

Lecture_1

Introduction to hematology

Fifth year students
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Anatomy and physiology

- Blood flows throughout the body in the vascular system, and
- Blood makes up **~8%** of total body weight
- **~55%** plasma **~45%** formed elements
- **4 - 5 L** for average
- Plasma: 90% are water and 10% (salt and minerals)
- Formed Elements
 - Red Blood Cells (RBCs, erythrocytes)
 - White Blood cells (WBCs, leukocytes)
 - Platelets (thrombocytes)
- RBCs make up about **45%** of blood volume WBCs and platelets make up about **1%**

Haematopoiesis

- Is the process of formation of blood cells in order maintain normal numbers of circulating cells and be able to respond rapidly to increased demands such as bleeding or infection.
- During development, haematopoiesis occurs in the yolk sac, liver and spleen, and subsequently in red bone marrow in the medullary cavity of all bones.
- In childhood, red marrow is progressively replaced by fat (yellow marrow) so that, in adults, normal haematopoiesis is restricted to the vertebrae, pelvis, sternum, ribs, clavicles, skull, upper humeri and proximal femora
- However, red marrow can expand in response to increased demands for blood cells.

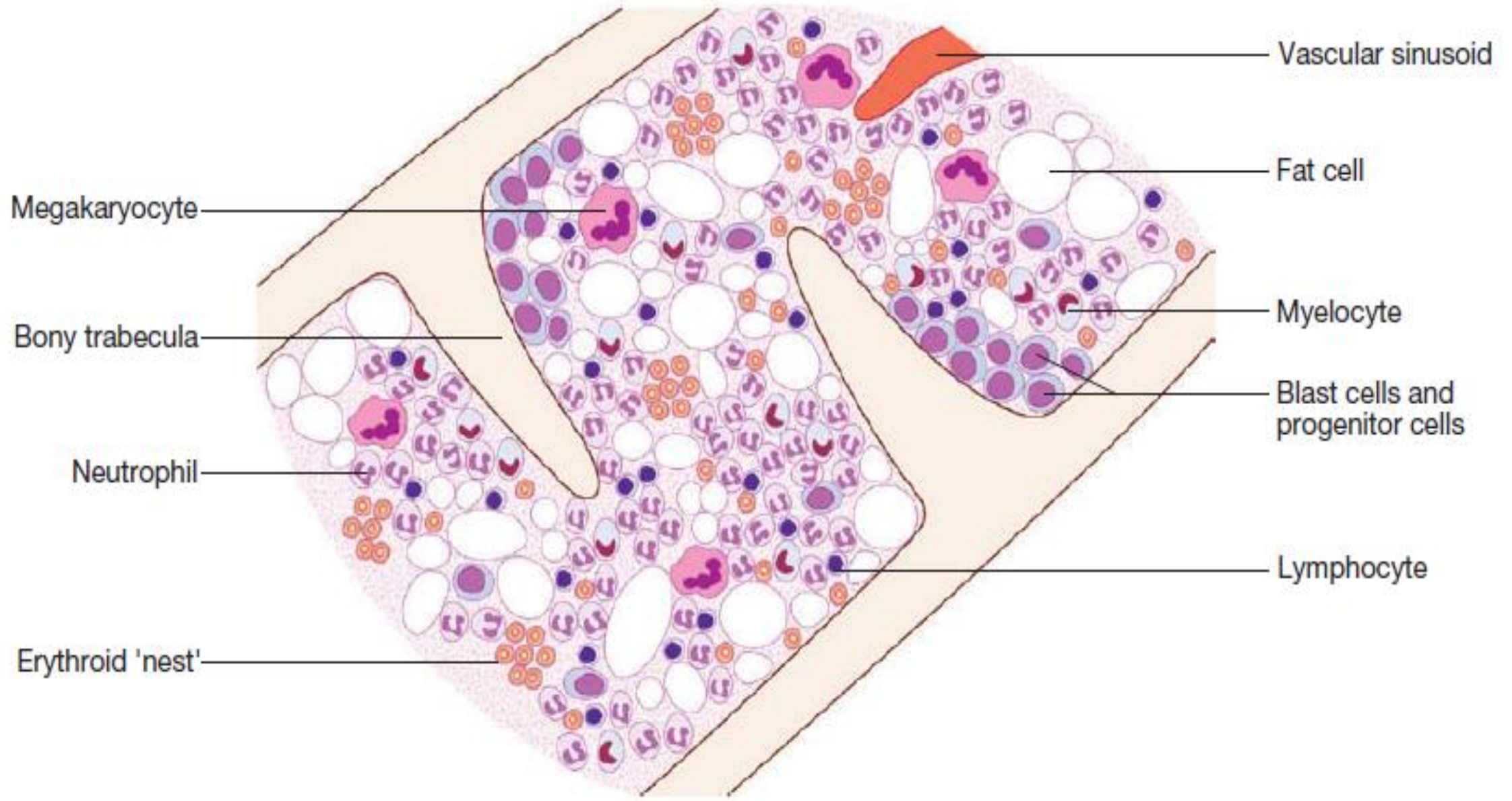
- BM contains a range of immature haematopoietic precursor cells and a storage pool of mature cells for release at times of increased demand.
- In normal marrow
- **RBC precursors** cluster around a central macrophage, which provides iron and also phagocytoses nuclei from red cells prior to their release into the circulation.
- **Megakaryocytes** are large cells that produce and release platelets into vascular sinuses.
- **WBC precursors** are clustered next to the bone trabeculae; maturing cells migrate into the marrow spaces towards the vascular sinuses
- **Plasma cells** are antibody-secreting mature B cells, represent < 5% of the marrow population

Stem cells

- Pluripotent haematopoietic stem cells comprise **0.01%** of the total BM, they can self-renew or differentiate to produce a hierarchy of lineage-committed progenitor cells.
- The resulting primitive progenitor cells cannot be identified morphologically, so they are named according to the types of cell (or colony) they form during cell culture experiments.
- **CFU–GM** (colony-forming unit – granulocyte, monocyte) is a progenitor cell that produces granulocytic and monocytic lines
- **CFU–E** produce erythroid cells
- **CFU–Meg** produce megakaryocytes and ultimately platelets.

- Growth factors, produced in bone marrow and elsewhere, control the survival, proliferation, differentiation and function of stem cells and their progeny, such as:
 - **interleukin-3 (IL-3)**
 - **stem cell factor (SCF)**
 - **Granulocyte macrophage–colony-stimulating factor (GM–CSF)**
 - **Erythropoietin**
 - **granulocyte–colony-stimulating factor (G–CSF)**
 - **thrombopoietin (Tpo), are lineage-specific.**
- act on a wide number of cell types at various stages of differentiation.

