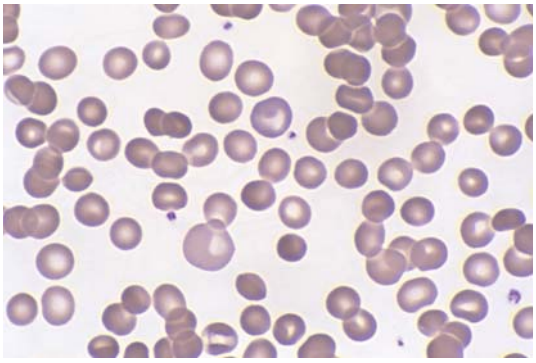


Figure A3-5
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing auto-agglutination, spherocytes and reticulocytes. (x 1000)

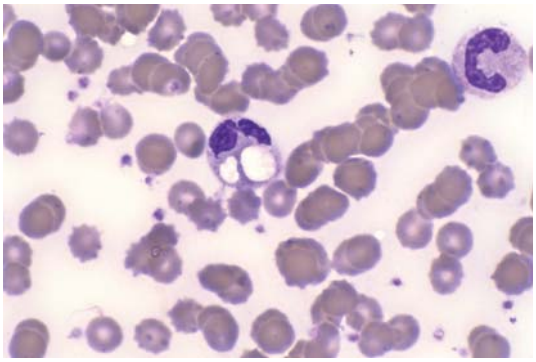


Figure A3-6
Paroxysmal cold haemoglobinuria: Donath Landsteiner antibody positive haemolytic anaemia. Peripheral blood film showing granulocytic erythrophagocytosis. (x 1000)

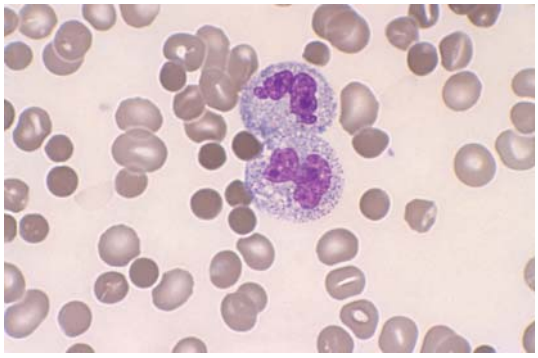


Figure A3-7
Clostridial sepsis: peripheral blood film from a case of *C. Perfringens* septicaemia showing toxic granulation in the neutrophils and microspherocytes. (x 1000)

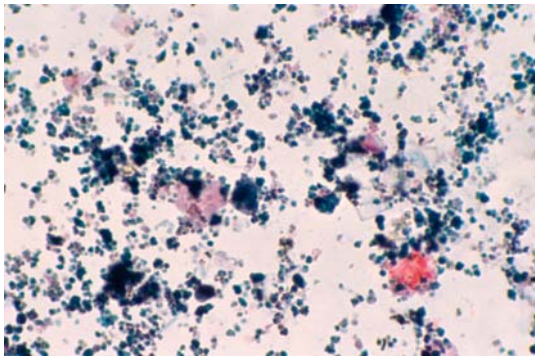


Figure A3-8
Clostridial sepsis: Perl's Prussian blue stain on the urine deposit from the case in Figure A3-7 showing a strongly positive urinary haemosiderin indicative of intravascular haemolysis. (x 1000)

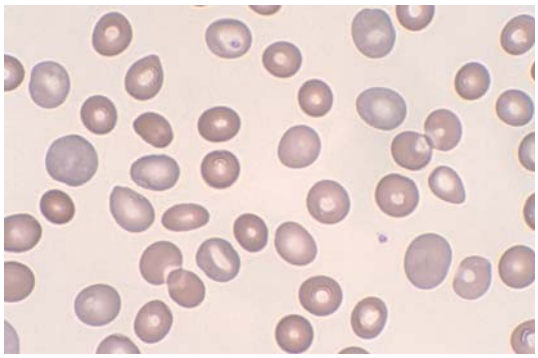


Figure A3-9
Paroxysmal nocturnal haemoglobinuria: peripheral blood film showing a macrocytic anaemia together with increased polychromasia or reticulocytes. (x 1000)

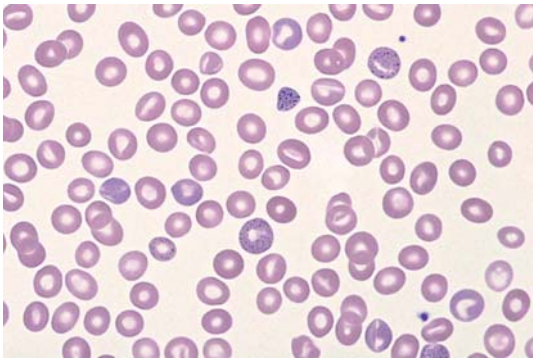


Figure A3-10
Lead poisoning: peripheral blood film showing coarse basophilic stippling in the red cells of a patient with a lead level of 5.95 µmol/l. (x 1000)

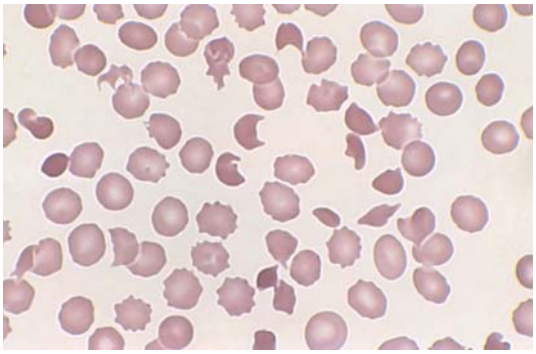


Figure A3-11
Haemolytic uraemic syndrome: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

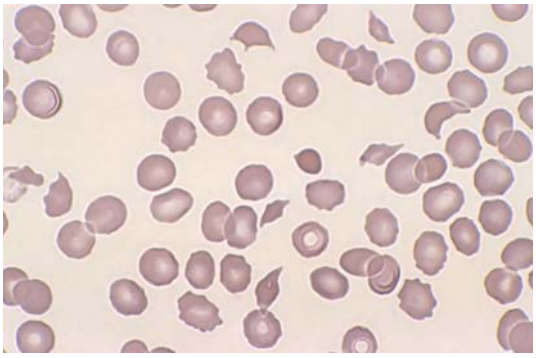


Figure A3-12
Thrombotic thrombocytopenic purpura: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

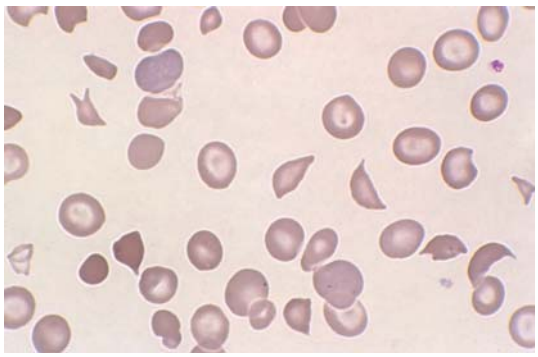


Figure A3-13
HIV infection: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

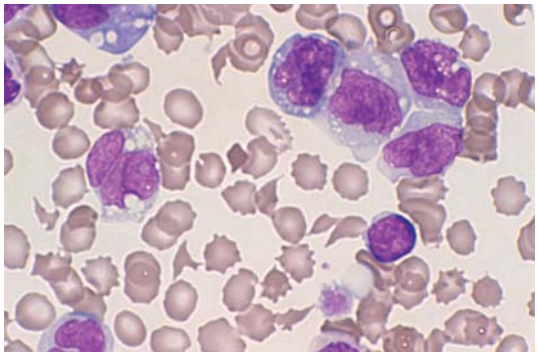


Figure A3-14
Disseminated intravascular coagulation: peripheral blood film from a patient with chronic myelomonocytic leukaemia (CMML) showing a marked number of schistocytes and thrombocytopenia. (x 1000)

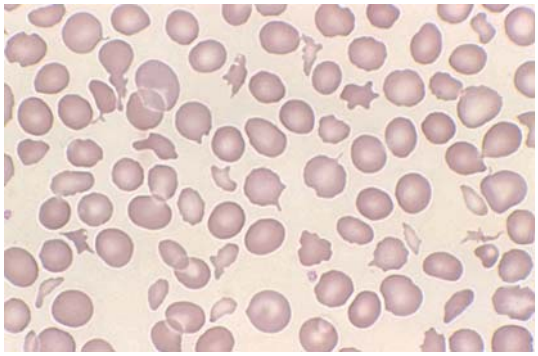


Figure A3-15
Valvular heart disease: peripheral blood film showing the presence of schistocytes. The platelet count is usually normal in valvular heart disease. (x 1000)

