Blood/ Hematology

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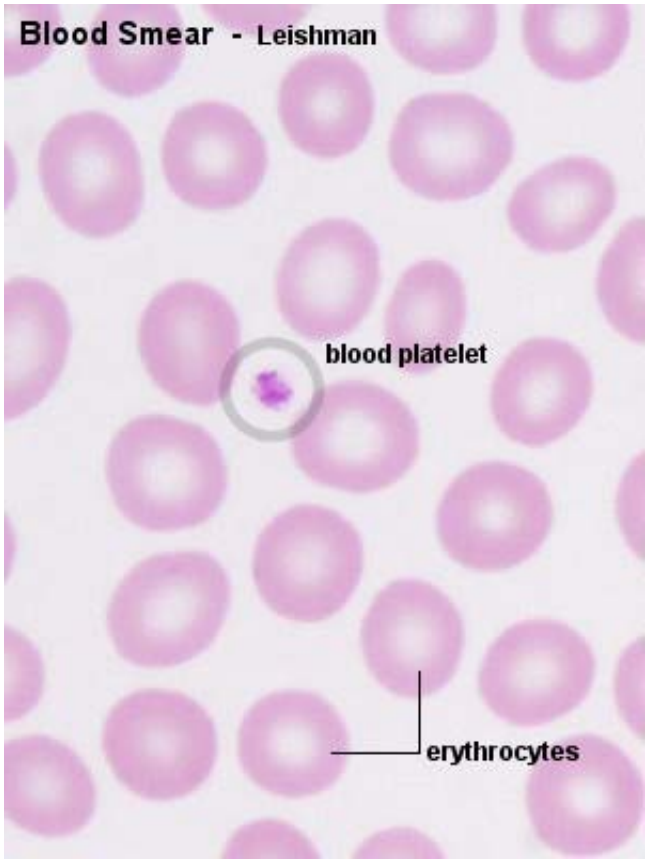
HTTP://Sinoemedicalassociation.or/AP2/

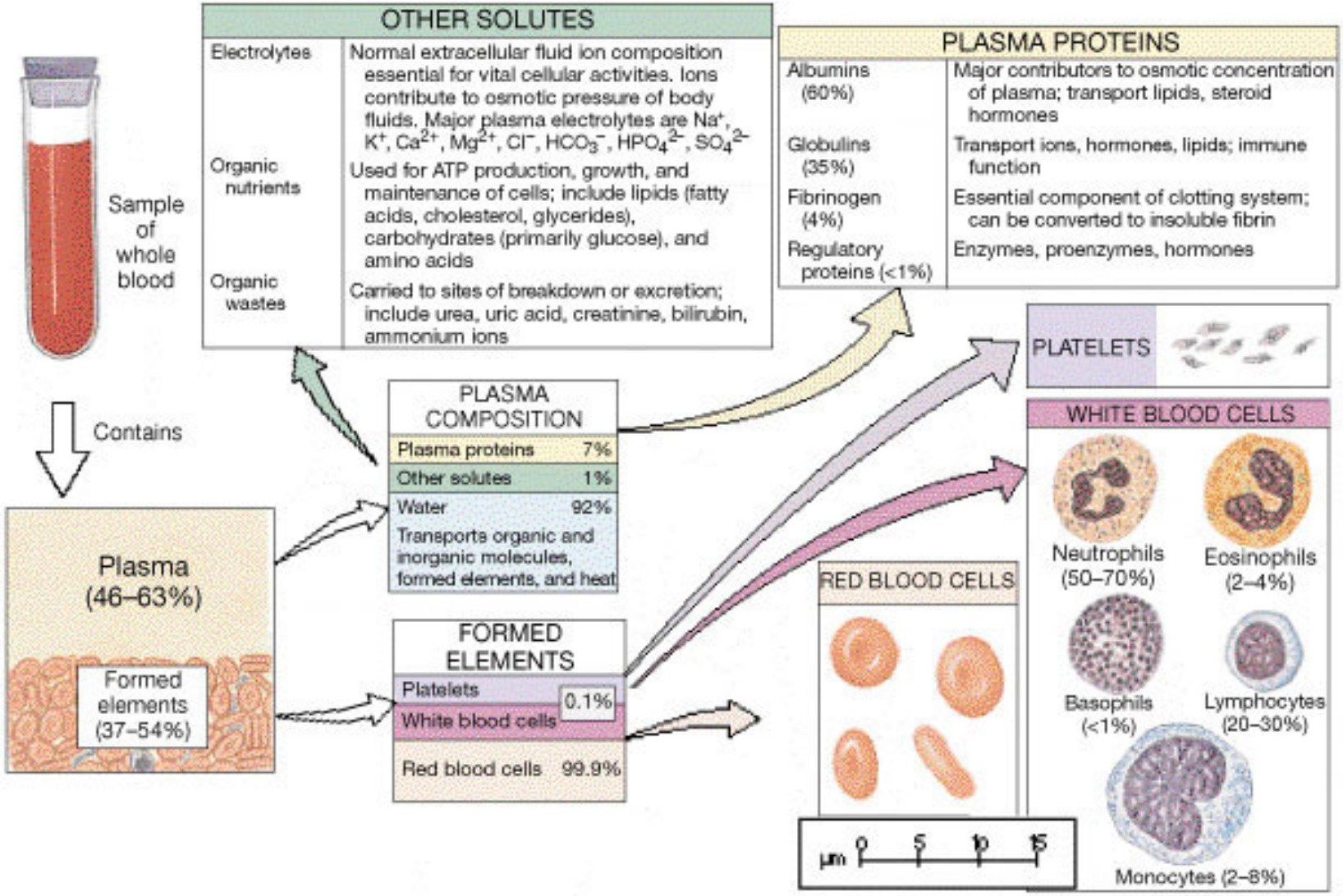
Blood is a connective tissue whose matrix is fluid.
It is composed of:

1. red corpuscles,
2. white cells,
3. platelets,
4. blood plasma.

It is transported throughout the body within blood vessels.

- Blood is sometimes considered to be a fluid connective tissue because of the mesenchymal origin of its cells and a low ratio of cells to liquid intercellular substance, the blood plasma.
- In human adults about 5 liter of blood contribute 7-8 % to the body weight of the individual.
- The contribution of red blood cells (erythrocytes) to the total volume of the blood (haematocrit) is about 43%.
- Erythrocytes are the dominant (99%) but not the only type of cells in the blood.
- We also find leukocytes and, in addition, blood platelets. Erythrocytes, leukocytes and blood platelets are also being referred to as the formed elements of the blood.
- Erythrocytes and blood platelets perform their functions exclusively in the blood stream.
- In contrast, leukocytes reside only temporarily in the blood.
- Leukocytes can leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.




OTHER SOLUTES
Electrolytes
Normal extracellular fluid ion composition essential for vital cellular activities. Ions contribute to osmotic pressure of body fluids. Major plasma electrolytes are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}, SO_4^{2-}
Organic nutrients
Used for ATP production, growth, and maintenance of cells; include lipids (fatty acids, cholesterol, glycerides), carbohydrates (primarily glucose), and amino acids
Organic wastes
Carried to sites of breakdown or excretion; include urea, uric acid, creatinine, bilirubin, ammonium ions
PLASMA PROTEINS
Albumins (60%)
Major contributors to osmotic concentration of plasma; transport lipids, steroid hormones
Globulins (35%)
Transport ions, hormones, lipids; immune function
Fibrinogen (4%)
Essential component of clotting system; can be converted to insoluble fibrin
Regulatory proteins (<1%)
Enzymes, proenzymes, hormones
Sample of whole blood
Contains
Plasma (46–63%)
Formed elements (37–54%)
PLASMA COMPOSITION
Plasma proteins 7%
Other solutes 1%
Water 92%
Transports organic and inorganic molecules, formed elements, and heat
FORMED ELEMENTS
Platelets
0.1%
White blood cells
Red blood cells 99.9%
PLATELETS
WHITE BLOOD CELLS
Neutrophils (50–70%)
Eosinophils (2–4%)
Basophils (<1%)
Lymphocytes (20–30%)
Monocytes (2–8%)
RED BLOOD CELLS
μm 0 5 10 15

Element	Diameter (in um)	Number (per mm^3)	Scientific notation (per mm^3)	Main function
 red blood cells	7 - 8	4,500,000 - 52500,000	4.5×10^6 5.5×10^6	oxygen transport
 white blood cells	9 - 12	7,000 - 10,000	7×10^3 1×10^4	defense against microorganisms
 platelets	2 - 4	300,000	3×10^5	blood-clotting

Blood facts

- Approximately 8% of an adult's body weight is made up of blood.
- Females have around 4-5 litres, while males have around 5-6 litres. This difference is mainly due to the differences in body size between men and women.
- Its mean temperature is 38 degrees Celcius.
- It has a pH of 7.35-7.45, making it slightly basic (less than 7 is considered acidic).
- Whole blood is about 4.5-5.5 times as viscous as water, indicating that it is more resistant to flow than water.
- This viscosity is vital to the function of blood because if blood flows too easily or with too much resistance, it can strain the heart and lead to severe cardiovascular problems.
- Blood in the arteries is a brighter red than blood in the veins because of the higher levels of oxygen found in the arteries.
- An artificial substitute for human blood has not been found.