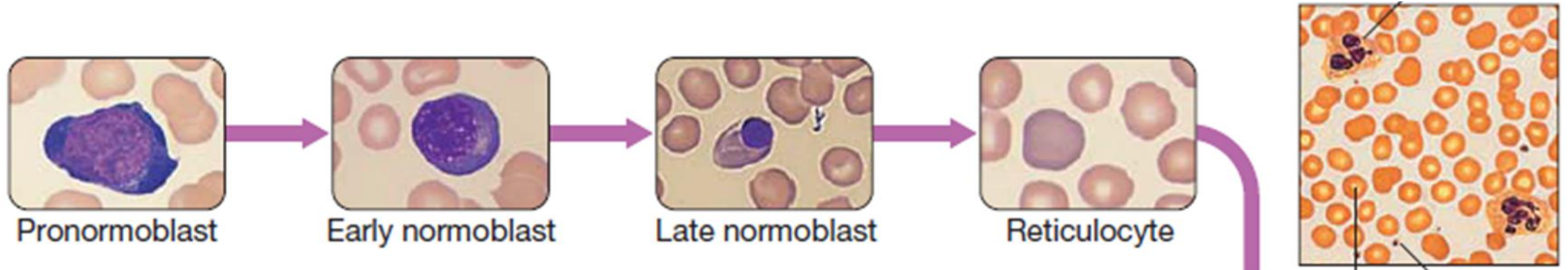
- Many of these growth factors are now synthesised by recombinant DNA technology and used as treatments:
 - Erythropoietin to correct renal anaemia
 - G-CSF to hasten neutrophil recovery after chemotherapy
- In addition BM contains stem cells that can differentiate into non-haematological cells. Mesenchymal stem cells differentiate into skeletal muscle, cartilage, cardiac muscle, and fat cells while others differentiate into nerves, liver and blood vessel endothelium.



Blood cells and their functions

1) Red cells

- Red cell precursors formed in the BM from the erythroid (CFU–E) progenitor cells are called **erythroblasts or normoblasts**, which will divide and acquire Hb, later these cells will lose the nucleus to form the **reticulocyte**. which still contains ribosomal material in the cytoplasm, giving these large cells a faint blue tinge (‘polychromasia’).