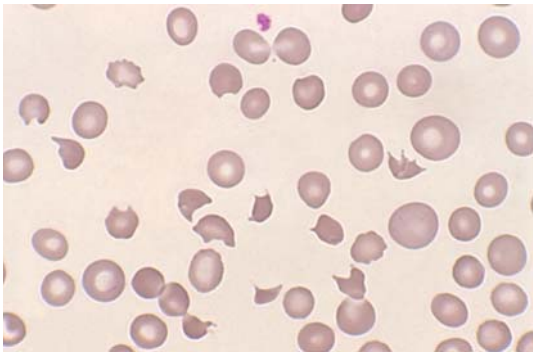


Figure A3-16
Mucin-secreting cancer of the stomach: peripheral blood film showing schistocytes and thrombocytopenia. (x 1000)

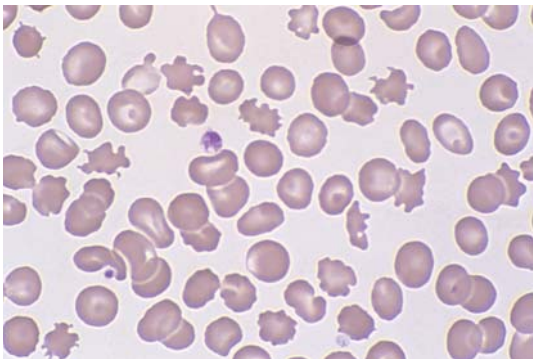


Figure A3-17
Acute renal failure: peripheral blood film showing increased numbers of burr cells. (x 1000)

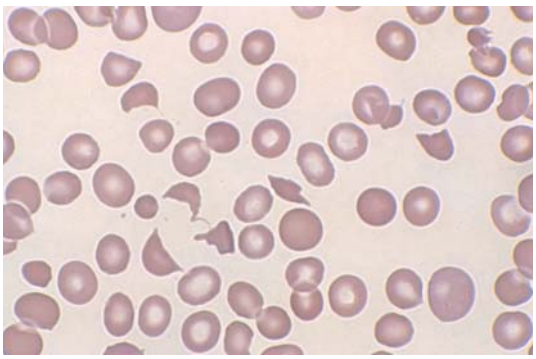


Figure A3-18
Cyclosporin-induced microangiopathic haemolytic anaemia following a peripheral blood stem cell transplant showing the presence of schistocytes and thrombocytopenia. (x 1000)

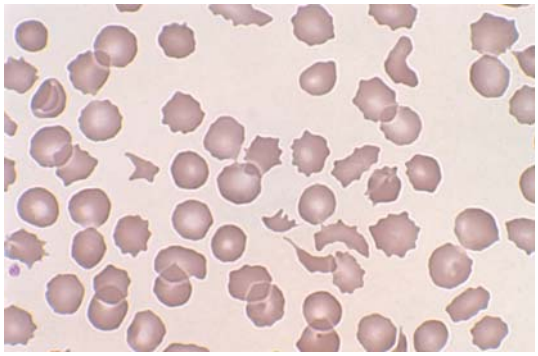


Figure A3-19
Necrotising enterocolitis: peripheral blood film in a premature neonate showing schistocytes and thrombocytopenia. (x 1000)

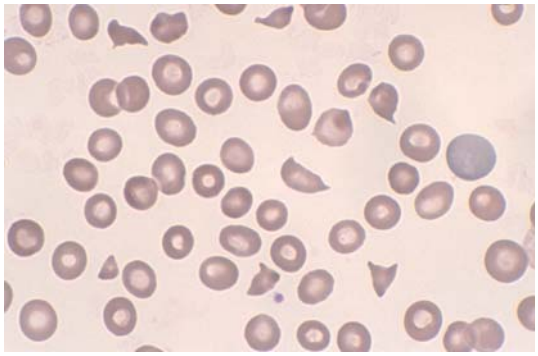


Figure A3-20
HELLP syndrome: peripheral blood film showing the presence of schistocytes in a primiparous woman in the third trimester of pregnancy. (x 1000)

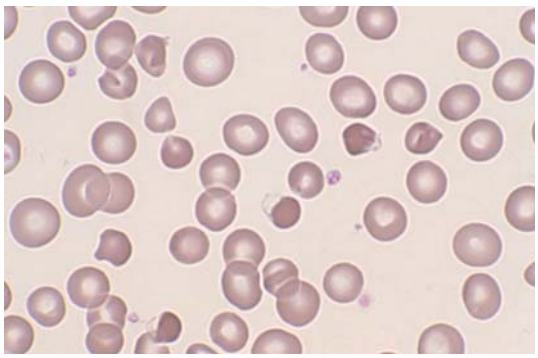


Figure A3-21
Oxidant drug (dapsone)-induced haemolytic anaemia: peripheral blood film showing the presence of bite and blister cells. (x 1000)

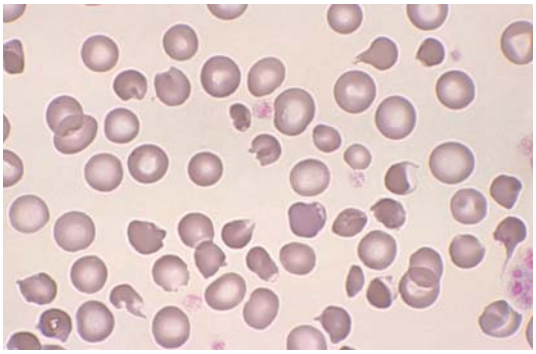


Figure A3-22
Naphthalene-induced haemolytic anaemia: peripheral blood film from a 19-day-old neonate showing bite and blister cells with a normal level of G-6-PD. (x 1000)

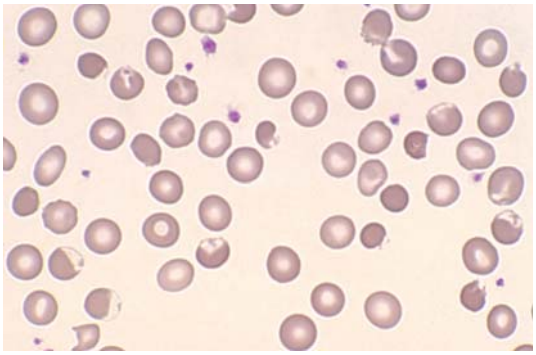


Figure A3-23
G-6-PD deficiency: peripheral blood film showing the presence of bite and blister cells following ingestion of fava beans (favism). (x 1000)

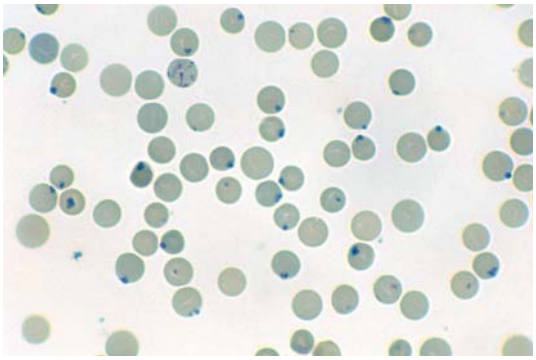


Figure A3-24
Heinz bodies in the peripheral blood film of a splenectomised patient who is being treated with the oxidant drug dapsone. New methylene blue stain. (x 1000)

A4 Haemoglobin disorders

The thalassaemias are hereditary anaemias that occur as a result of a mutation that affects the synthesis of normal haemoglobin. Normal haemoglobin consists of two pairs of dissimilar polypeptide chains, α-like and non-α(β, γ or δ). Each chain encloses an iron-containing porphyrin known as haem. The normal haemoglobins are:

- Haemoglobin A, consisting of two α- and two β-globin chains
- Haemoglobin A_2, consisting of two α- and two δ-globin chains
- Haemoglobin F, consisting of two α- and two γ-globin chains.

α-Thalassaemia is characterised by a reduction or total lack of α-globin chains and β-thalassaemia by a reduction or total lack of β-globin chains. A microcytic hypochromic anaemia is associated with both α- and β-thalassaemia.