There are three major categories of plasma proteins, and each individual type of proteins has its own specific properties and functions in addition to their overall collective role:

1. **Albumins**, which are the smallest and most abundant plasma proteins. Reductions in plasma albumin content can result in a loss of fluid from the blood and a gain of fluid in the interstitial space (space within the tissue), which may occur in nutritional, liver and kidney disease. Albumin also helps many substances dissolve in the plasma by binding to them, hence playing an important role in plasma transport of substances such as drugs, hormones and fatty acids.
2. **Globulins**, which can be subdivided into three classes from smallest to largest in molecular weight into alpha, beta and gamma globulins. The globulins include high density lipoproteins (HDL), an alpha-1 globulin, and low density lipoproteins (LDL), a beta-1 globulin. HDL functions in lipid transport carrying fats to cells for use in energy metabolism, membrane reconstruction and hormone function. HDLs also appear to prevent cholesterol from invading and settling in the walls of arteries. LDL carries cholesterol and fats to tissues for use in manufacturing steroid hormones and building cell membranes, but it also favours the deposition of cholesterol in arterial walls and thus appears to play a role in disease of the blood vessels and heart. HDL and LDL therefore play important parts in the regulation of cholesterol and hence have a large impact on cardiovascular disease.
3. **Fibrinogen**, which is a soluble precursor of a sticky protein called fibrin, which forms the framework of blood clot. Fibrin plays a key role in coagulation of blood, which is discussed later in this article under Platelets.

Amino acids These are formed from the break down of tissue proteins or from the digestion of digested proteins.

Nitrogenous waste Being toxic end products of the break down of substances in the body, these are usually cleared from the bloodstream and are excreted by the kidneys at a rate that balances their production.

Nutrients Those absorbed by the digestive tract are transported in the blood plasma. These include glucose, amino acids, fats, cholesterol, phospholipids, vitamins and minerals.

Gases Some oxygen and carbon dioxide are transported by plasma. Plasma also contains a substantial amount of dissolved nitrogen.

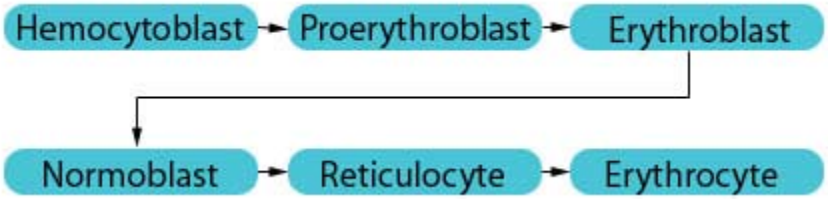
Electrolytes The most abundant of these are sodium ions, which account for more of the blood's osmolarity than any other solute.

Hemopoiesis

- During fetal development, the formation of blood cells (hemopoiesis) commences in wall of the yolk sac.
- After the second month of fetal development, the liver, and, slightly later, the spleen, become the dominant sites of hemopoiesis .
- From the 6th month, and dominating from the 7th month onwards, the formation of blood cells occurs in bone marrow, which is the major site of formation blood cells in normal adult humans.
- Yellow bone marrow, which harbours mainly adipocytes, dominates in the hollow of the diaphysis of adult long bones.
- Hemopoiesis occurs in red bone marrow, which is typically found between the trabeculae of spongy bone in the epiphysis of adult long bones.
- Both age and demands on hemopoiesis may effect the relative amounts of red and yellow bone marrow.
- Hemopoietic cells surround the vascular sinusoids and are supported by reticular connective tissue.
- In addition to the endothelial cells of the sinusoids and the reticulocytes of the connective tissue, macrophages are frequent in red bone marrow.

Erythropoiesis	Leukopoiesis	Thrombopoiesis
Erythropoiesis, the process of making erythrocytes, begins with the formation of proerythroblasts from hemopoietic stem cells. Over three to five days, several stages of development follow as ribosomes proliferate and hemoglobin is synthesized. Finally, the nucleus is ejected, producing the depression in the center of the cell. Young erythrocytes, called reticulocytes, still containing some ribosomes and endoplasmic reticulum, pass into the bloodstream and develop into mature erythrocytes after another	Leukopoiesis, the process of making leukocytes, is stimulated by various colony-stimulating factors (CSFs), hormones produced by mature white blood cells. The development of each kind of white blood cell begins with the division of themopoietic stem cells into one of the following “blast” cells. • Myeoblasts divide to form eosinophilic, neutrophilic, or basophilic myelocytes, which lead to the development of the three kinds of granulocytes. • Monoblasts lead to the development of monocytes.	Thrombopoiesis, the process of making platelets, begins with the formation of megakaryoblasts from hemopoietic stem cells. The megakaryoblasts divide without cytokinesis to become megakaryocytes, huge cells with a large, multilobed nucleus. The megakaryocytes then fragment into segments as the plasma membrane infolds into the cytoplasm.

one or two days. **Erythroprotein** Erythroprotein (EPO), a hormone produced mostly by the kidneys, stimulates bone marrow to produce erythrocytes. When inadequate amounts of oxygen are delivered to body cells, a condition called hypoxia, the kidneys increase EPO secretion, which, in turn, stimulates an increase in erythrocyte production. The average production rate of erythrocytes in healthy individuals is two million cells per second. Normal production requires adequate amounts of iron and vitamin B12 and folic acid.	• Lymphoblasts lead to the development of lymphocytes.	



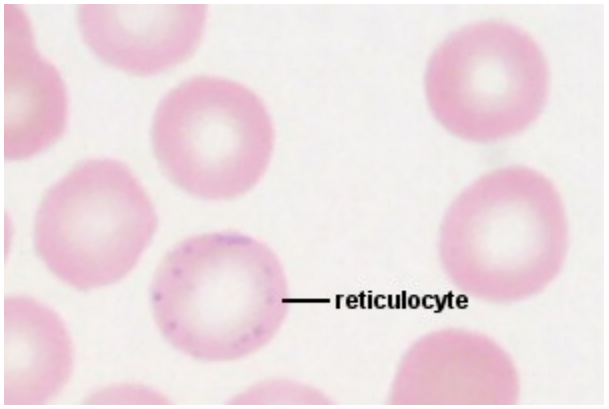
Hemopoietic Cells

The basis of hemopoiesis is a small population of self-replicating stem cells, which ultimately can generate all types of blood cells. Their progeny may develop into either lymphocytic stem cells or pluripotent haemal stem cells (colony-forming unit - stem cell - CFU-S). The latter type gives rise to stem cells which can form the major groups of blood cells other than lymphocytes. Depending on their progeny it is possible to differentiate

- burst-forming unit of the erythroid line (BFU-E),
- colony-forming unit - granulocytes and macrophages (CFU-G/M), and
- colony-forming unit - megakaryocytes (CFU-Mk).

Erythrocytes

- The first identifiable stage of erythropoiesis is the proerythroblast - a large, slightly basophilic cell, which contains a large, lightly stained nulceus.
- Proerythroblasts proliferate to generate a sequence of cells which show a gradual decrease in size and condensation of their chromatin.
- They are named after changes in the staining characteristic of their cytoplasm (basophilic erythroblast, polychromatophilic and orthochromic normoblasts).
- The nucleus is finally extruded from the normoblast.
- The cell enters circulation as a reticulocyte, which still contains some organelles.
- Reticulocytes remain for a few days in either the bone marrow or the spleen to mature to erythrocytes.



- In some blood smears reticulocytes may be recognisable because of a very slight basophilic staining - either homogeneous or in the form of a basophilic stippling.

Granulocytes

- Myeloblast appear light-microscopically similar to proerythroblast.
- They proliferate to generate promyelocytes. Promyelocytes begin to accumulate nonspecific granules, but they are still able to divide.
- The maturation of their progeny, the myelocytes, is characterised by the accumulation of specific granules and changes in nuclear morphology. Metamyelocytes have a C-shaped nucleus.

Blood Platelets (Thrombocytes)

- are, as mentioned above, fragments of the cytoplasm of megakaryocytes.
- Megakaryocytes are very large cells (up to 160 µm in diameter), which contain very large, highly lobulated, polyploid nuclei.
- Megakaryocytes are in turn the product of the differentiation of basophilic megakaryoblasts.

Precursors of blood cells which are usually only found in the bone marrow can be found in peripheral blood in a variety of pathological conditions.
If a Rh-negative mother has been immunised by erythrocytes of a Rh-positive foetus, a condition called Erythroblastosis fetalis may develop during subsequent pregnancies.

It would show itself in the foetus or newborn by the presence of erythrocyte precursors in peripheral blood - although other, more severe symptoms should be obvious.

Chronic myeloid leukemia is another condition - in this case showing itself by the presence of all types of granulocyte precursors in peripheral blood.

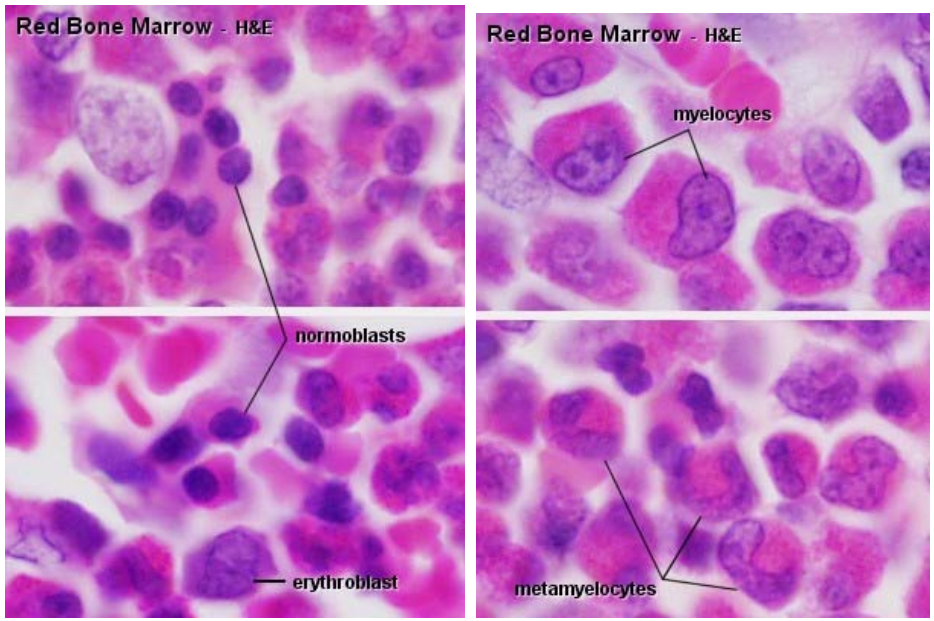
- The nomenclature employed for haemopoietic cells (but not the number of stages recognized) is somewhat variable across texts.
- Note also that these cell types refer to stages of development along a morphologically more or less continuous spectrum.
- Most of the haemopoietic cells visible will be of the erythroblastic line. The only cell type of this line which is easy to distinguish in H&E stained sections are normoblasts.
  A very condensed nucleus is seen in late (orthochromic) normoblast. Granulocyte and erythrocyte precursors will mostly intermingle, but may be distinguished by nuclear morphology and/or size. A bent nucleus is found in metamyelocytes - this shape is very pronounced in the last, immature form of neutrophils, which are also called stab or band cells. If the cell

(1) is large, with a distinct "clearing" in the otherwise pink cytoplasm and

(2) has an ovoid or slightly indented nucleus, it is likely to be a myelocyte. Cells with large light nuclei and almost unstained cytoplasm are either reticulocytes or macrophages.