Myeloblast



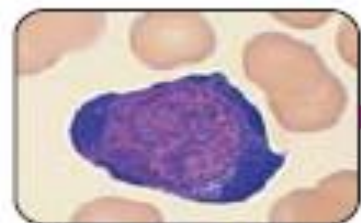
Promyelocyte



Myelocyte



Metamyelocyte



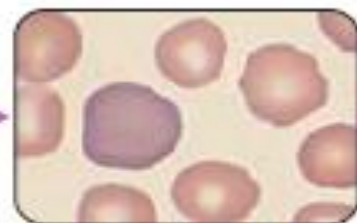
Pronormoblast



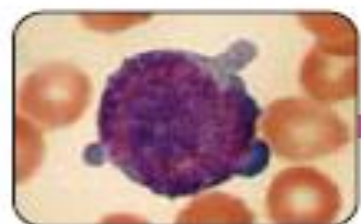
Early normoblast



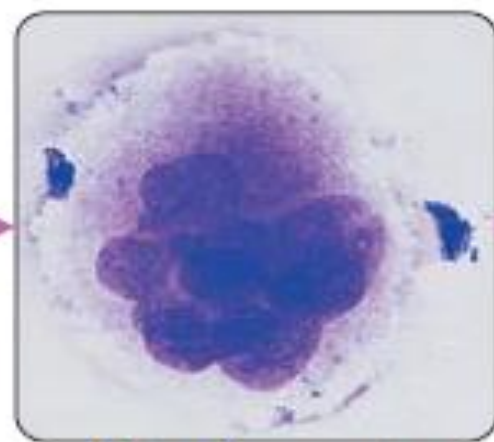
Late normoblast



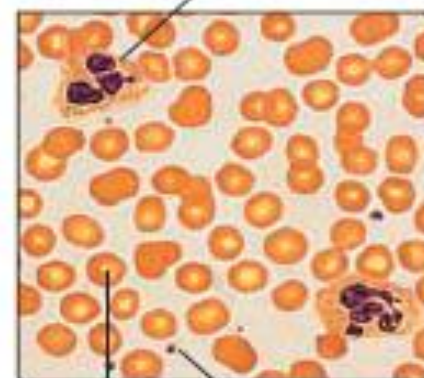
Reticulocyte



Megakaryoblast



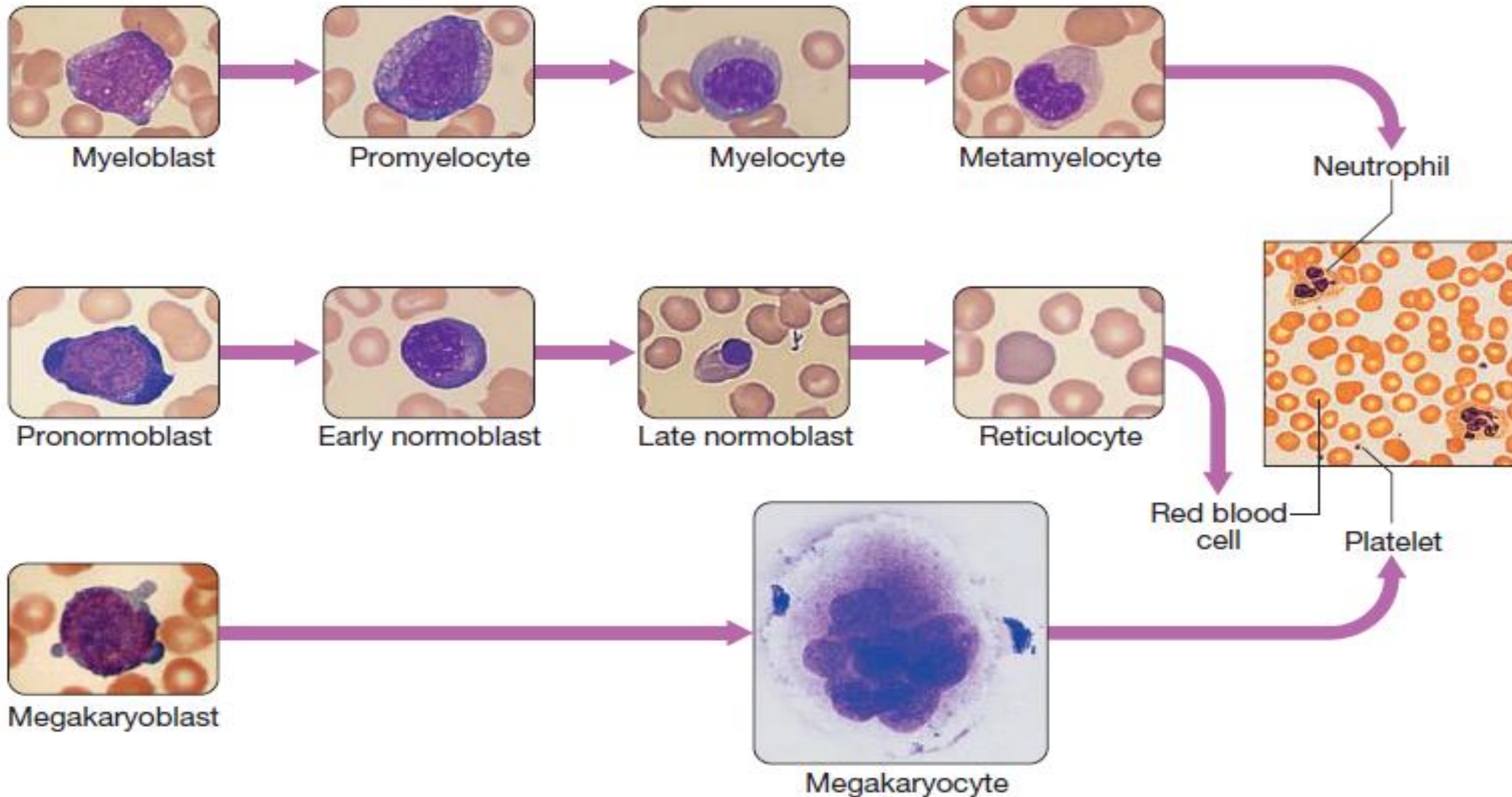
Megakaryocyte



Neutrophil

Red blood cell

Platelet



- **Reticulocytes** lose their ribosomal material and mature over 3 days, then released into the circulation.
- (reticulocytosis) reflect increased erythropoiesis
- **Erythropoietin**, is a polypeptide hormone produced by **renal interstitial peritubular cells** in response to hypoxia. It stimulate Proliferation and differentiation of red cell precursors. Failure of erythropoietin production in patients with renal failure causes anaemia, which can be treated with exogenous recombinant erythropoietin or similar pharmacological agents called erythropoiesis-stimulating agents, e.g. darbepoetin.

- Normal RBC life span in the circulation is only 120 days.
- Unnucleated, **8 μm** biconcave discs, filled with Hb, which delivers O₂ to the tissues.
- It has a deformable membrane so it can pass through the smallest capillaries, it consist of lipid bilayer to with ‘skeleton’ of proteins
- Inherited abnormalities of any of these proteins can affect the cells membrane when they pass through the spleen, or can lead to abnormally shaped red cells ex: spherocytes or elliptocytes



- RBC are exposed to osmotic stress in the pulmonary and renal circulation
- in order to maintain homeostasis, the membrane contains ion pumps, which control intracellular levels of sodium, potassium, chloride and bicarbonate.
- In the absence of mitochondria, RBCS get their energy **anaerobic glycolysis and the pentose phosphate pathway** in the cytosol.
- Membrane glycoproteins also form the antigens recognised by blood grouping as ABO and Rhesus systems.

Hemoglobin

is a protein specially adapted for oxygen transport. It is composed of four globin chains, each surrounding an iron-containing porphyrin pigment molecule termed haem.

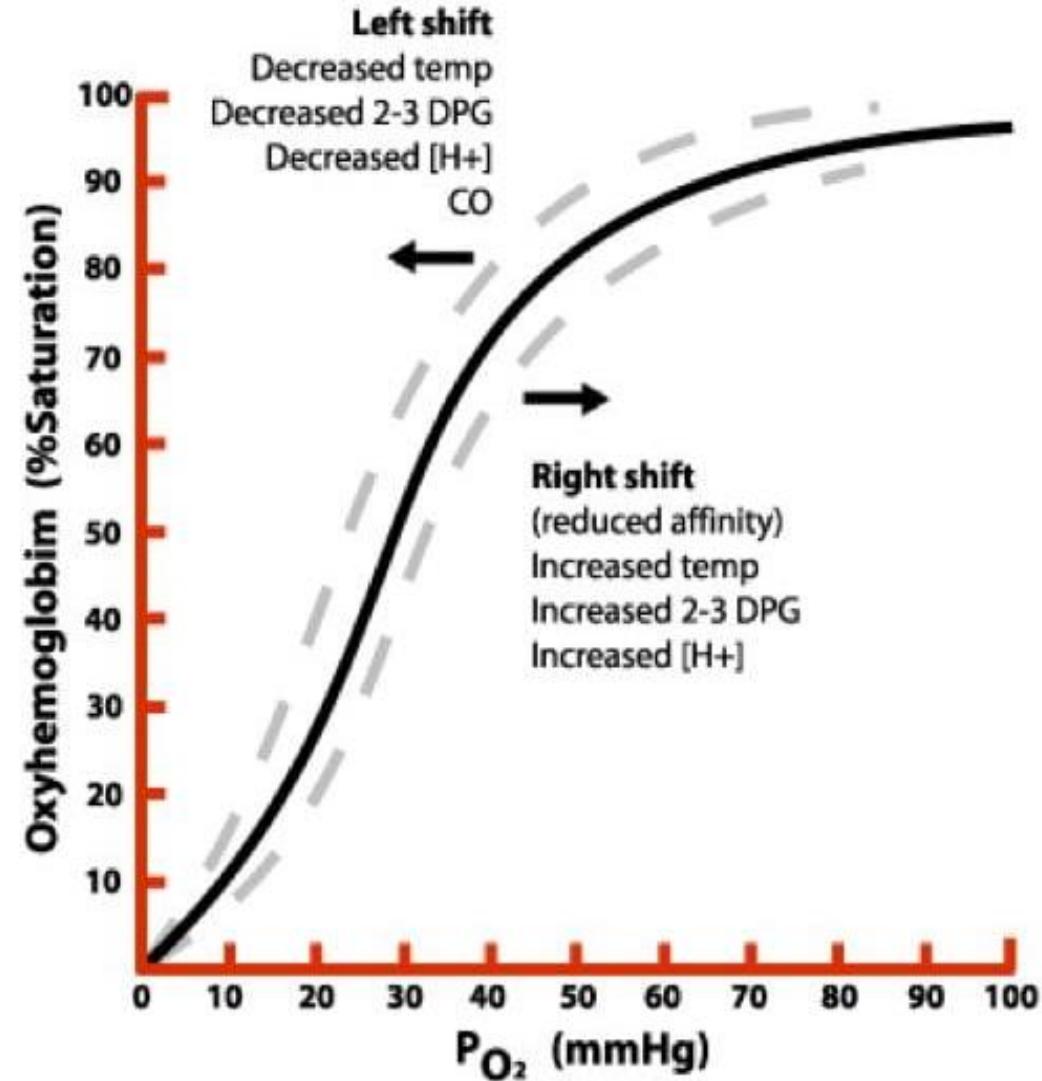
Globin chains are a combination of two alpha and two non-alpha chains;

- Hb A ($\alpha\alpha/\beta\beta$) \geq 90% of adult Hb
- Hb F ($\alpha\alpha/\gamma\gamma$) is the predominant Hb in the fetus

Each haem molecule contains a ferrous ion (Fe^{2+}), to which oxygen reversibly binds; the affinity for O_2 increases as successive oxygen molecules bind.