THE α-THALASSAEMIAS

α-Thalassaemia commonly occurs in populations from South-East Asia, the Mediterranean, Africa and China. It can arise when any number of the four α-globin genes are either reduced or absent and hence can be divided into four groups.

Number of genes affected	Condition
1	Silent carrier α-thalassaemia trait
2	α-Thalassaemia trait
3	Haemoglobin H (HbH) disease
4	Hydrops fetalis

Silent carrier α-thalassaemia trait

Silent carrier α-thalassaemia trait is characterised by minimal or no haematological changes, and the haemoglobin level and the mean cell volume (MCV) are low normal. There is no obvious microcytosis seen on the blood film. The diagnosis of silent carrier α-thalassaemia trait is made by family studies and/or gene analysis.

α-Thalassaemia trait

This trait is characterised by a microcytic hypochromic blood film; the average MCV is 68 fL and the average mean cell haemoglobin (MCH) is 22 pg. HbH inclusion bodies (β_4) are present after the blood is incubated at 37°C for 2 hours with a supravital stain such as brilliant cresyl blue. These inclusion bodies represent precipitates of HbH and give the cell a golfball-like appearance. Haemoglobin electrophoresis is normal in α-thalassaemia trait; thus it is vital to detect the occasional HbH cell whose presence enables the diagnosis of α-thalassaemia trait to be made. Refer to Fig A4-1.

Haemoglobin H disease

HbH disease is characterised by a markedly microcytic hypochromic blood film with increased numbers of target cells and red cell fragments. The average MCV is 57 fL and the average MCH is 21 pg. HbH inclusions are present in the great majority of red cells. Refer to Figs A4-2 and A4-3.

Hydrops fetalis

Infants with hydrops fetalis are delivered stillborn at 30–40 weeks. The hydrops is due to a failure to produce α-globin chains. If a blood film can be obtained from the stillborn, it will show a characteristic population of large hypochromic macrocytes, marked polychromasia, basophilic stippling and increased numbers of nucleated red cells. Refer to Fig A4-4.

Haemoglobin constant spring

Haemoglobin H disease can be associated with a haemoglobin known as Haemoglobin Constant Spring (HbCS). HbCS is an alpha chain variant rather than a deletion. The alpha chain is elongated by 31 additional amino acid residues at the C-terminal end making it very unstable. The presence of HbCS causes the red cells to break down faster than usual giving rise to a severe anaemia.

The red cells of HbCS are large and different from those seen in any of the other forms of thalassaemia. They are markedly overhydrated relative to those of the deletional forms of alpha thalassaemia. Coarse basophilic stippling is a characteristic feature of HbCS. Refer to Fig A4-5.

THE β-THALASSAEMIAS

β-Thalassaemia commonly occurs in populations of Mediterranean and African origin, as well as in the Middle East, India, Pakistan, China and South-East Asia.

The β-thalassaemias include four syndromes: silent carrier β-thalassaemia trait, β-thalassaemia trait, β-thalassaemia intermedia and β-thalassaemia major.

Silent carrier β-thalassaemia trait

Silent carrier β-thalassaemia trait is characterised by minimal or no haematological changes. The haemoglobin level and the MCV are low normal and there is no obvious microcytosis seen on the blood film. Characteristically, silent carriers of β-thalassaemia have normal levels of HbA_2. Diagnosis of silent carrier β-thalassaemia trait is made by family studies and/or gene analysis.

β-Thalassaemia trait

This trait is characterised by a microcytic hypochromic red cell picture together with target cells, elliptocytes, and basophilic stippling. The average MCV is 63 fL and the average MCH is 20 pg.

In β-thalassaemia trait, the HbA2 level is increased above 3.5% and may be as high as 8.0%, while the HbF level is elevated in approximately 50% of patients, ranging from less than 1% to 5%. Refer to Fig A4-6.

β-Thalassaemia intermedia

This is a more severe form of β-thalassaemia trait but less severe than β-thalassaemia major. At the most severe end of the scale patients are transfusion dependent while at the less severe end they are transfusion independent. The red cell changes are more severe than those found in β-thalassaemia trait, with increased numbers of red cell poikilocytes. Teardrop poikilocytes are a prominent feature. Refer to Fig A4-7.

β-Thalassaemia major

As the neonate has substantial HbF at birth, anaemia in these patients usually develops during the first few months of life and becomes progressively worse in time. These infants will be transfusion dependent by the end of the first year of life; a later onset of the condition would suggest a case of thalassaemia intermedia.

β-Thalassaemia major is characterised by Hb levels as low as 30 g/L and variable amounts of HbF according to the transfusion status at the time of measurement. The acid elution or Kleihauer test shows that the HbF is evenly distributed among the red cells. The blood film shows marked red cell poikilocytosis, microcytosis and hypochromasia, target cells, basophilic stippling, Pappenheimer bodies (siderotic granules) and a reticulocytosis with increased numbers of nucleated red cells. As a result of frequent transfusions, the blood picture is often dimorphic, and consequently the MCV and MCH are difficult to define. Refer to Fig A4-8.

ABNORMAL HAEMOGLOBINS

Haemoglobins C, E and S (HbC, HbE and HbS) are abnormal haemoglobins characterised by an amino acid substitution in the β-globin chain.

Haemoglobin C

HbC ($\alpha_2\beta_2^{6Glu \rightarrow Lys}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with lysine at the sixth position on the β chain. It is found in West Africans, particularly from Ghana and the Upper Volta.

HBC TRAIT

Individuals with HbC trait are clinically normal. Target cells are present on an otherwise normal blood film.

HBCC DISEASE

HbCC disease is associated with a haemolytic anaemia; the haemoglobin level ranges from 80 to 120 g/L. The blood film shows marked numbers of target cells, red cell fragments and microspherocytes. Upon careful examination, the red cells will be seen to contain intraerythrocytic crystals that dissolve readily when oxygen is released to the tissues. The MCV and MCH are slightly reduced as a result of the marked number of target cells that result from potassium efflux from the red cells, shrinking their contents with dehydration, leading to an increased ratio of surface area to volume. Refer to Fig A4-10.

IN VITRO TEST FOR DETECTION OF HBC

An in vitro test to demonstrate the presence of HbC crystals may be performed by adding 3% NaCl to the red cells and examining a wet preparation under a coverslip after 4 hours or longer. Hypertonic dehydration of the red cells produces tetrahedral crystals in up to 75% of the cells. Refer to Fig A4-11.

HAEMOGLOBIN E

HbE ($\alpha_2\beta_2^{26Glu \rightarrow Lys}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with lysine at position 26 on the β chain. It is found in South-East Asians.

HBE TRAIT

Individuals with HbE trait are asymptomatic, with haemoglobin levels of 120 g/L average MCV of 74 fL and an average MCH of 25 pg. Occasional target cells may be present. Refer to Fig A4-12.

HBEE DISEASE

HbEE disease is also asymptomatic; the haemoglobin level is rarely less than 100 g/L. The red cell indices are distinctly abnormal, with an average MCV of 58 fL and an average MCH of 20 pg. The red cells are markedly microcytic and hypochromic, with a marked number of target cells present. Refer to Fig A4-13.

Haemoglobin S

HbS ($\alpha_2\beta_2^{6Glu \rightarrow Val}$) is an abnormal haemoglobin produced by the replacement of glutamic acid with valine at the sixth position on the β chain, and is found in the African as well as in the American black population.

HBS TRAIT

Individuals with HbS trait are clinically asymptomatic, with normal haemoglobin levels and a normal blood picture.

HBSS DISEASE

HbSS disease is characterised by a mild to moderate normochromic anaemia. The blood picture shows a reticulocytosis with a varying number of sickle cells. Sickle cells are biconcave discs that, upon deoxygenation, change shape to become sickle-shaped. Sickling is associated with the formation of liquid crystals or tactoids of HbS that run parallel to the long axis of the cell and cause the characteristic sickle shape. When the cells containing HbS enter the fine capillaries of the body, they become deoxygenated, change shape and thus block off those capillaries. This process gives rise to small infarcts throughout the body, especially in the spleen. As a result of this process, blood films of patients with sickle cell disease demonstrate features of autosplenectomy, namely Howell-Jolly bodies, Pappenheimer bodies, target cells and nucleated red cells. Refer to Fig A4-17.