- Macrophages are frequently associated with normoblasts, and together these cells form erythroblastic islands. The name for macrophages in these islands, nurse cells, may tell you a bit about their function in addition to the scavenging of the expelled nuclei.

- If you still have some time and are desperate to get frustrated try to hunt up a nice basophilic erythroblast - a basophilic cell with homogeneously staining nucleus that is somewhat smaller than the nuclei of granulocyte precursors.



The formation of blood cells (cell types and acronyms are defined below)

All the various types of blood cells

- are produced in the **bone marrow** (some 10^{11} of them each day in an adult human!).
- arise from a single type of cell called a **hematopoietic stem cell** — an "adult" multipotent stem cell.

These stem cells

- are very rare (only about one in 10,000 bone marrow cells);
- are attached (probably by adherens junctions) to osteoblasts lining the inner surface of bone cavities;
- express a cell-surface protein designated **CD34**;
- produce, by mitosis, two kinds of progeny:
    - more stem cells (A mouse that has had all its blood stem cells killed by a lethal dose of radiation can be saved by the injection of a single living stem cell!).
    - cells that begin to differentiate along the paths leading to the various kinds of blood cells.

Which path is taken is regulated by

- the need for more of that type of blood cell which is, in turn, controlled by appropriate cytokines and/or hormones.

For example, **Interleukin-7** (**IL-7**) is the major cytokine in stimulating bone marrow stem cells to start down the "lymphoid" path leading to the various **lymphocytes** (mostly B cells and T cells).

Some of the cytokines that drive the differentiation of the "myeloid" leukocytes are

- **Erythropoietin** (**EPO**), produced by the kidneys, enhances the production of **red blood cells** (RBCs).
- **Thrombopoietin** (**TPO**), assisted by Interleukin-11 (**IL-11**), stimulates the production of **megakaryocytes**. Their fragmentation produces **platelets**.
- **Granulocyte-macrophage colony-stimulating factor** (**GM-CSF**), as its name suggests, sends cells down the path leading to both those cell types. In due course, one path or the other is taken.
    - Under the influence of **granulocyte colony-stimulating factor** (**G-CSF**), they differentiate into **neutrophils**.
    - Further stimulated by interleukin-5 (**IL-5**) they develop into **eosinophils**.
    - Interleukin-3 (**IL-3**) participates in the differentiation of most of the white blood cells but plays a particularly prominent role in the formation of **basophils** (responsible for some allergies).
    - Stimulated by **macrophage colony-stimulating factor** (**M-CSF**) the granulocyte/macrophage progenitor cells differentiate into **monocytes, macrophages**, and dendritic cells (DCs).

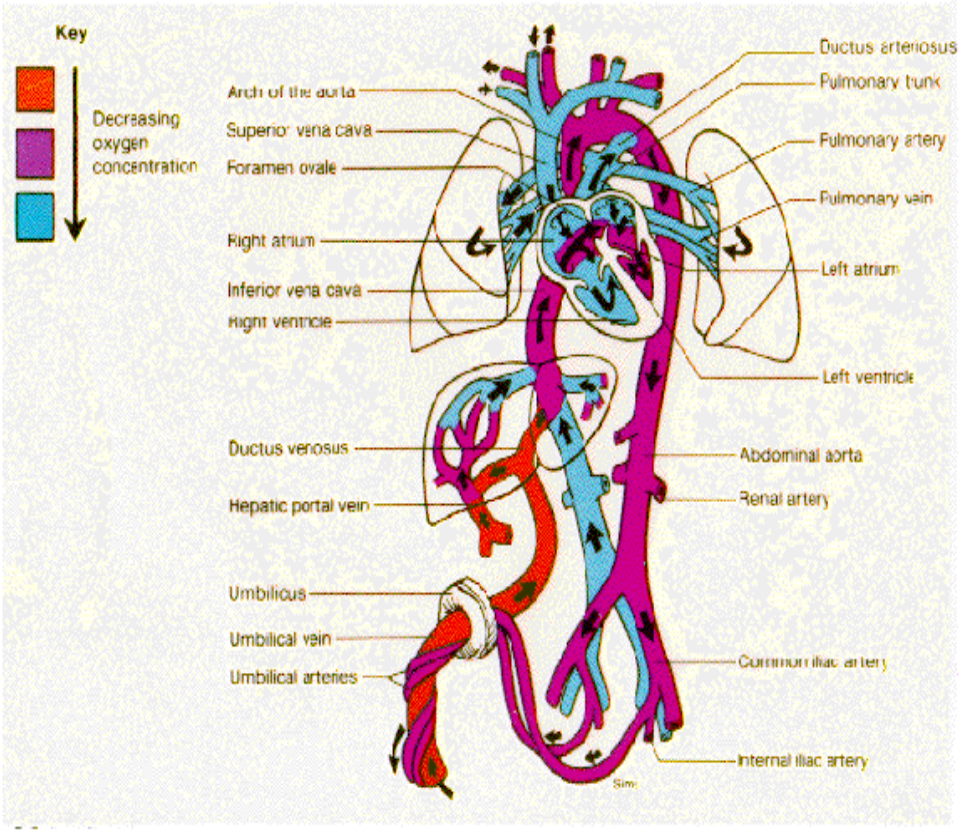
HEMATOPOIETIC FACTORS

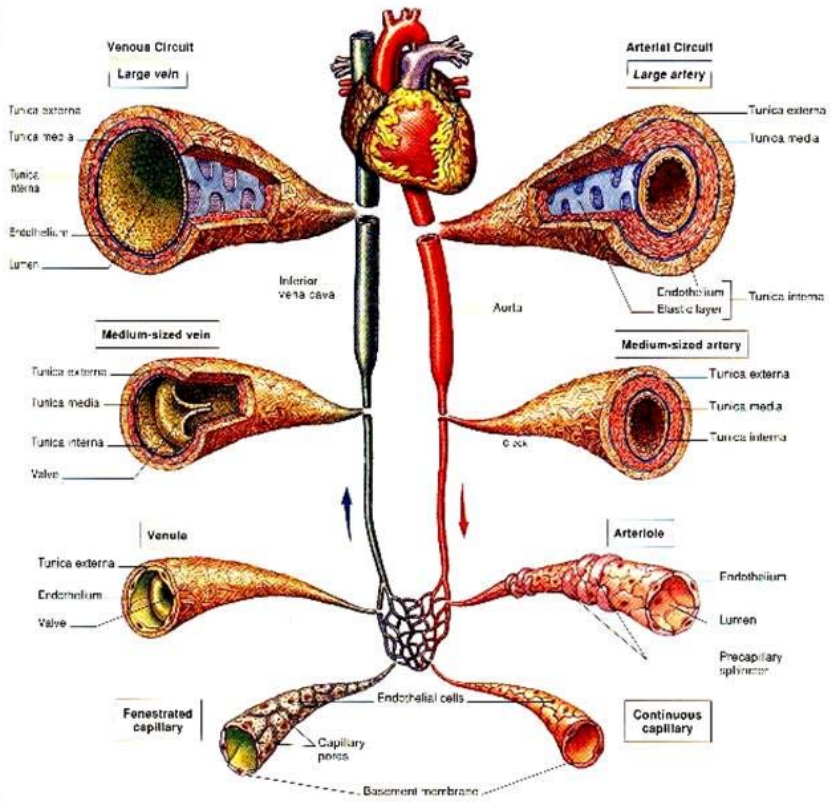
NAME	CELLULAR SOURCE	CELL TYPES PRODUCED IN INCREASED NUMBERS
Erythropoietin (EPO)	Kidney cells, Kupffer cells	rbc
G-CSF	Monocytes, fibroblasts, endothelial cells	n
M-CSF	Monocytes, fibroblasts, endothelial cells	m
GM-GSF	T cells, monocytes, fibroblasts, endothelial cells	n, m, e, meg, rbc
IL-1	Macrophages, endothelial cells, fibroblasts	n, m, e, b, meg, rbc
IL-3	T cells	n, m, e, b, meg, rbc
IL-4	T cells	b
IL-5	T cells	e
IL-6	Macrophages, endothelial cells, fibroblasts	n, m, e, b, meg, rbc

n, neutrophils; m, monocytes; e, eosinophils; b, basophils; meg, megakaryocytes; rbc, red blood cells
IL – interleukin , CSF – colony stimulating factor

Overview of Blood Circulation [see cardiovascular lectures]

- Blood leaves the heart via arteries that branch repeatedly until they become capillaries
- Oxygen (O_2) and nutrients diffuse across capillary walls and enter tissues
- Carbon dioxide (CO_2) and wastes move from tissues into the blood
- Oxygen-deficient blood leaves the capillaries and flows in veins to the heart
- This blood flows to the lungs where it releases CO_2 and picks up O_2
- The oxygen-rich blood returns to the heart