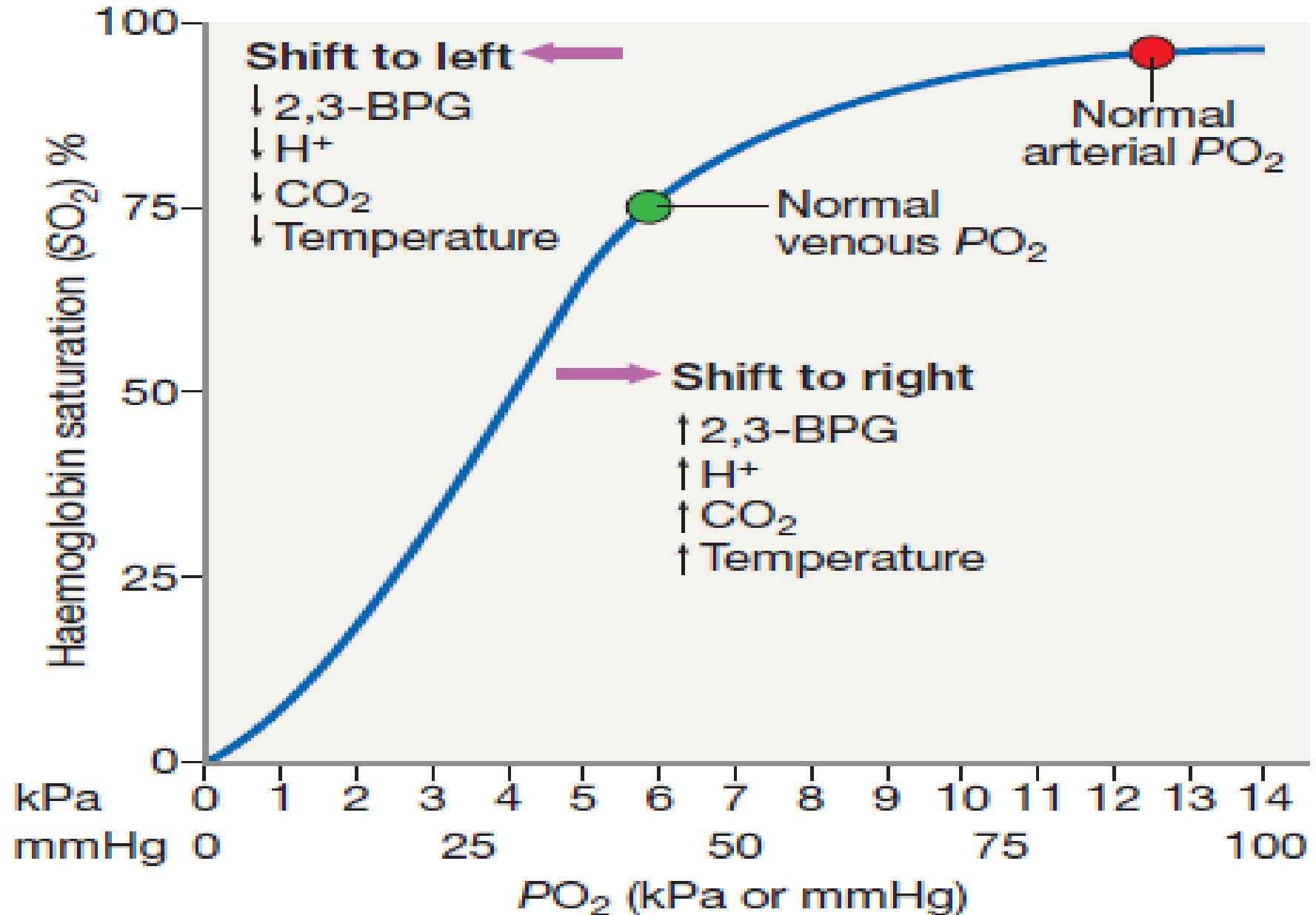
When 2,3-bisphosphoglycerate (2,3-BPG), a product of red cell metabolism, binds to the Hb it lowers oxygen affinity (more oxygen released from blood)

- Haemoglobin F is unable to bind 2,3-BPG and has a left-shifted oxygen dissociation (less oxygen released), which, together with the low pH of foetal blood, ensures foetal O₂
- Strong oxidising agents, such as dapsone, can convert ferrous iron in haemoglobin to its ferric state (Fe₃₊).
- The resultant methaemoglobin also has a left-shifted oxygen dissociation curve, which can result in tissue hypoxia