IN VITRO SICKLING TEST FOR DETECTION OF HBS

The reducing agent sodium dithionite induces red cells containing HbS to sickle. A mixture of red cells and sodium dithionite placed on a glass slide and sealed with a coverslip will reveal the presence of sickle-shaped cells within 1–12 hours. Refer to Fig A4-18.

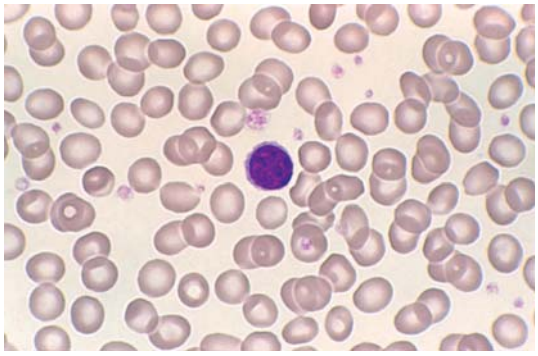


Figure A4-1
α-Thalassaemia trait: peripheral blood film showing a homogeneous population of microcytic hypochromic red cells. (x 1000)

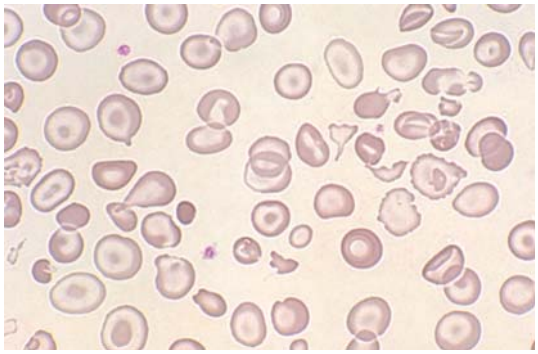


Figure A4-2
HbH disease: peripheral blood film showing microcytes, hypochromasia, target cells and fragmented cells. Cells resembling 'bite' cells may be present. These cells are a feature of an unstable Hb and are not indicative of an oxidant drug. (x 1000)

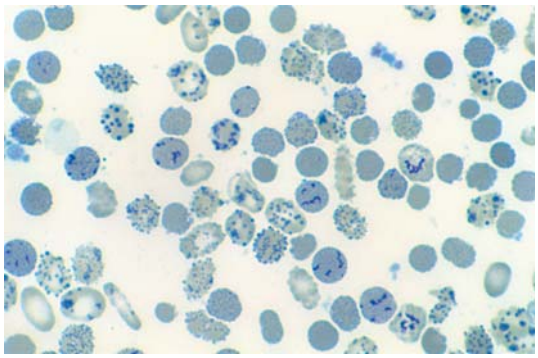


Figure A4-3
HbH disease: cresyl blue stain of peripheral blood film showing HbH inclusion bodies (β4) that are precipitates of HbH as a result of redox action of the cresyl blue dye. (x 1000)

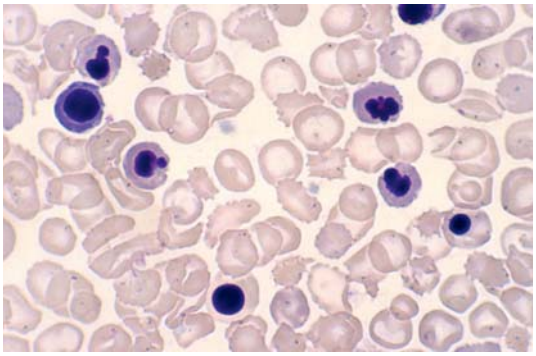


Figure A4-4
Hydrops fetalis: peripheral blood film from a stillborn infant showing hypochromic macrocytes, polychromasia, basophilic stippling and increased numbers of dysplastic nucleated red cells. (x 1000)

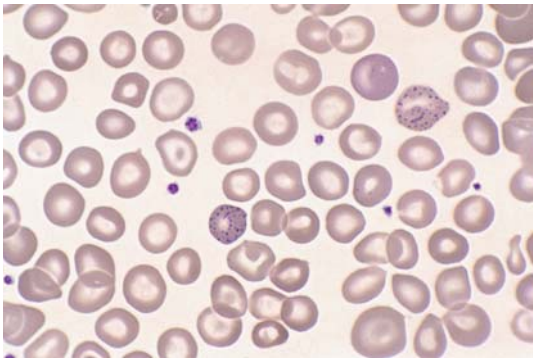


Figure A4-5
HbHCS: peripheral blood film showing microcytes, hypochromasia and coarse basophilic stippling. (x 1000)

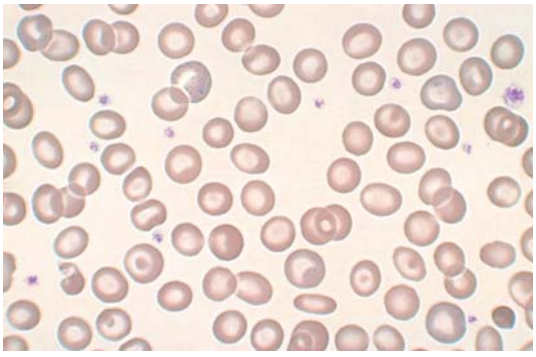


Figure A4-6
β-Thalassaemia trait: peripheral blood film showing microcytes, hypochromasia, target cells and basophilic stippling (insoluble aggregates of free α chains). (x 1000)

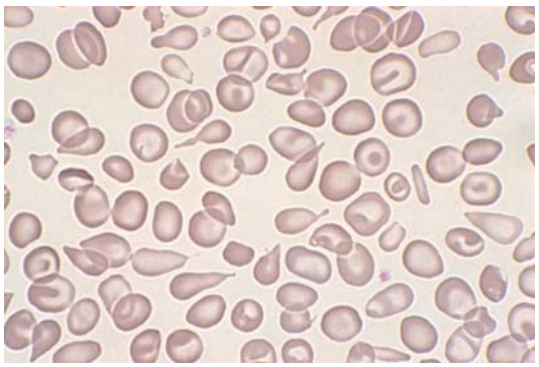


Figure A4-7
β-Thalassaemia intermedia: peripheral blood film showing microcytes, hypochromasia and increased red cell changes including teardrops. (x 1000)

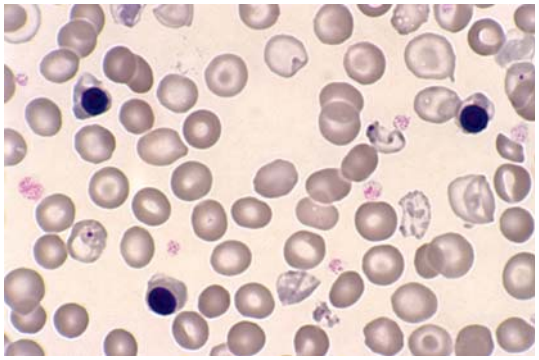


Figure A4-8
β-Thalassaemia major: peripheral blood film from a patient following splenectomy showing marked red cell changes, microcytes, hypochromasia, target cells, fragments, Howell-Jolly bodies, Pappenheimer bodies, reticulocytes and nucleated red blood cells. (x 1000)

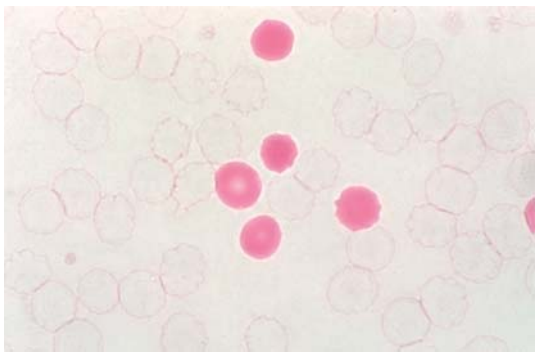


Figure A4-9
Kleihauer (acid-elution test) demonstrating the presence of HbF in fetal red cells. The cells containing adult Hb appear as ghost cells. (x 1000)

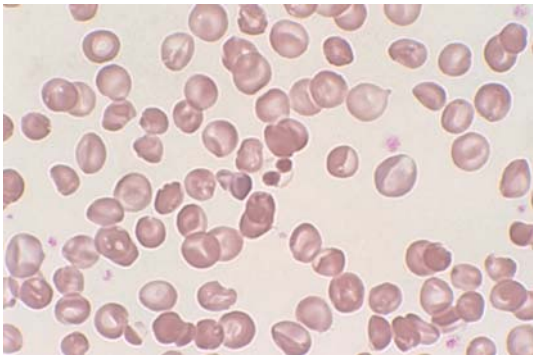


Figure A4-10
HbCC disease: peripheral blood film showing occasional microcytes and target cells. Note the intraerythrocytic crystals within some of the red cells. (x 1000)