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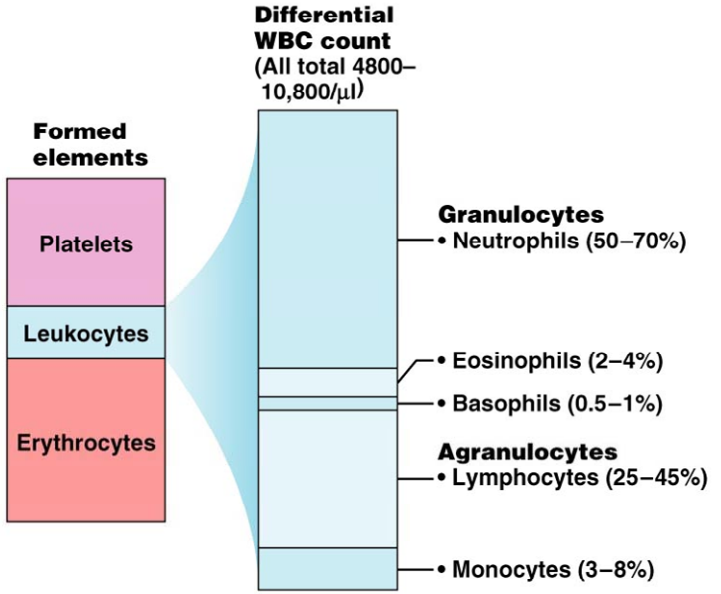
Composition of Blood

- Blood is the body's only fluid tissue
- It is composed of liquid plasma and formed elements
- Formed elements include:
  - Erythrocytes, or red blood cells (RBCs)
  - Leukocytes, or white blood cells (WBCs)
  - Platelets
- Hematocrit – the percentage of RBCs out of the total blood volume



Components of Whole Blood

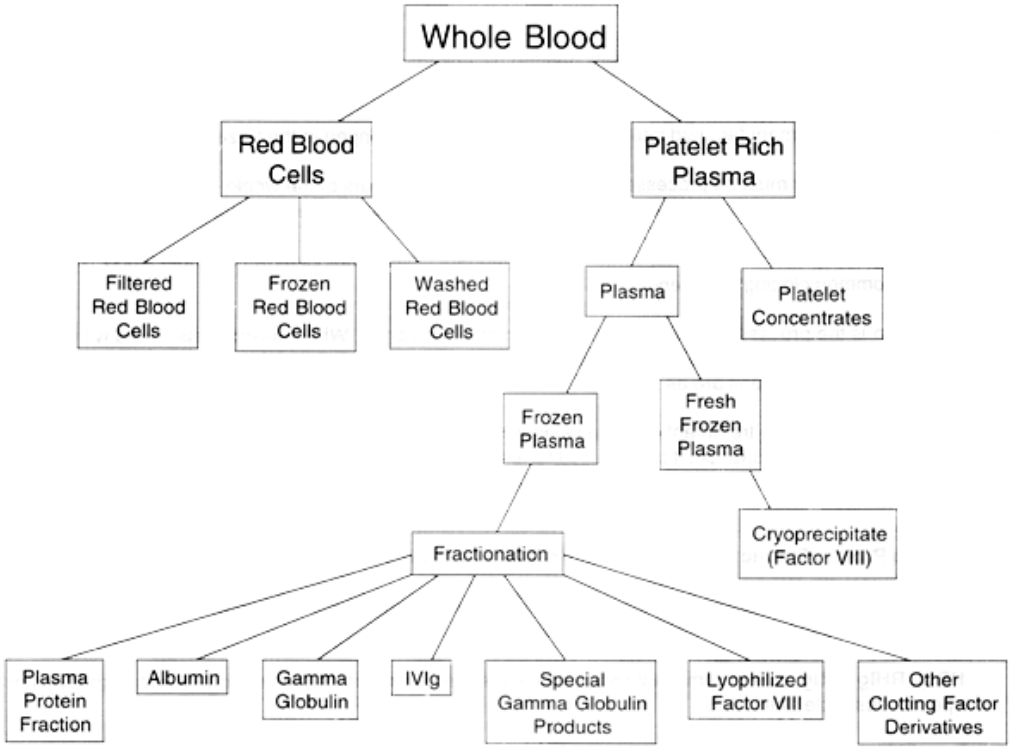
Whole blood is a living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues. Whole blood contains red blood cells, white blood cells, and platelets suspended in a fluid called plasma.

Physical Characteristics and Volume

- Blood is a sticky, opaque fluid with a metallic taste
- Color varies from scarlet to dark red
- The pH of blood is 7.35–7.45
- Temperature is 38°C
- Blood accounts for approximately 8% of body weight
- Average volume: 5–6 L for males, and 4–5 L for females

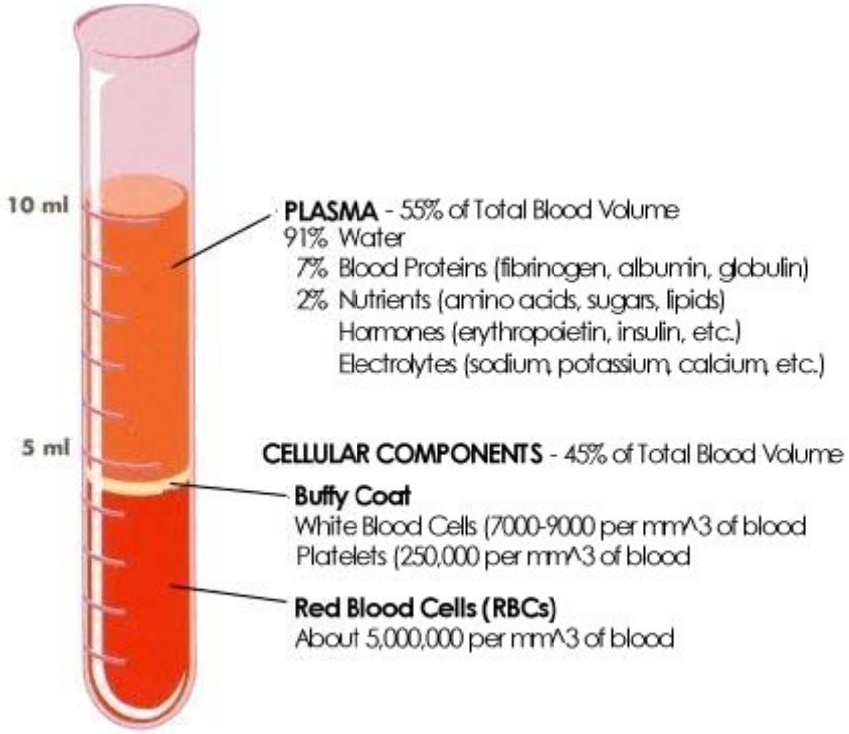
Blood may be transfused as whole blood or as one of its components.
Because patients seldom require all of the components of whole blood, it makes sense to transfuse only that portion needed by the patient for a specific condition or disease.
This treatment, referred to as "blood component therapy," allows several patients to benefit from one unit of donated whole blood.
Blood components include red blood cells, plasma, platelets, and cryoprecipitated antihemophilic factor (AHF). Up to four components may be derived

from one unit of blood.



Functions of Blood

- Blood performs a number of functions dealing with:
  - Substance distribution
  - Regulation of blood levels of particular substances
  - Body protection



Functions of Blood

1. **Transports:**
   - Dissolved gases (e.g. oxygen, carbon dioxide);
   - Waste products of metabolism (e.g. water, urea);
   - Hormones;

- Enzymes;
- Nutrients (such as glucose, amino acids, micro-nutrients (vitamins & minerals), fatty acids, glycerol);
- Plasma proteins (associated with defence, such as blood-clotting and anti-bodies);
- Blood cells (incl. white blood cells 'leucocytes', and red blood cells 'erythrocytes').

2. **Maintains Body Temperature**

3. **Controls pH**

   The pH of blood must remain in the **range 6.8 to 7.4,** otherwise it begins to damage cells.

4. **Removes toxins from the body**

   The kidneys filter all of the blood in the body (approx. 8 pints), 36 times every 24 hours. Toxins removed from the blood by the kidneys leave the body in the urine.
   (Toxins also leave the body in the form of sweat.)

5. **Regulation of Body Fluid Electrolytes**

   Excess salt is removed from the body in urine, which may contain around 10g salt per day
   (such as in the cases of people on western diets containing more salt than the body requires).

Functions of Blood

- Primary
  - Transportation
  - Exchange
- Secondary
  - Immunity
  - Thermoregulation
  - Fluid volume balance
  - pH balance

Distribution

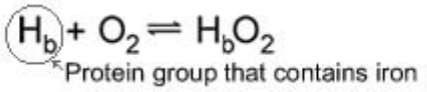
- Blood transports:
  - Oxygen from the lungs and nutrients from the digestive tract
  - Metabolic wastes from cells to the lungs and kidneys for elimination
  - Hormones from endocrine glands to target organs

Regulation

- Blood maintains:
  - Appropriate body temperature by absorbing and distributing heat
  - Normal pH in body tissues using buffer systems
  - Adequate fluid volume in the circulatory system

Protection

- Blood prevents blood loss by:
  - Activating plasma proteins and platelets
  - Initiating clot formation when a vessel is broken
- Blood prevents infection by:
  - Synthesizing and utilizing antibodies
  - Activating complement proteins
  - Activating WBCs to defend the body against foreign invaders

Erythrocytes (Red blood cells)	Immature erythrocytes have a nucleus but mature erythrocytes have no nucleus. ***Heme*** Erythrocytes have a "prosthetic group" (meaning "in addition to" - in this case, in addition to the cell). The active component of this prosthetic group is Heme. Heme relies on the presence of iron (Fe). Heme combines with oxygen to form oxyhaemoglobin:  . Erythrocytes are eventually broken down by the spleen into the blood pigments bilinubin and bilviridin, and iron. These components are then transported by the blood to the liver where the iron is re-cycled for use by new erythrocytes, and the blood pigments form bile salts. (Bile breaks down fats.) Have a longevity of approx. 120 days. There are approx. 4.5 - 5.8 million erythrocytes per micro-litre of healthy blood (though there are variations between racial groups and men/women).	Carry oxygen
Leucocytes (White blood cells)	There are different types of leucocytes classified as: Granular: e.g. Neutrophils, Eosinophils, Basophils. Agranular (do not contain granules): e.g. Monocytes, Lymphocytes.	Major part of the immune system.
Trombocytes (Platelets)	There are approx. 5,000 - 10,000 leucocytes per micro-litre of blood. Have a longevity of a few hours to a few days (but some can remain for many years).	To facilitate blood clotting - the purpose of which is to prevent loss of body fluids.
Plasma	Normal blood plasma is 90-92 % water. This is the straw-coloured fluid in which the blood cells are suspended, and consists of: 1. Dissolved substances including electrolytes such as sodium, chlorine, potassiun, manganese, and calcium ions; 2. Blood plasma proteins (albumin, globulin, fibrinogen); 3. Hormones.	The medium in which the blood cells are transported around the body (by the blood vessels) and are able to operate effectively. Helps to maintain optimum body temperature throughout the organism.