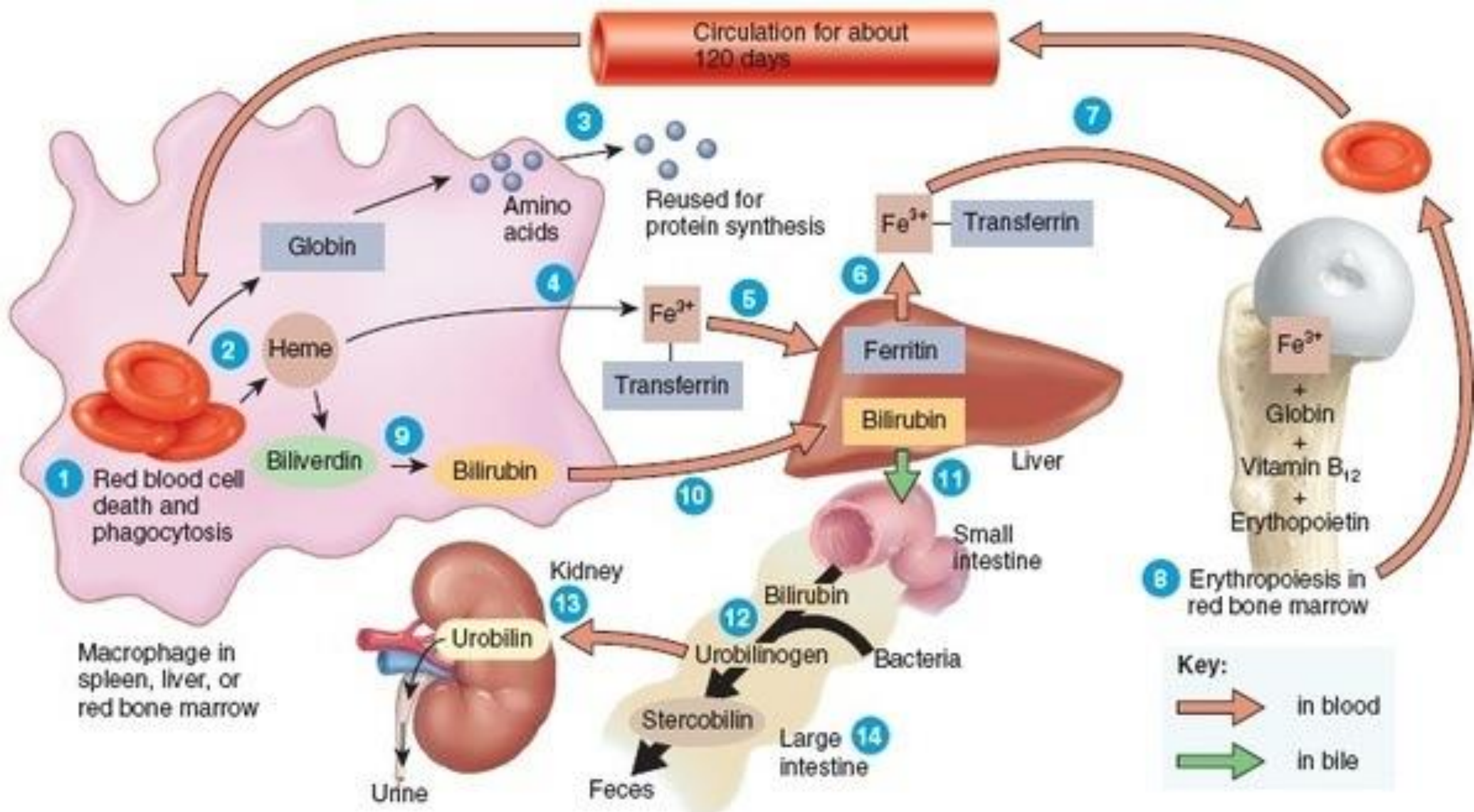
- Genetic mutations affecting the haem-binding pockets of globin result in haemoglobinopathies or unstable haemoglobins.
- Alpha globin chains are produced by two genes on chromosome 16, and beta globin chains by a single gene on chromosome 11;
- imbalance in the production of globin chains results in the thalassaemia
- Defects in haem synthesis cause the porphyria

The haemoglobin–oxygen dissociation curve



Destruction

- At the end of RBCs lifespan they will be phagocytosed by the reticulo-endothelial system (RES)
- Amino acids from globin chains are recycled and iron is removed from haem for reuse in Hb synthesis.
- The remnant haem structure is degraded to bilirubin and conjugated with glucuronic acid and excreted in bile.
- In the small bowel, bilirubin is converted to stercobilin and excreted in feces, but a small amount is excreted by the kidney as urobilinogen.
- haemolysis or ineffective haematopoiesis results in jaundice and increased urobilinogen.
- Free intravascular Hb is toxic and is normally bound by haptoglobins, a proteins produced by the liver



Circulation for about 120 days

Globin

Amino acids

Reused for protein synthesis

Fe^{3+} - Transferrin

Heme

Fe^{3+} - Transferrin

Fe^{3+}

Ferritin

Bilirubin

Liver

Fe^{3+} + Globin + Vitamin B₁₂ + Erythropoietin

Biliverdin

Bilirubin

Bilirubin

Small intestine

Urobilinogen

Bacteria

Large intestine

Kidney

Urobilin

Urine

Stercobilin

Feces

Key:



in blood



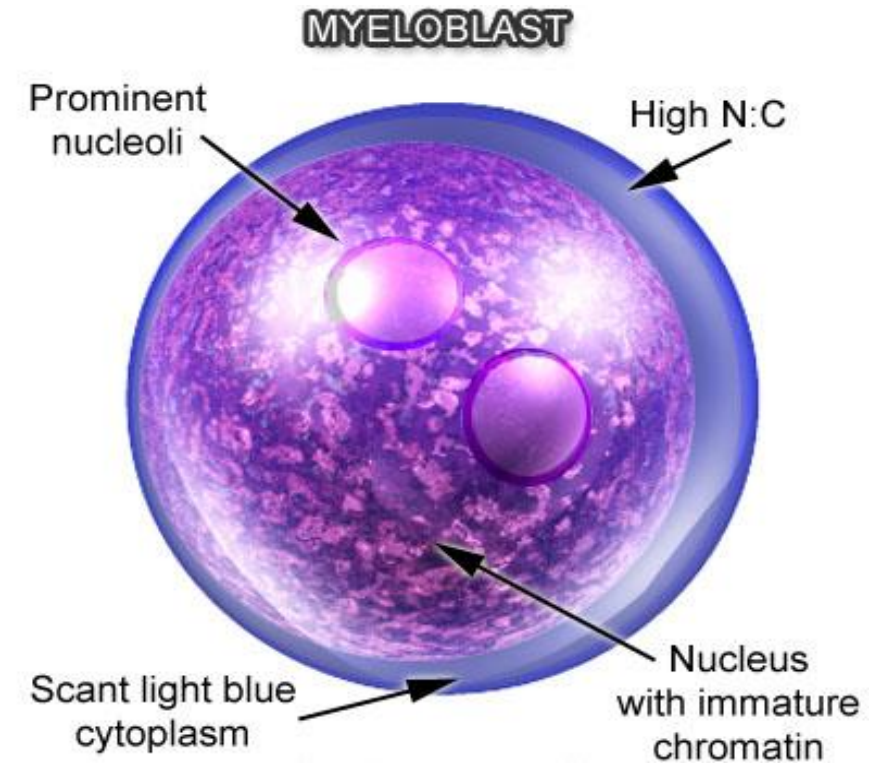
in bile

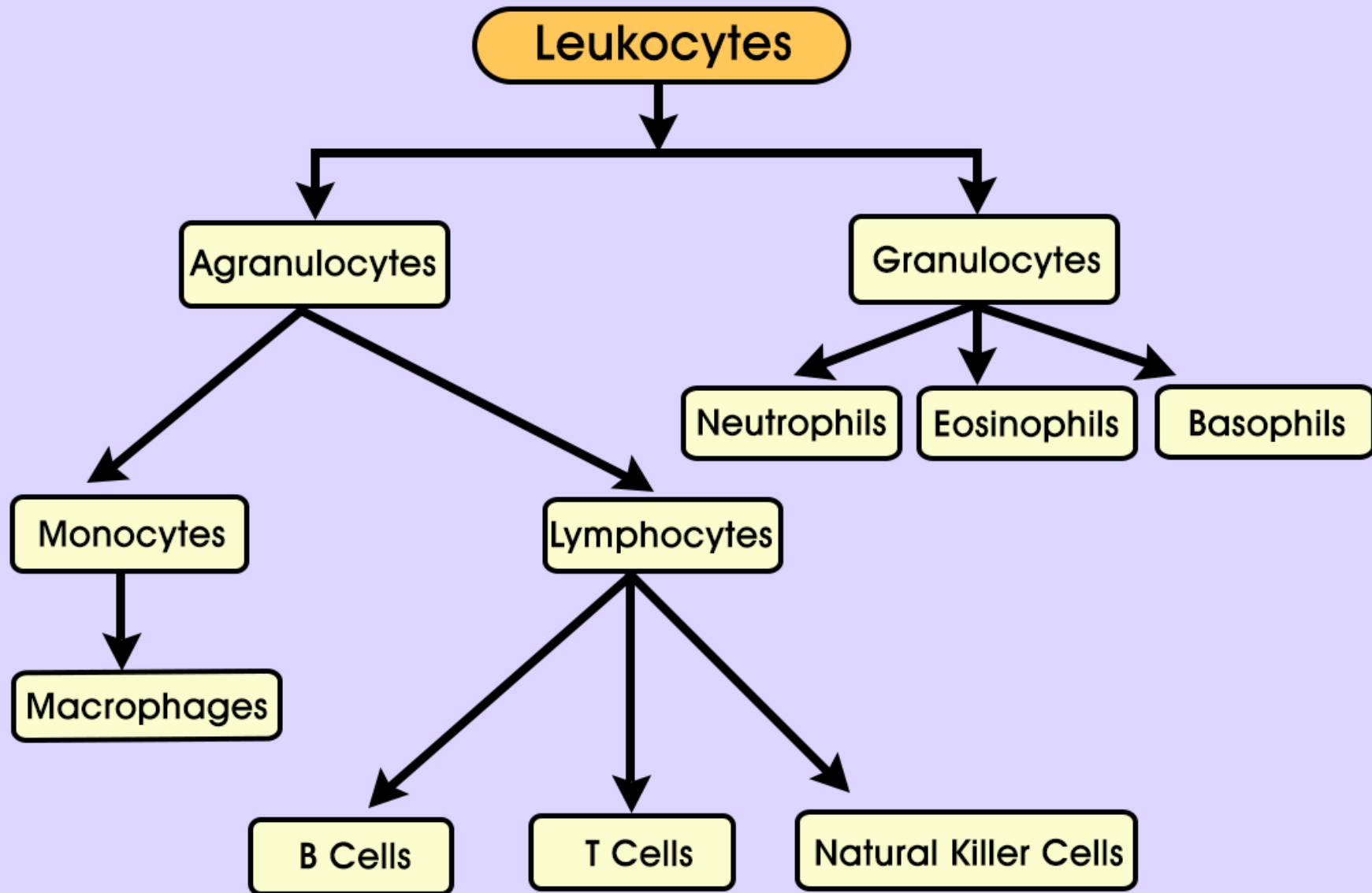
White cells (leucocytes)

- are the cells of the immune system, involved in protecting the body against both infectious disease and foreign invaders.
- It consist of :
 - **granulocytes** (neutrophils, eosinophils and basophils)
 - **agranulocytes** (monocytes and lymphocytes)
- Granulocytes and monocytes are formed from CFU–GM cells during myelopoiesis.

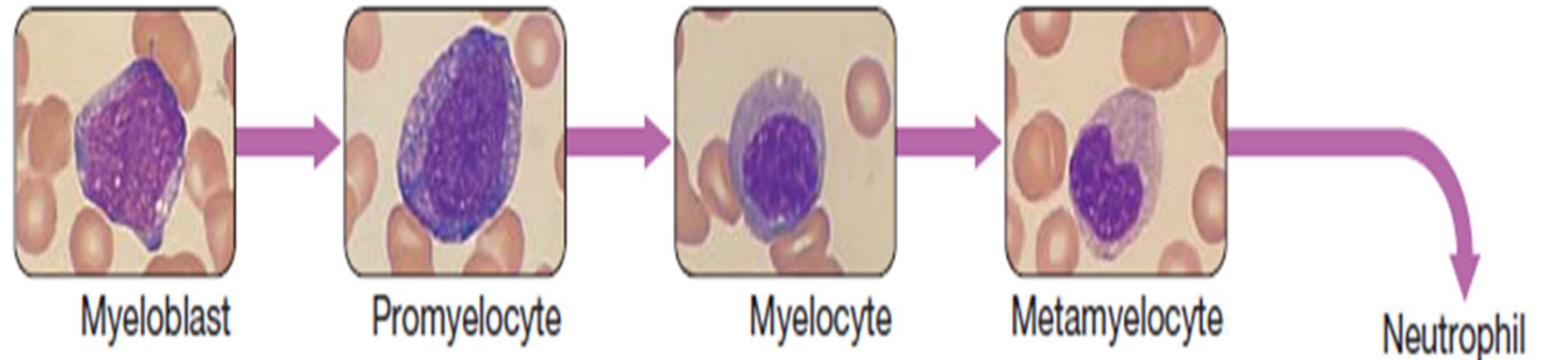
- The first recognisable granulocyte in the marrow is the **myeloblast**, a large cell with a small amount of basophilic cytoplasm and a primitive nucleus with open chromatin and nucleoli.
- As the cells divide and mature, the nucleus segments and the cytoplasm acquires specific neutrophilic, eosinophilic or basophilic granules

This takes about 14 days.



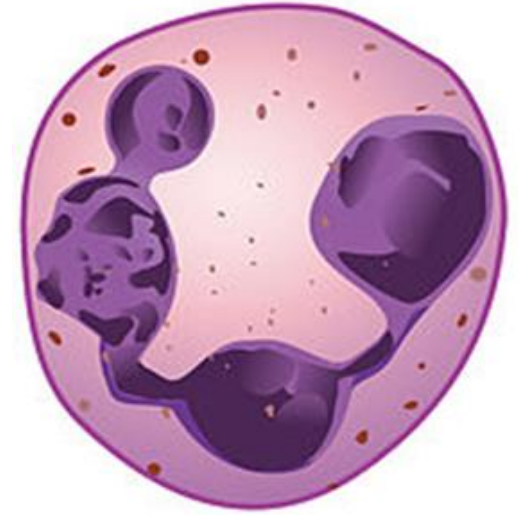


- The cytokines G-CSF, GM-CSF and M-CSF are involved in the production of myeloid cells
- G-CSF can be used clinically to hasten recovery of blood neutrophil counts after chemotherapy
- Myelocytes or metamyelocytes are normally found **only** in the BM but may appear in the circulation in infection or toxic states.
- The appearance of more primitive myeloid precursors in the blood is often associated with the presence of nucleated red cells and is termed a 'leucoerythroblastic' picture; this indicates a serious disturbance of BM function.