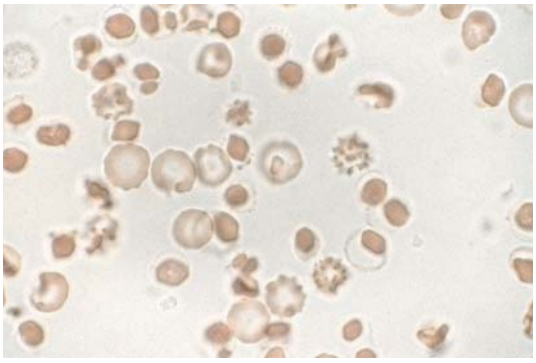


Figure A4-11
HbCC disease: in vitro demonstration of tetrahedral crystals of HbCC in peripheral blood. (x 1000)

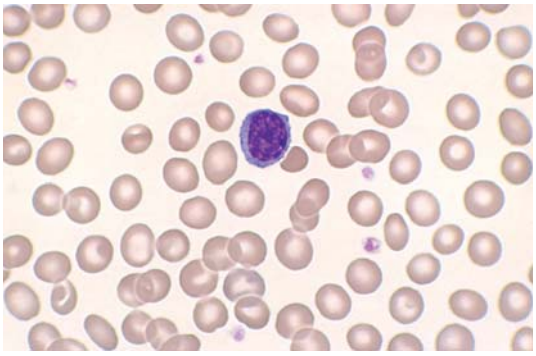


Figure A4-12
HbE trait: peripheral blood film showing a homogeneous population of microcytic hypochromic red cells. (x 1000)

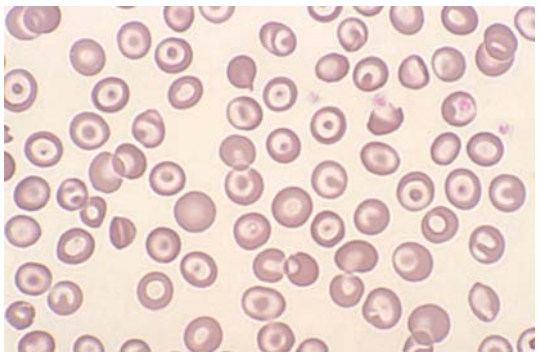


Figure A4-13
HbEE disease: peripheral blood film showing microcytic hypochromic red cells with a marked number of target cells. (x 1000)

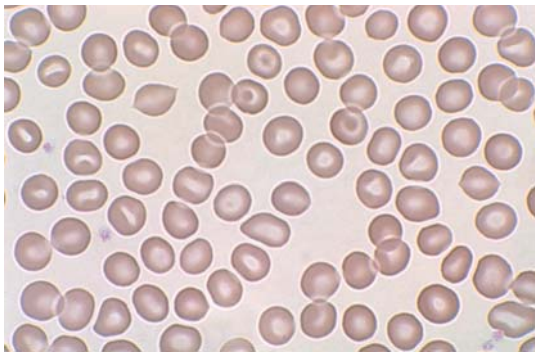


Figure A4-14
HbE/α-thalassaemia trait: peripheral blood film showing microcytic hypochromic red cells with an occasional target cell. (x 1000)

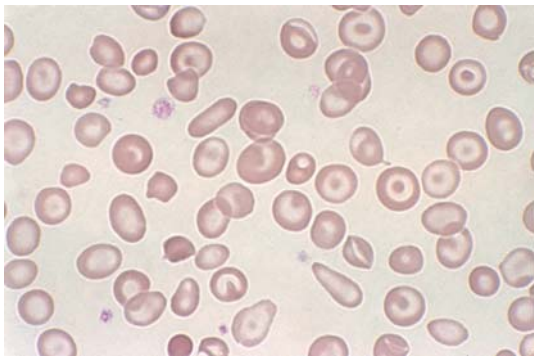


Figure A4-15
HbE/β-thalassaemia trait: peripheral blood film showing microcytic hypochromic red cells with elliptocytes and target cells. (x 1000)

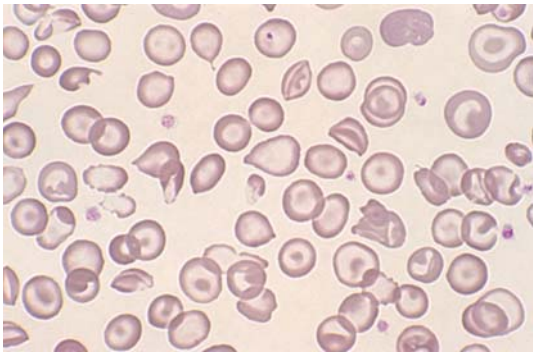


Figure A4-16
HbE/HbH disease: peripheral blood film showing marked red cell changes, microcytic hypochromic red cells, target cells and fragments. (x 1000)

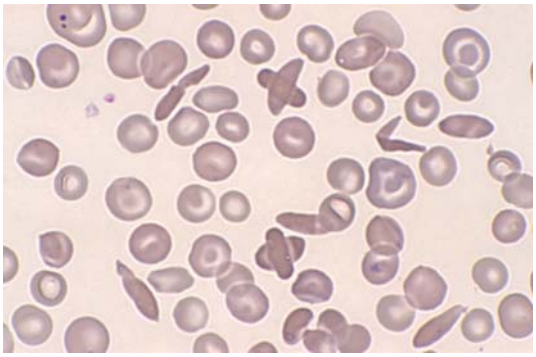


Figure A4-17
HbSS disease: peripheral blood film showing sickle cells, an occasional Howell-Jolly body and reticulocyte. (x 1000)

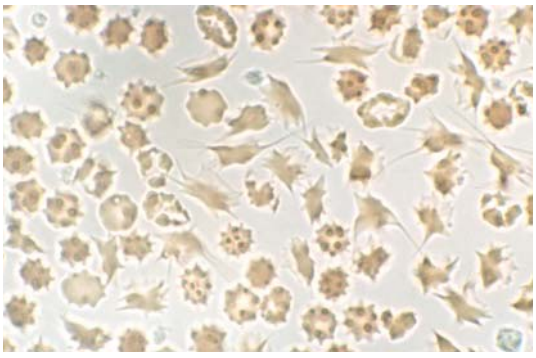


Figure A4-18
HbS: in vitro sodium dithionite preparation showing sickle cells in the peripheral blood. (x 1000)

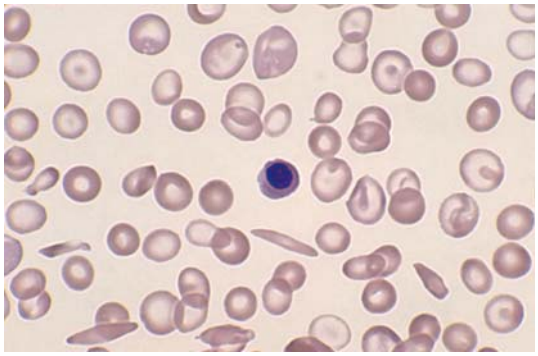


Figure A4-19
HbS/β-thalassaemia trait: peripheral blood film showing microcytic hypochromic cells with target and sickle cells as well as an occasional nucleated red cell. (x 1000)

A5 Red cell membrane disorders

HEREDITARY SPHEROCYTOSIS (HS)

HS is characterised by red cells that lack an area of central pallor, have a smaller diameter than normal and are intensely haemoglobinised. These cells are known as spherocytes. The presence of spherocytes results in a raised mean cell haemoglobin concentration (MCHC) of about 380 g/L. The mean cell haemoglobin (MCH) and mean cell volume (MCV) are within the normal range.

Spherocytes result from an intracorpuscular red cell membrane defect. Deficiency of spectrin, ankyrin and band 3 protein leads to uncoupling of the skeletal lipid bilayer resulting in membrane loss in the form of microvesicles. This loss of surface area leads to the formation of spherocytes.

HS is associated with a raised reticulocyte count and erythroid hyperplasia of the marrow. Spherocytes haemolyse more readily in hypotonic salt solutions, resulting in a tail on the osmotic fragility curve. Refer to Figs A5-1 and A5-2.