		Helps to control the pH of the blood and the body tissues, maintaining this within a range at which the cells can thrive. Helps to maintain an ideal balance of electrolytes in the blood and tissues of the body.

HEMATOCRIT

The hematocrit (Ht or HCT) or packed cell volume (PCV) or erythrocyte volume fraction (EVF) is the proportion of blood volume that is occupied by red blood cells.

The volume of RBCs refers to the amount of space that the RBCs occupy within the blood.

If whole blood (the cellular portion together with the plasma) is placed in a special hematocrit tube (a small test tube) and then spun very rapidly in a centrifuge, the heavier components will quickly settle to the bottom of the tube

When the centrifuge spins, the RBCs are forced to the bottom of the tube because they are the heaviest element in the blood. The WBCs and platelets are lighter so, as the hematocrit tube spins, they come to rest on top of the heavier RBCs in a layer called the buffy coat.

Above the buffy coat rests the plasma. From the hematocrit tube, one can approximate the percentage of space that the RBCs occupy in the total sample.

At sea level, the hematocrit of a normal adult male averages about 47, which means that 47% of the blood volume is RBCs, while that of a

normal adult female is 42.

The normal ranges for hematocrit are dependent on age and, after adolescence, the sex of the individual. The normal ranges are:

- Newborns: 55%-68%
- One (1) week of age: 47%-65%
- One (1) month of age: 37%-49%
- Three (3) months of age: 30%-36%
- One (1) year of age: 29%-41%
- Ten (10) years of age: 36%-40%
- Adult males: 42%-54%
- Adult women: 38%-46%

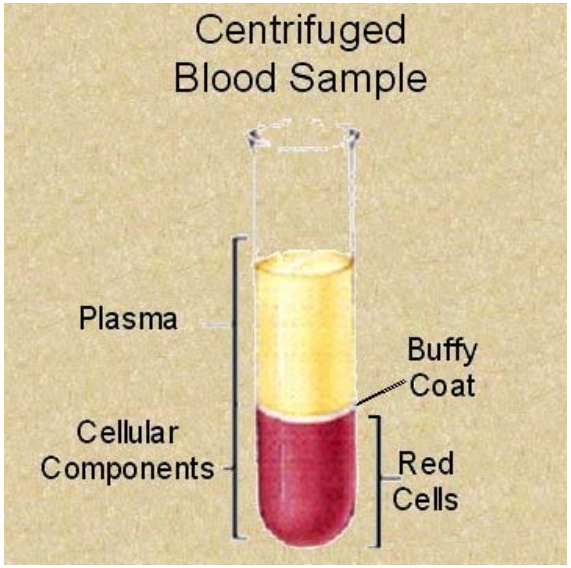
What does a low hematocrit mean?

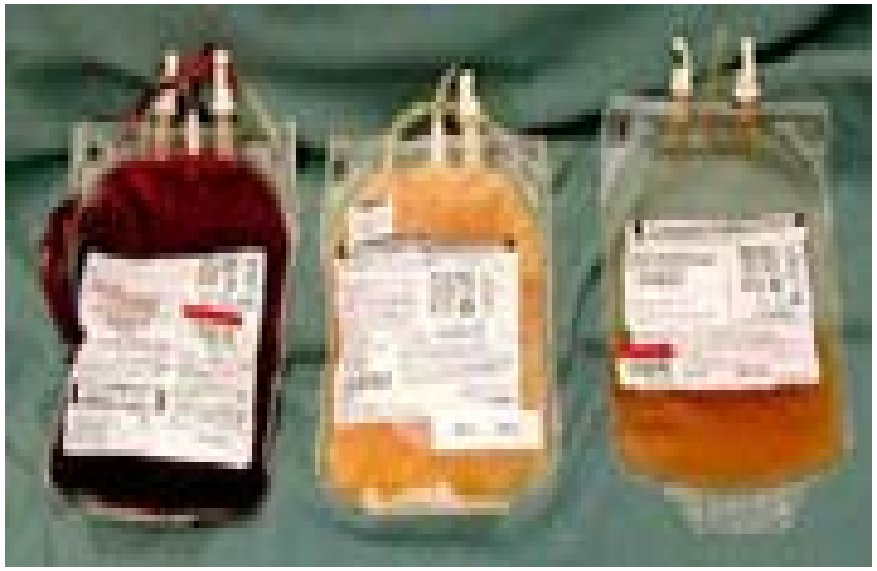
- **A low hematocrit is referred to as being anemic.**
- **There are many reasons for anemia.**
- **Some of the more common reasons are loss of blood (traumatic injury, surgery, bleeding colon cancer),**
- **nutritional deficiency (iron, vitamin B12, folate),**
- **bone marrow problems (replacement of bone marrow by cancer,**
- **suppression by chemotherapy drugs, kidney failure),**
- **and abnormal hematocrit (sickle cell anemia).**

What does a high hematocrit mean?

- **Higher than normal hematocrit levels can be seen in people living at high altitudes and in chronic smokers.**
- **Dehydration produces a falsely high hematocrit that disappears when proper fluid balance is restored.**
- **Some other infrequent causes of elevated hematocrit are lung disease, certain tumors, a disorder of the bone marrow known as polycythemia rubra vera,**
- **and abuse of the drug erythropoietin (Epogen) by athletes for blood doping purposes.**

The following table includes further general information about the constituents of blood.





Blood Plasma

Plasma is the straw-colored liquid in which the blood cells are suspended.

Composition of blood plasma	
Component	**Percent**
Water	~92
Proteins	6–8
Salts	0.8
Lipids	0.6
Glucose (blood sugar)	0.1

- **Blood plasma contains over 100 solutes, including:**
  - **Proteins – albumin, globulins, clotting proteins, and others**
  - **Lactic acid, urea, creatinine**
  - **Organic nutrients – glucose, carbohydrates, amino acids**
  - **Electrolytes – sodium, potassium, calcium, chloride, bicarbonate**
  - **Respiratory gases – oxygen and carbon dioxide**

Formed Elements

- **Erythrocytes, leukocytes, and platelets make up the formed elements**
  - **Only WBCs are complete cells**
  - **RBCs have no nuclei or organelles, and platelets are just cell fragments**
- **Most formed elements survive in the bloodstream for only a few days**
- **Most blood cells do not divide but are renewed by cells in bone marrow**

Plasma transports materials needed by cells and materials that must be removed from cells:

- **various ions (Na^+, Ca^{2+}, HCO_3^-, etc.**
- **glucose and traces of other sugars**
- **amino acids**
- **other organic acids**
- **cholesterol and other lipids**
- **hormones**
- **urea and other wastes**

Most of these materials are in transit from a place where they are added to the blood (a "source")

- **exchange organs like the intestine**
- **depots of materials like the liver**

to places ("sinks") where they will be removed from the blood.

- **every cell**
- **exchange organs like the kidney, and skin.**

TABLE 17.1 Composition of Plasma

CONSTITUENT	DESCRIPTION AND IMPORTANCE
Water	90% of plasma volume; dissolving and suspending medium for solutes of blood; absorbs heat
Solutes	
Plasma proteins	8% (by weight) of plasma volume; all contribute to osmotic pressure, maintaining water balance in blood and tissues; all have other functions (transport, enzymatic, etc.)
▪ Albumin	60% of plasma proteins; produced by liver; main contributor to osmotic pressure
▪ Globulins	36% of plasma proteins
alpha, beta	Produced by liver; mostly transport proteins that bind to lipids, metal ions, and fat-soluble vitamins
gamma	Antibodies released by plasma cells during immune response
▪ Fibrinogen	4% of plasma proteins; produced by liver; forms fibrin threads of blood clot
Nonprotein nitrogenous substances	By-products of cellular metabolism, such as urea, uric acid, creatinine, and ammonium salts
Nutrients (organic)	Materials absorbed from digestive tract and transported for use throughout body; include glucose and other simple carbohydrates, amino acids (digestion products of proteins), fatty acids, glycerol and triglycerides (fat products), cholesterol, and vitamins
Electrolytes	Cations include sodium, potassium, calcium, magnesium; anions include chloride, phosphate, sulfate, and bicarbonate; help to maintain plasma osmotic pressure and normal blood pH
Respiratory gases	Oxygen and carbon dioxide; oxygen mostly bound to hemoglobin inside RBCs; carbon dioxide transported bound to hemoglobin in RBCs or dissolved in plasma as bicarbonate ion or CO_2
Hormones	Steroid and thyroid hormones carried by plasma proteins

Blood plasma is the liquid component of blood, in which the blood cells are suspended.