Neutrophils

- The predominant WBCs in the blood of adults
- **10–14 μm** in diameter, with a multilobular nucleus containing **2–5** segments and granules in their cytoplasm
- Function: recognise, ingest and destroy foreign particles and microorganisms
- Daily $\sim 10^{11}$ neutrophils enter the circulation, where cells may be circulating freely or attached to endothelium in the marginating pool.
- These two pools are equal in size; factors such as exercise or catecholamines increase the number of cells flowing in the blood

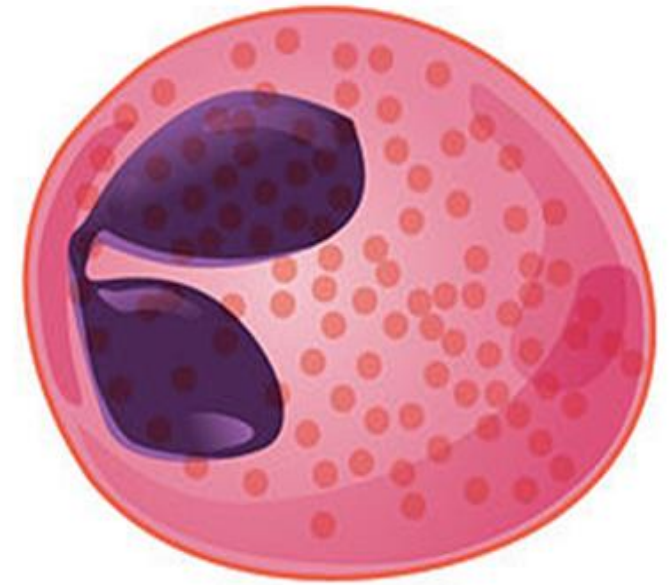


Neutrophil

- Neutrophils spend **6–10** hours in the circulation before being removed, principally by the spleen
- Alternatively, they pass into the tissues and either are consumed in the inflammatory process or undergo apoptotic cell death and phagocytosis by macrophages

Eosinophils

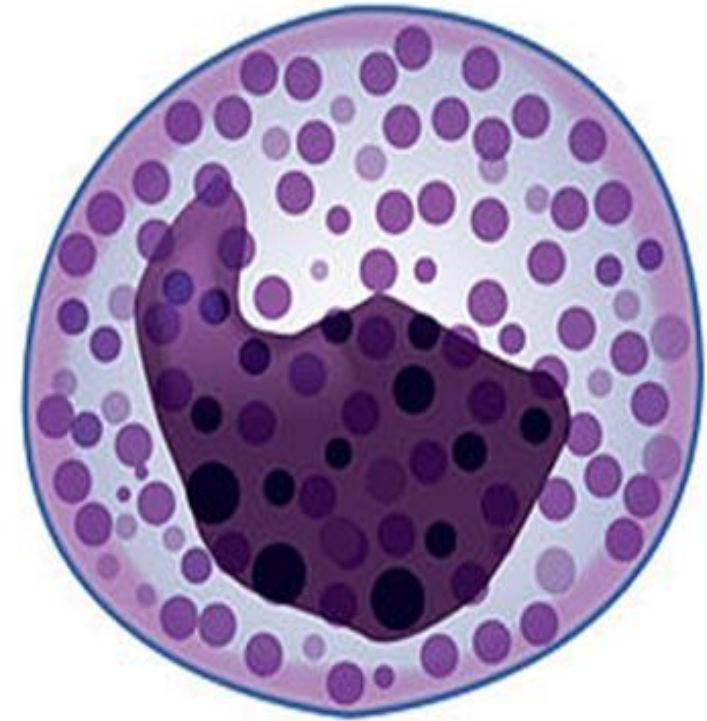
- represent **1–6%** of the circulating WBC.
- similar size to neutrophils but have a bilobed nucleus and prominent orange granules on Romanowsky staining
- Eosinophils are phagocytic and their granules contain a peroxidase capable of generating reactive oxygen species and proteins involved in the intracellular killing of protozoa and helminths
- They are also involved in allergic reactions (e.g. atopic asthma,)



Eosinophil

Basophils

- Representing **<1%** of circulating WBCs.
- With dense black granules that obscure the nucleus.
- Mast cells resemble basophils but are found only in the tissues.
- These cells are involved in hypersensitivity reactions



Basophil

Monocytes

- Monocytes are the largest of the white cells, with a diameter of **12–20 μm** and an irregular nucleus in abundant pale blue cytoplasm containing occasional cytoplasmic vacuoles
- These cells circulate for a few hours and then migrate into tissue, where they become macrophages, Kupffer cells or antigen-presenting dendritic cells
- They phagocytose debris, apoptotic cells and microorganisms



Lymphocytes

- There are two main types: T cells (which mediate cellular immunity) and B cells (which mediate humoral immunity)
- Lymphoid cells that migrate to the thymus develop into T cells, whereas B cells develop in the bone marrow.
- ~ **80%** of lymphocytes in the circulation are T cells
- Size heterogeneous, could be as small as RBCs as large and as neutrophils
- Small lymphocytes are circular with scanty cytoplasm but the larger cells are more irregular with abundant blue cytoplasm.
- Lymphocyte lifespan (a few days to many years).

- Cell surface antigens ('cluster of differentiation' (CD) antigens), which appear at different points of lymphocyte maturation and indicate the lineage and maturity of the cell, are used to classify lymphomas and lymphoid leukaemias.



lymphocyte

Hemostasis

Is the process of clot formation in case of vascular injury in order to prevent excessive bleeding

Successful haemostasis can be achieved by complex interactions between the

- vascular endothelium
- platelets
- von-Willebrand factor
- coagulation factors
- natural anticoagulants
- fibrinolytic enzymes

Dysfunction of any of these components may result in haemorrhage or thrombosis

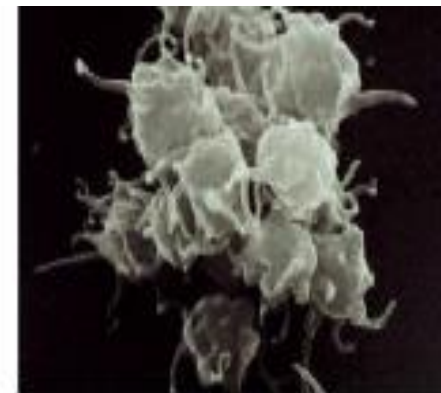
Platelets

- Platelets are derived from Megakaryocytic progenitor cells (CFU–Meg) divide to form megakaryoblasts then undergo maturation process to produce megakaryocytes, large numbers of platelets then fragment off from each megakaryocyte into the circulation.
- This process is stimulated by thrombopoietin produced in the liver.
Platelets
- Circulate for **8–10** days then destroyed in the RES
- ~**30%** of peripheral platelets are normally pooled in the spleen and do not circulate.