BURNS

Third-degree burns induce changes on the blood film that can be seen almost immediately after the event. Direct action of heat at 49°C denatures spectrin in the red cell membrane, giving rise to membrane budding, fragmentation, microcytes and microspherocytes. The presence of microcytes and microspherocytes falsely elevates the platelet number—hence the need for a manual count. Refer to Figs A5-3 and A5-4.

LIVER DISEASE

Obstructive liver disease is characterised by the presence of target cells and round macrocytes. Target cells have a characteristic distribution of haemoglobin in the cell centre as well as around the periphery. Their ratio of surface area to volume is greater than normal since the red cell membrane is expanded by the accumulation of lecithin and cholesterol from free exchange with plasma lipids. In obstructive jaundice and hepatitis with biliary obstruction, there is an increase in free cholesterol and lecithin in the plasma due to the bile salts that inhibit the activity of the enzyme lecithin-cholesterol acyl transferase, which normally esterifies cholesterol. Refer to Fig A5-6.

SPUR CELL ANAEMIA

Spur cell anaemia is seen in hepatocellular disease rather than obstructive liver disease; alcoholic liver disease is a classic example. Spur cells are produced in two stages. First, excess cholesterol produced by the patient's diseased liver increases the surface area of the red cell, resulting in a red cell with a scalloped or undulating periphery. In the second stage, these scalloped cells are converted to spur cells by a process of splenic conditioning. Over a period of a few days, the membrane lipids as well as the

increased surface area are lost and the cell becomes rigid and assumes the appearance of a spur cell.

The life span of a spur cell is shortened owing to splenic sequestration; thus patients with spur cell anaemia have a moderately severe haemolytic anaemia. Spur cell anaemia is usually seen in fulminant hepatocellular liver disease. Refer to Fig A5-7.

HEREDITARY ELLIPTOCYTOSIS (HE)

The elliptocyte (ovalocyte) is an oval biconcave disc that varies in shape from being slightly oval to a cylindrical elongated cell. The elliptocytes of HE demonstrate both quantitative and qualitative abnormalities in two major proteins comprising the membrane skeleton, namely spectrin and protein 4.1. There are various types of HE; the silent carrier, mild HE, HE with infantile poikilocytosis and chronic haemolytic HE. Mild HE is the type that is commonly seen in the laboratory. These patients usually have normal haemoglobin levels, but a mild compensated anaemia may be present. While approximately 5% of elliptocytes are seen on normal blood films, between 30% and 100% are seen on the blood film of mild HE. Refer to Fig A5-8.

SOUTH-EAST ASIAN OVALOCYTOSIS

This disorder is characterised by the presence of oval red cells, many of which contain one or two transverse bars that give the cells the appearance of double stomatocytes. This abnormality results from increased ankyrin binding and decreased protein 3 mobility, leading to the production of rigid red cells. This rigidity acts as a protective mechanism against all strains of malaria, including *Plasmodium falciparum*.

South-East Asian ovalocytosis is seen in up to 30% of people of Melanesian stock in Malaysia and Melanesia, particularly in the lowland tribes where malaria is endemic. Refer to Fig A5-9.

HEREDITARY STOMATOCYTOSIS (HYDROCYTOSIS)

The Na^+/K^+ ATPase pump is greatly increased in hereditary stomatocytosis. The influx of Na^+ into red cells exceeds the loss of K^+ exiting from red cells. This leads to an increase in monovalent cation content causing the movement of water into the red cells. Hence the red cells swell and are transformed from discocytes to bowl forms. These bowl forms are known as stomatocytes and have an increased MCV.

The defect in this disorder is due to the deficiency of a membrane protein known as protein 7.2b or stomatin. The function of this protein is to regulate membrane Na^+ permeability. Stomatocytes require increased energy to protect them against osmotic rupture. They are also vulnerable to splenic sequestration. Patients with hereditary stomatocytosis have haemolytic anaemia. They are jaundiced with splenomegaly and often develop pigment gallstones later in life. Splenectomy may diminish the rate of haemolysis in these patients.

Inheritance of stomatocytosis (hydrocytosis) is autosomal-dominant. Refer to Fig A5-10.

HEREDITARY XEROCYTOSIS

Hereditary xerocytosis is a rare autosomal-dominant haemolytic anaemia. The red cells are dehydrated as there is a loss of potassium exiting the cell compared with the amount of sodium entering the cell. Thus the intracellular cation content and water content is reduced. The enzyme involved in the passage of anions across the red cell membrane, glyceralderhyde-3-phosphate dehydrogenase, is increased.

The blood picture is that of a severe haemolytic anaemia. The MCV may be slightly increased due to the presence of increased reticulocytes. Target cells are prominent and there may be some 'puddling' of haemoglobin towards the periphery of some of the cells. Refer to A5-11.

ABETALIPOPROTEINAEMIA

Abetalipoproteinaemia is a rare autosomal-recessive disorder characterised by the presence of acanthocytic red cells on the peripheral blood film. The primary defect is due to a mutation and lack of activity in the microsomal triglyceride transfer protein needed to bind lipids to the β-apolipoprotein in plasma. The plasma triglycerides are almost absent and the plasma cholesterol is markedly decreased. There is an increase in sphingomyelin in the outer half of the red cell membrane bilayer, increasing the surface layer of the cell. This β-apolipoprotein defect leads to the production of acanthocytic red cells, about 50–90% of the red cells being acanthocytes. The sphingomyelin accumulates with cell ageing; hence the nucleated precursor red cells and the reticulocytes are not affected.

Abetalipoproteinaemia is characterised clinically by ataxic neurologic disease, retinitis pigmentosa (often leading to blindness) and fat malabsorption. The neurologic abnormalities present between 5 and 10 years of age and continue until death in the second or third decade. Despite the marked acanthocytosis seen in these patients, anaemia and haemolysis are not seen. The haemoglobin levels are normal. Refer to Fig A5-12.

HEREDITARY PYROPOIKILOCYTOSIS (HPP)

HPP is an extremely rare disorder presenting in infancy and characterised by the presence of extreme poikilocytosis with red cell budding, triangular fragments, spherocytes and elliptocytes. The MCV is significantly reduced owing to the presence of a large number of fragments.

Whereas normal red cells fragment at 49°C, the red cells in HPP fragment at 45–46°C. Prolonged heating at 37°C will also induce fragmentation; thus these patients suffer from a severe microangiopathic anaemia that is partially corrected by splenectomy. Refer to Fig A5-13.

VITAMIN E DEFICIENCY

Vitamin E (α-tocopherol) is a fat-soluble vitamin that appears to serve as an antioxidant in humans. Nutritional deficiency of vitamin E is extremely rare as α-tocopherol occurs in many food products and the daily requirement is only 5–7 mg. Vitamin E deficiency in humans is virtually limited to the neonatal period and to pathologic states associated with chronic fat malabsorption. Low birthweight infants are born with low serum and tissue concentrations of vitamin E. When these infants are fed a diet unusually rich in polyunsaturated fatty acids and inadequate vitamin E, a haemolytic anaemia will develop by 4–6 weeks of age. The anaemia is associated with morphologic alterations of the red cell membrane; a red cell similar to a spur cell is produced. A haemolytic anaemia due to increased splenic sequestration follows. Treatment with vitamin E produces a prompt reversal of this process. Modifications of infant formulas have all but eliminated vitamin E deficiency in the preterm infant. Refer to Fig A5-14.