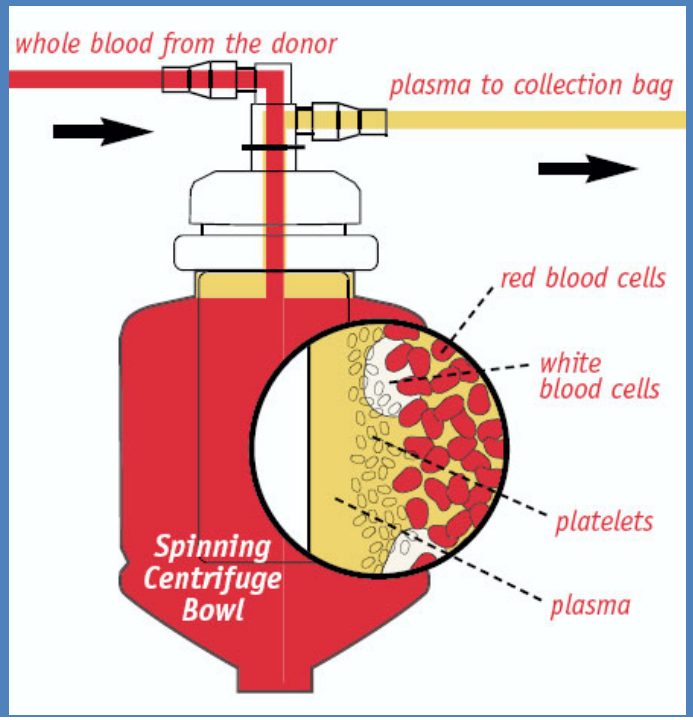
It makes up about 55% of total blood volume.

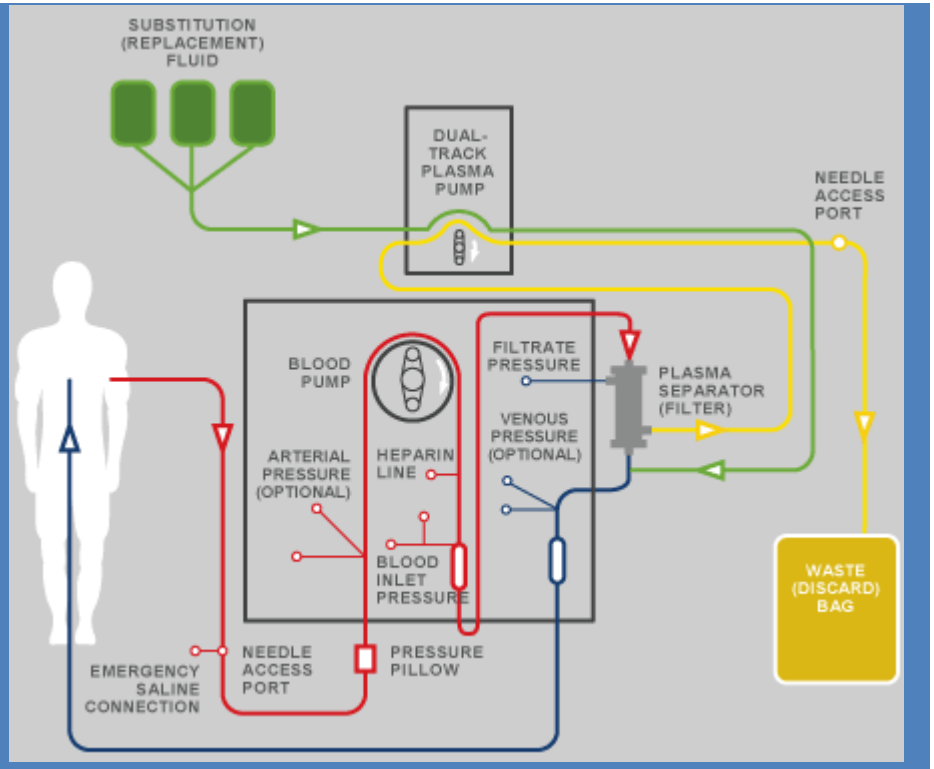
It is composed of mostly water (90% by volume), and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide (plasma being the main medium for excretory product transportation).

Blood plasma is prepared simply by spinning a tube of fresh blood in a centrifuge until the blood cells fall to the bottom of the tube.

The blood plasma is then poured or drawn off. Blood serum is blood plasma without fibrinogen or the other clotting factors.

Plasmapheresis is a type of medical therapy involving separation of plasma from red blood cells.




SUBSTITUTION (REPLACEMENT) FLUID
DUAL-TRACK PLASMA PUMP
NEEDLE ACCESS PORT
BLOOD PUMP
FILTRATE PRESSURE
PLASMA SEPARATOR (FILTER)
VENOUS PRESSURE (OPTIONAL)
ARTERIAL PRESSURE (OPTIONAL)
HEPARIN LINE
BLOOD INLET PRESSURE
WASTE (DISCARD) BAG
EMERGENCY SALINE CONNECTION
NEEDLE ACCESS PORT
PRESSURE PILLOW

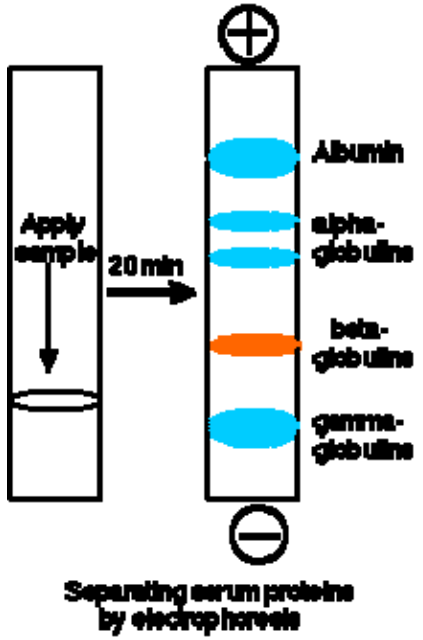
Serum Proteins

Proteins make up 6–8% of the blood. They are about equally divided between **serum albumin** and a great variety of **serum globulins**.

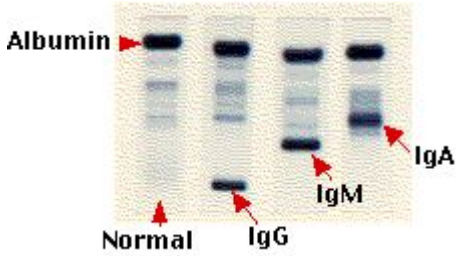
After blood is withdrawn from a vein and allowed to clot, the clot slowly shrinks. As it does so, a clear fluid called serum is squeezed out. Thus:

Serum is blood plasma without fibrinogen and other clotting factors.

The serum proteins can be separated by **electrophoresis**.



- A drop of serum is applied in a band to a thin sheet of supporting material, like paper, that has been soaked in a slightly-alkaline salt solution.
- At pH 8.6, which is commonly used, all the proteins are negatively charged, but some more strongly than others.
- A direct current can flow through the paper because of the conductivity of the buffer with which it is moistened.
- As the current flows, the serum proteins move toward the positive electrode.
- The stronger the negative charge on a protein, the faster it migrates.
- After a time (typically 20 min), the current is turned off and the proteins stained to make them visible (most are otherwise colorless).
- The separated proteins appear as distinct bands.
- The most prominent of these and the one that moves closest to the positive electrode is **serum albumin**.
- Serum albumin
  - is made in the liver
  - binds many small molecules for transport through the blood
  - helps maintain the osmotic pressure of the blood
- The other proteins are the various serum globulins.
- They migrate in the order
  - **alpha globulins** (e.g., the proteins that transport thyroxine and retinol [vitamin A])
  - **beta globulins** (e.g., the iron-transporting protein **transferrin**)
  - **gamma globulins**.
    - Gamma globulins are the least negatively-charged serum proteins. (They are so weakly charged, in fact, that some are swept in the flow of buffer back toward the negative electrode.)
    - Most antibodies are gamma globulins.
    - Therefore gamma globulins become more abundant following infections or immunizations.



If a precursor of an antibody-secreting cell becomes cancerous, it divides uncontrollably to generate a clone of plasma cells secreting a **single kind of antibody molecule**. The image (courtesy of Beckman Instruments, Inc.) shows — from left to right — the electrophoretic separation of:

1. **normal** human serum with its diffuse band of gamma globulins;
2. serum from a patient with **multiple myeloma** producing an **IgG** myeloma protein;

3. serum from a patient with Waldenström's macroglobulinemia where the cancerous clone secretes an **IgM** antibody;
4. serum with an **IgA** myeloma protein.

- Gamma globulins can be harvested from donated blood (usually pooled from several thousand donors) and injected into persons exposed to certain diseases such as chicken pox and hepatitis. Because such preparations of **immune globulin** contain antibodies against most common infectious diseases, the patient gains temporary protection against the disease.

Serum Lipids

Because of their relationship to cardiovascular disease, the analysis of serum lipids has become an important health measure.

The table shows the range of typical values as well as the values above (or below) which the subject may be at increased risk of developing atherosclerosis.

LIPID	Typical values (mg/dl)	Desirable (mg/dl)
Cholesterol (total)	170–210	<200
LDL cholesterol	60–140	<100
HDL cholesterol	35–85	>40
Triglycerides	40–160	<160

- Total cholesterol is the sum of
  - HDL cholesterol
  - LDL cholesterol and
  - 20% of the triglyceride value
- Note that
  - **high LDL** values are **bad**, but
  - **high HDL** values are **good**.
- Using the various values, one can calculate a **cardiac risk ratio** = **total cholesterol** divided by **HDL cholesterol**
- A cardiac risk ratio greater than 7 is considered a warning.