- The platelets are discoid, with a diameter of **2–4 μm** The surface membrane invaginates to form a tubular network, the canalicular system, which provides a conduit for the discharge of the granule content following platelet activation.
- Drugs that inhibit platelet function and thrombosis ex: aspirin, clopidogrel, and ticagrelor, dipyridamole and the glycoprotein IIb/IIIa inhibitors abciximab, tirofiban and eptifibatide(which prevent fibrinogen binding;



Resting platelets



Activated platelets

Clotting factors

- The coagulation system consists of a cascade of soluble inactive zymogen proteins, When activated, each is capable of activating one or more components of the cascade.
- Activated factors are designated by the suffix 'a'. Some of these reactions require phospholipid and calcium.
- Coagulation occurs by two pathways: it is initiated by the extrinsic (or tissue factor) pathway and amplified by the 'intrinsic pathway'

- Clotting factors are synthesised by the liver, although factor V is also produced by platelets and endothelial cells.
- Factors II, VII, IX and X require post-translational carboxylation to allow them to participate in coagulation.
- The carboxylase enzyme responsible for this in the liver is vitamin K-dependent
- This production can be inhibited by warfarin, which is the basis of the anticoagulant effect of coumarins
- Congenital (e.g. haemophilia) and acquired (e.g. liver failure) causes of coagulation factor deficiency are associated with bleeding

Intrinsic system

XII → XIIa

XI → XIa

IX → IXa + VIIIa

X → Xa + Va

Prothrombin → Thrombin

Fibrinogen → Fibrin

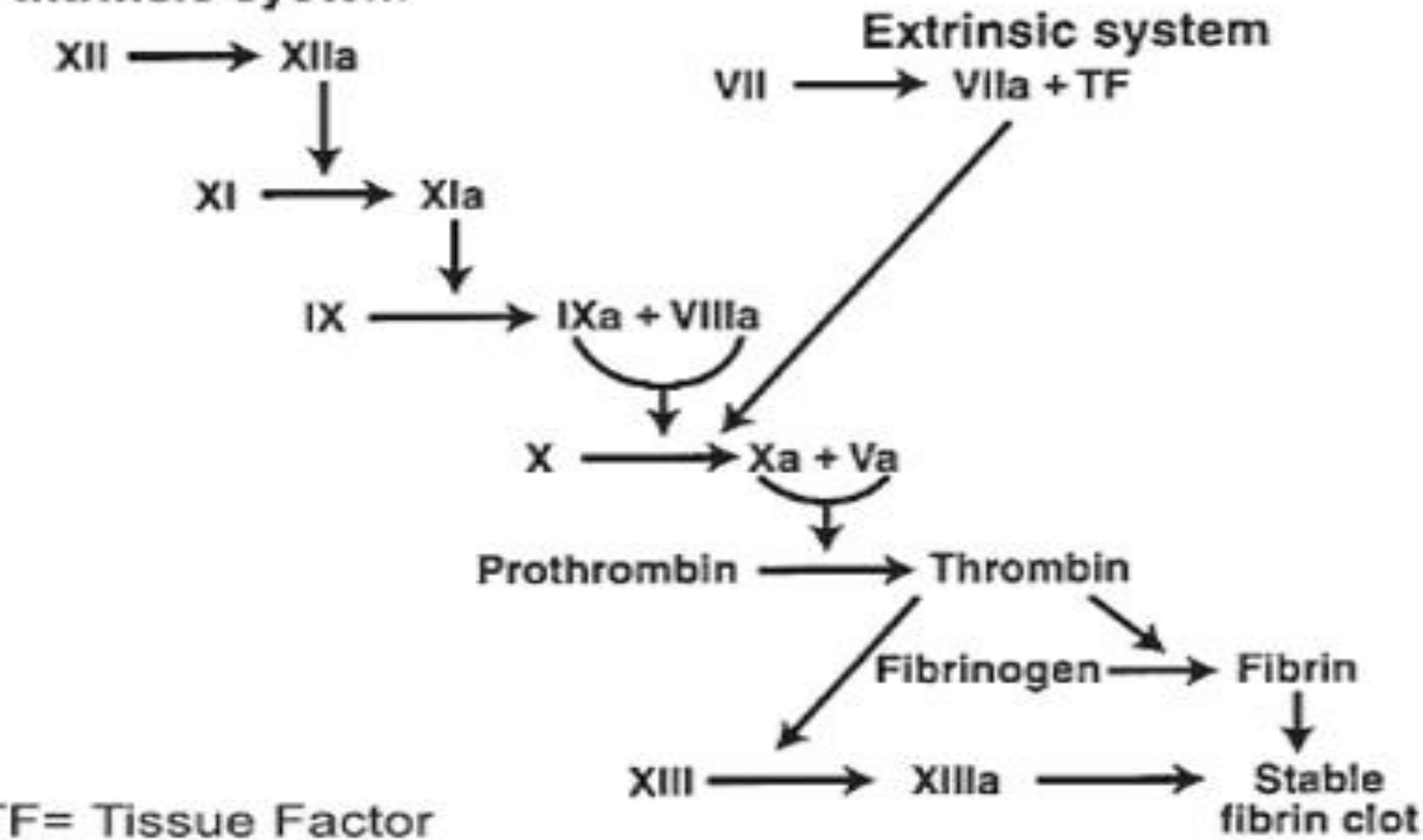
XIII → XIIIa

XIIIa → Stable fibrin clot

Extrinsic system

VII → VIIa + TF

TF = Tissue Factor



23.3 Coagulation screening tests

Investigation	Reference range ²	Situations in which tests may be abnormal
Platelet count	150–400 × 10 ⁹ /L	Thrombocytopenia
Prothrombin time (PT)	9–12 secs	Deficiencies of factors II, V, VII or X Severe fibrinogen deficiency
Activated partial thromboplastin time (APTT)	26–36 secs	Deficiencies of factors II, V, VIII, IX, X, XI, XII Severe fibrinogen deficiency Unfractionated heparin therapy Antibodies against clotting factors Lupus anticoagulant Multiple factor deficiency (e.g. DIC)
Fibrinogen concentration	1.5–4.0 g/L	Hypofibrinogenaemia, e.g. liver failure, DIC

¹N.B. International normalised ratio (INR) is used only to monitor coumarin therapy and is not a coagulation screening test. ²Ranges are approximate and may vary between laboratories.
(DIC = disseminated intravascular coagulation)

Thank you