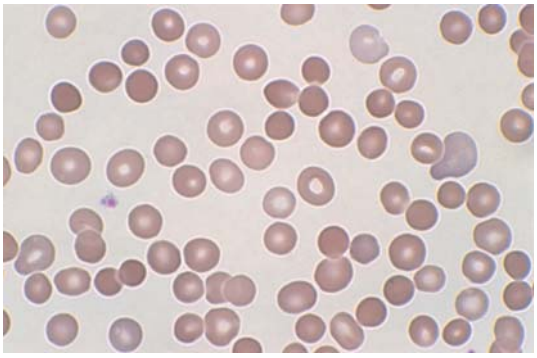


Figure A5-1
Hereditary spherocytosis: peripheral blood film showing spherocytes and increased numbers of reticulocytes. (x 1000)

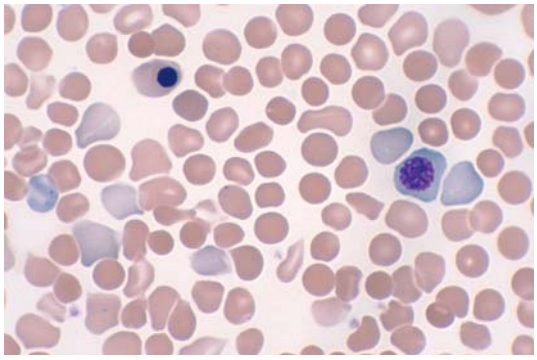


Figure A5-2
Hereditary spherocytosis in a 1-day-old neonate showing marked numbers of spherocytes, polychromasia and nucleated red cells. (x 1000)

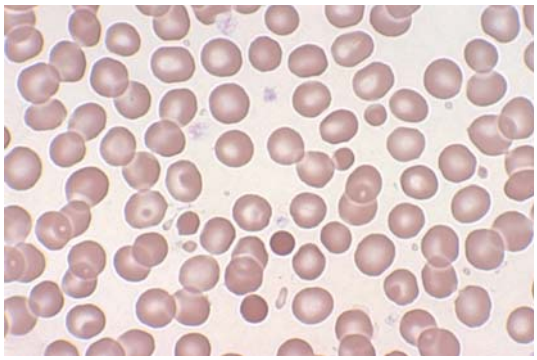


Figure A5-3
Burns: peripheral blood film showing spherocytes and microspherocytes as well as red cell budding and microcytes. (x 1000)

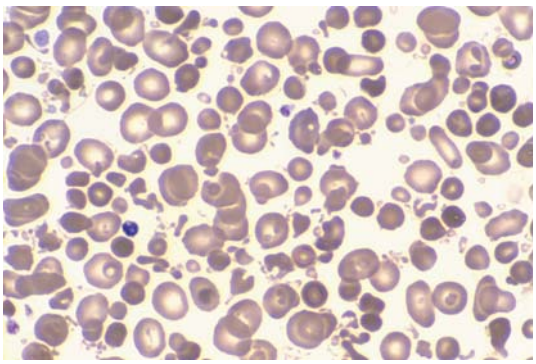


Figure A5-4
Burns: peripheral blood film showing marked numbers of spherocytes, microspherocytes, microcytes and marked red cell budding. (x 1000)

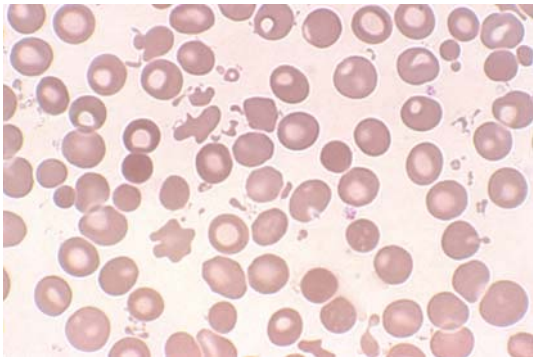


Figure A5-5
Blood film from a specimen left in a courier van on a very hot day. Note the increased number of red cell fragments; the platelet count was falsely elevated due to the red cell fragmentation. (x 1000)

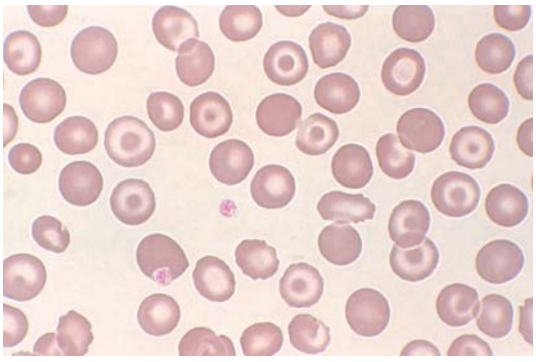


Figure A5-6
Liver disease: peripheral blood film showing the presence of target cells and round macrocytes. (x 1000)

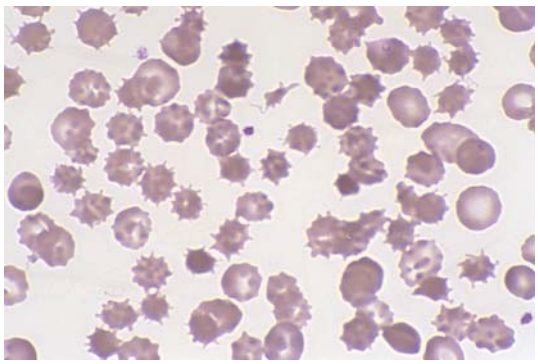


Figure A5-7
Spur cell anaemia: spur cells in the peripheral blood in fulminant liver disease secondary to alcohol. (x 1000)

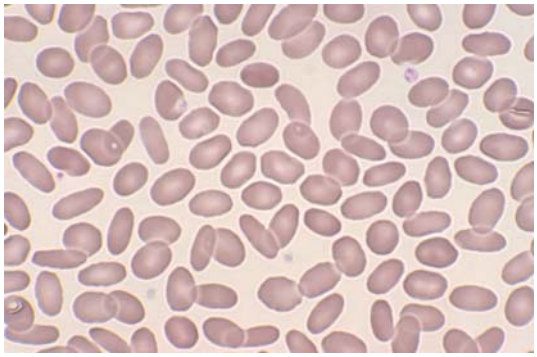


Figure A5-8
Hereditary elliptocytosis: peripheral blood film showing characteristic oval and elongated elliptocytes with rounded ends. (x 1000)

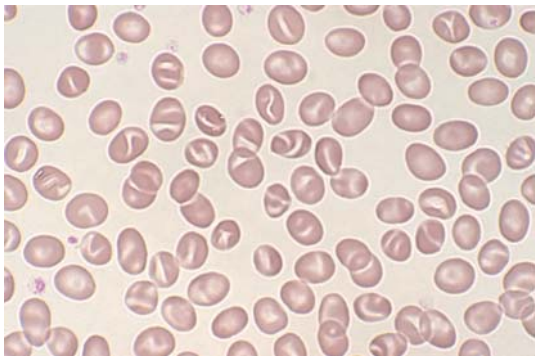


Figure A5-9
South-East Asian ovalocytosis: peripheral blood film showing oval-shaped stomatocytes, some with two transverse slits. (x 1000)

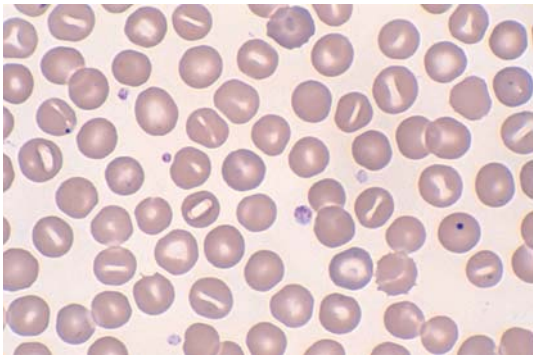


Figure A5-10
Hereditary stomatocytosis (hydrocytosis): macrocytic red cells with a single slit instead of the characteristic area of central pallor. This is a post-splenectomy picture with an MCV of 130.6 fL. (x 1000)

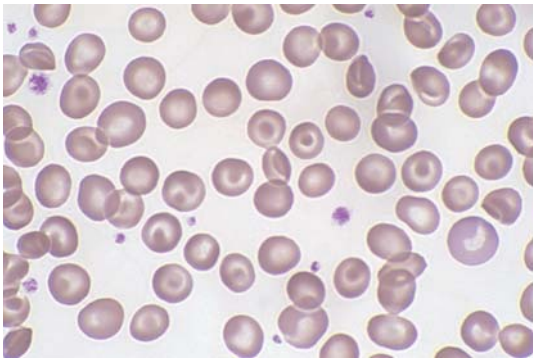


Figure A5-11
Hereditary xerocytosis: peripheral blood film showing target cells as well as 'puddling' of haemoglobin towards the periphery of some red cells. (x 1000)

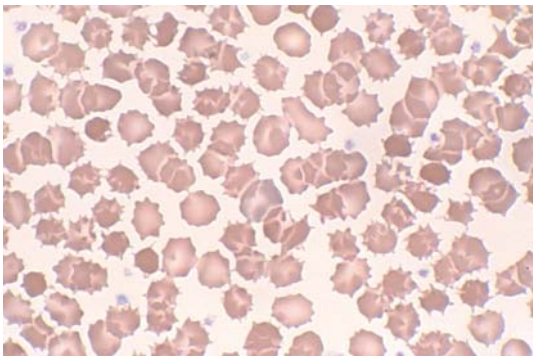


Figure A5-12
Abetalipoproteinaemia: peripheral blood film showing marked numbers of acanthocytes. (x 1000)