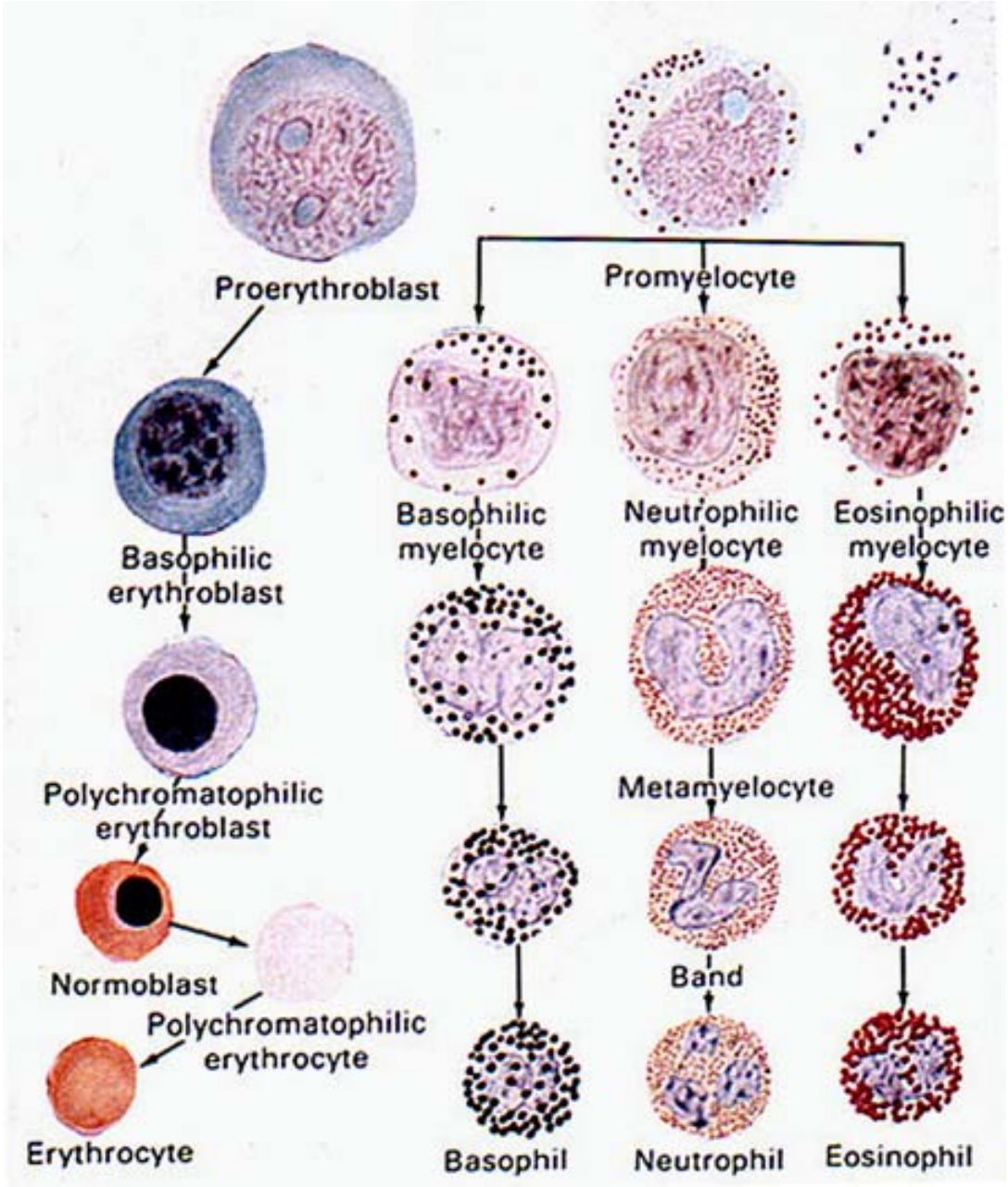
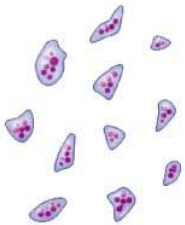

Proerythroblast
Promyelocyte
Basophilic erythroblast
Basophilic myelocyte
Neutrophilic myelocyte
Eosinophilic myelocyte
Polychromatophilic erythroblast
Metamyelocyte
Normoblast
Band
Polychromatophilic erythrocyte
Erythrocyte
Basophil
Neutrophil
Eosinophil

TABLE 17.2 Summary of Formed Elements of the Blood

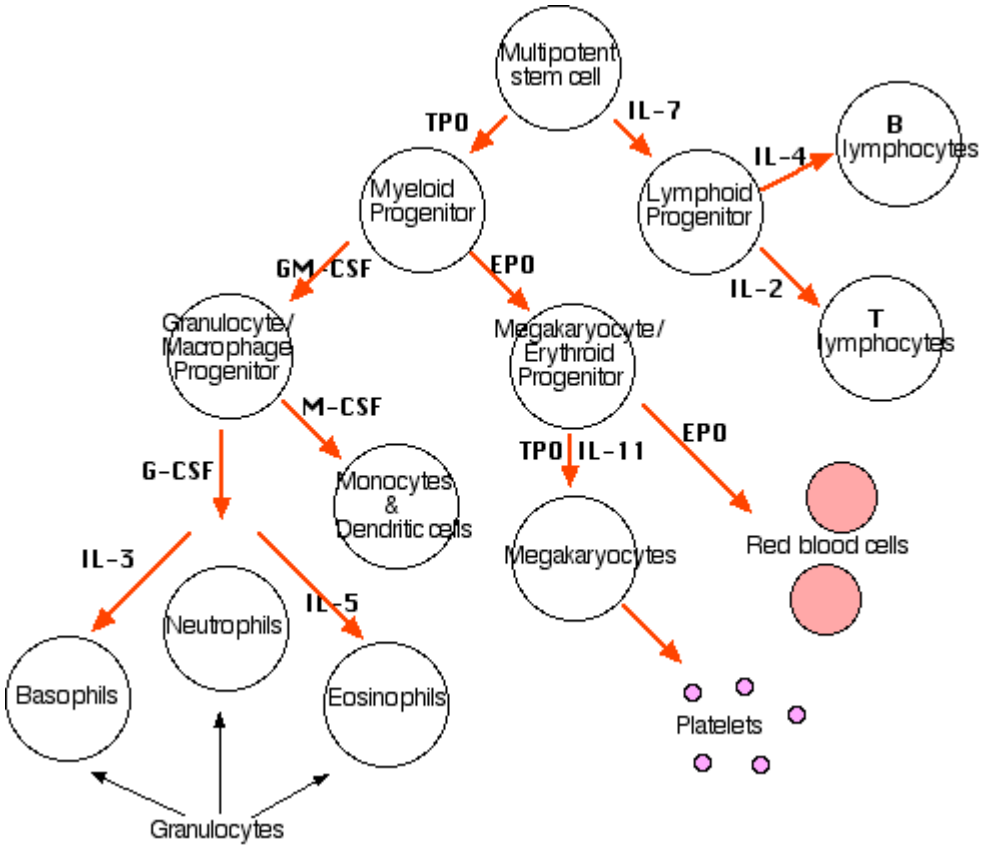
CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 μm	4–6 million	D: about 15 days LS: 100–120 days	Transport oxygen and carbon dioxide
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Granulocytes					
▪ Neutrophil		Nucleus multilobed; inconspicuous cytoplasmic granules; diameter 10–12 μm	3000–7000	D: about 14 days LS: 6 hours to a few days	Phagocytize bacteria
▪ Eosinophil		Nucleus bilobed; red cytoplasmic granules; diameter 10–14 μm	100–400	D: about 14 days LS: about 5 days	Kill parasitic worms; destroy antigen-antibody complexes; inactivate some inflammatory chemicals of allergy
▪ Basophil		Nucleus lobed; large purplish-black cytoplasmic granules; diameter 10–14 μm	20–50	D: 1–7 days LS: a few hours to a few days	Release histamine and other mediators of inflammation; contain heparin, an anticoagulant

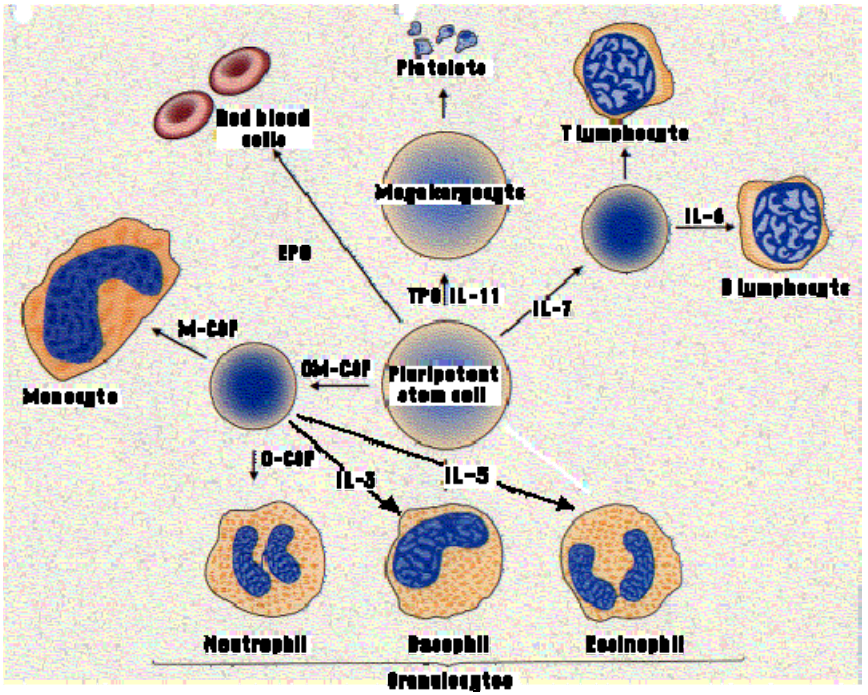
*Appearance when stained with Wright's stain.

TABLE 17.2 Summary of Formed Elements of the Blood *(continued)*

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Agranulocytes					
▪ Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 μm	1500–3000	D: days to weeks LS: hours to years	Mount immune response by direct cell attack or via antibodies
▪ Monocyte		Nucleus U or kidney shaped; gray-blue cytoplasm; diameter 14–24 μm	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 μm	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

*Appearance when stained with Wright's stain.