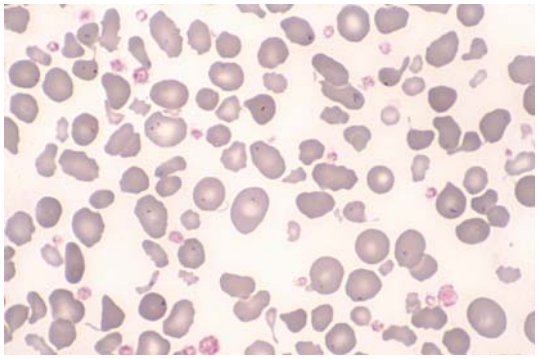


Figure A5-13
Hereditary pyropoikilocytosis: peripheral blood film showing the presence of spherocytes, extreme poikilocytosis with fragments, triangulocytes and bizarre red cells shapes. (x 1000)

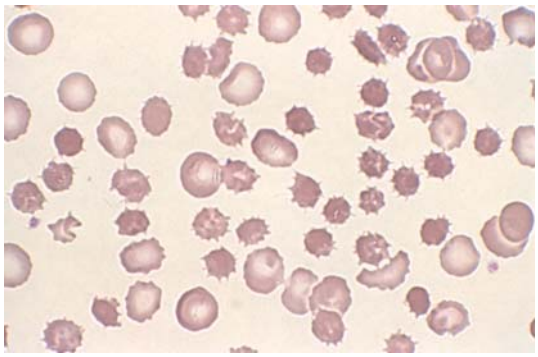


Figure A5-14
Vitamin E deficiency: peripheral blood film showing red cells similar in appearance to those of spur cell anaemia. Vitamin E is an antioxidant. (x 1000)

A6 Miscellaneous red cell abnormalities

SPLENECTOMY

Post-splenectomy blood films show the following features: target cells, acanthocytes, spherocytes, nucleated red cells, Howell-Jolly bodies, Pappenheimer bodies and Heinz bodies. This presence of intracellular remnants within the red cells attests to the culling or pitting function of the spleen. The spleen is also responsible for the surface remodelling of red cells, that is, it removes surplus membrane from red cells such as target cells that have an increased ratio of surface area to volume—hence the appearance of target cells in the first weeks following splenectomy. Refer to Figs A6-1 to A6-4.

LIPAEMIC BLOOD (GHOST CELLS)

Hyperlipidaemia may be detected on the blood film by the presence of ghost-like red cells. It is thought that excess lipid coats the red cell membrane, preventing complete methanol fixation and producing a ghost cell or a cell with an indistinct membrane. Refer to Fig A6-5.

CRYOPROTEIN

The prefix 'cryo' designates a property of precipitating in the cold and redissolving when warmed. Cryoproteins are either immunoglobulins (IgM, IgG or IgA) (cryoglobulins in plasma or serum) or cryofibrinogen (in plasma only). They occur in disorders such as plasma cell myeloma, Waldenström macroglobulinaemia, rheumatoid arthritis, Sjögren's syndrome, renal disease and hepatic disease. Cryoproteins may interfere with some laboratory tests such as the erythrocyte sedimentation rate (ESR) and the white and red cell count. Blood samples from patients with a cryoprotein should be prewarmed to 37°C prior to processing through the blood cell analyser.

Cryofibrinogen may be recognised on the blood film by the presence of aggregates of amorphous material occurring throughout the film and especially towards the tail. Refer to Fig A6-6.

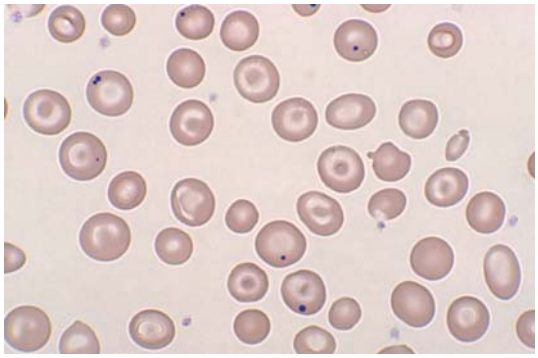


Figure A6-1
Splenectomy: peripheral blood film showing target cells and Howell-Jolly bodies (nuclear remnants). (x 1000)

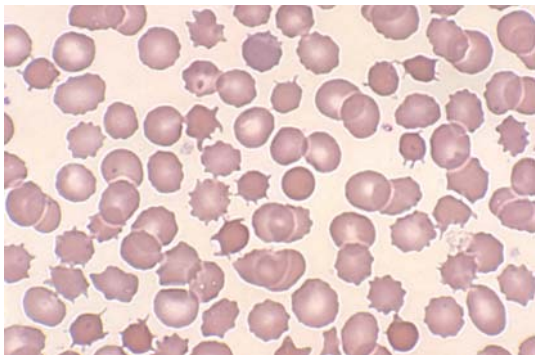


Figure A6-2
Splenectomy: peripheral blood film showing increased numbers of acanthocytes (hyperchromic red cells with an irregular number of fine spines). (x 1000)

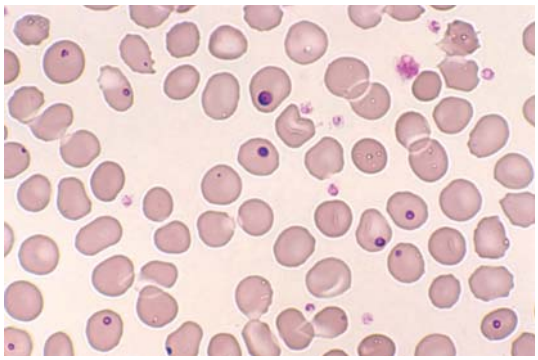


Figure A6-3
Splenectomy: peripheral blood film showing increased numbers of Howell-Jolly bodies. (x 1000)

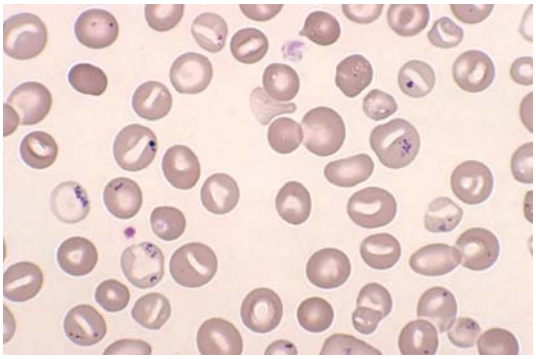


Figure A6-4
Splenectomy: peripheral blood film showing many Pappenheimer bodies. (x 1000)

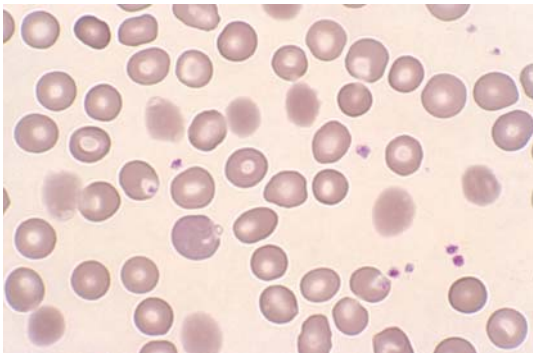


Figure A6-5
Hyperlipidaemia: peripheral blood film showing the presence of ghost cells with an indistinct cell membrane. (x 1000)

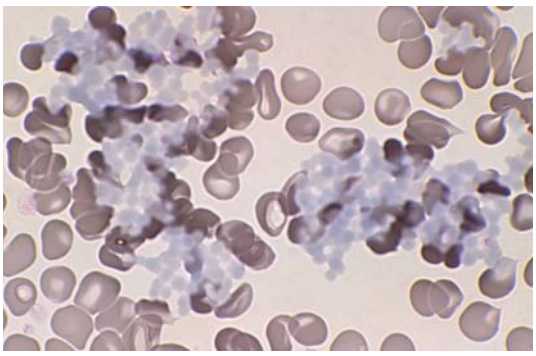


Figure A6-6
Cryoprotein: peripheral blood film showing amorphous aggregates of cryofibrinogen in a patient with plasma cell myeloma. (x 1000)