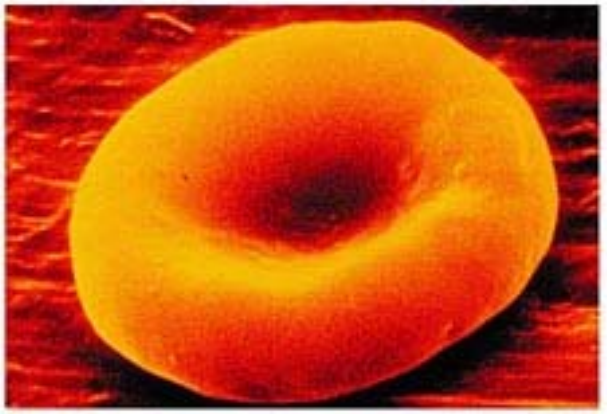


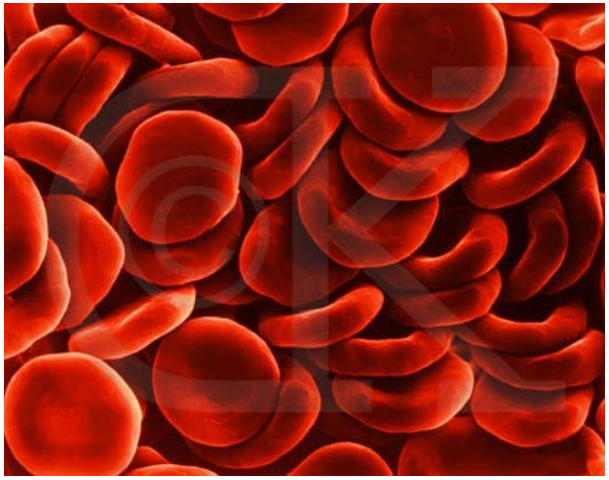
ERYTHROCYTES

Erythrocyte: Usually biconcave and circular outline, devoid of a nucleus. Number in man varies between 5 and 5.5 million per cubic mm of blood. Erythrocytes carry oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

Erythrocytes (RBCs)

- Biconcave discs, anucleate, essentially no organelles
- Filled with hemoglobin (Hb), a protein that functions in gas transport
- Contain the plasma membrane protein spectrin and other proteins that:
  - Give erythrocytes their flexibility
  - Allow them to change shape as necessary
- Erythrocytes are an example of the complementarity of structure and function
- Structural characteristics contribute to its gas transport function
  - Biconcave shape has a huge surface area relative to volume
  - Erythrocytes are more than 97% hemoglobin
  - ATP is generated anaerobically, so the erythrocytes do not consume the oxygen they transport





Life cycle

1. erythropoiesis
   a. negative - feedback regulation
      1. erythropoietin
2. 120 days ---> destruction
3. recycling
   a. bilirubin; jaundice

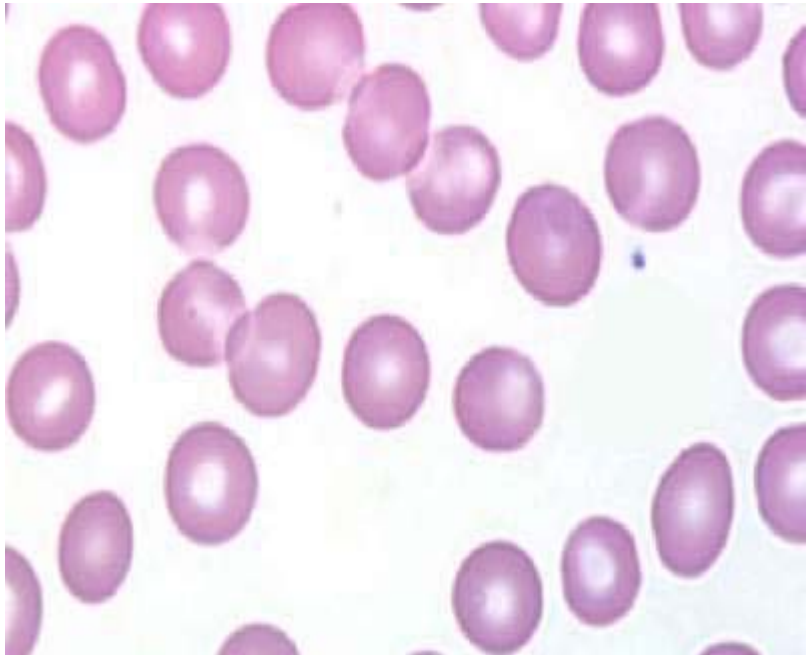
Mature erythrocytes lack a nucleus and most cellular organelles, thereby maximizing the cell's volume and thus its ability to carry hemoglobin and to transport O_2.

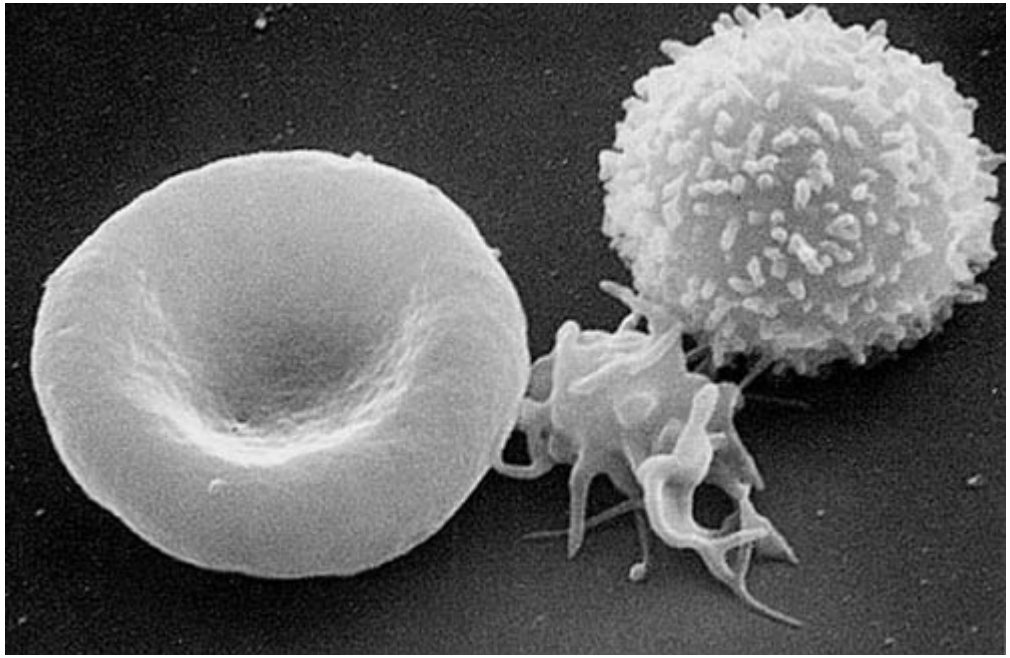
Erythrocytes are shaped like flattened donuts with a depressed center (rather than a donut hole). Their flattened shape maximizes surface area for the exchange of O_2 and CO_2 and allows flexibility that permits their passage through narrow capillaries.

RBC precursors mature in the bone marrow closely attached to a macrophage.

- They manufacture hemoglobin until it accounts for some 90% of the dry weight of the cell.
- The nucleus is squeezed out of the cell and is ingested by the macrophage.
- No-longer-needed proteins are expelled from the cell in vesicles called exosomes.

Exosomes
Antigen-presenting cells like • dendritic cells and • B cells can also present antigen to T cells by means of exosomes. These are tiny membrane-enclosed vesicles released by the cell. Their surface is studded with MHC-peptide complexes. Antigen-presentation by exosomes may in some cases inhibit — rather than stimulate — an immune response.





Erythrocyte Function

- RBCs are dedicated to respiratory gas transport

- Hb reversibly binds with oxygen and most oxygen in the blood is bound to Hb
- Hb is composed of the protein globin, made up of two alpha and two beta chains, each bound to a heme group
- Each heme group bears an atom of iron, which can bind to one oxygen molecule
- Each Hb molecule can transport four molecules of oxygen

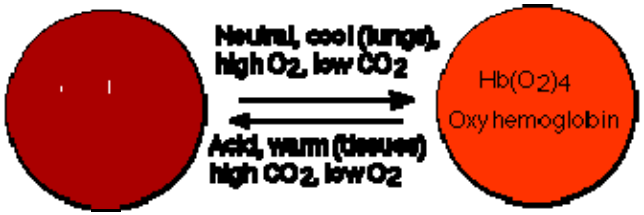
Oxygen Transport

In adult humans the hemoglobin (Hb) molecule

- **consists of four polypeptides:**
  - **two** alpha (α) chains **of 141 amino acids and**
  - **two** beta (β) chains **of 146 amino acids**
- **Each of these is attached the prosthetic group** heme**.**
- **There is one atom of iron at the center of each heme.**
- **One molecule of oxygen can bind to each heme.**

The reaction is reversible.

- **Under the conditions of lower temperature, higher pH, and increased oxygen pressure in the capillaries of the lungs, the reaction proceeds to the right. The purple-red deoxygenated hemoglobin of the venous blood becomes the bright-red** oxyhemoglobin **of the arterial blood.**
- **Under the conditions of higher temperature, lower pH, and lower oxygen pressure in the tissues, the reverse reaction is promoted and oxyhemoglobin gives up its oxygen.**



Carbon Dioxide Transport

Carbon dioxide (CO_2) combines with water forming carbonic acid, which dissociates into a hydrogen ion (H^+) and a bicarbonate ions:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

95% of the CO_2 generated in the tissues is carried in the red blood cells:

- It probably enters (and leaves) the cell by diffusion through the plasma membrane assisted by facilitated diffusion through transmembrane channels in the plasma membrane. (One of the proteins that forms the channel is the **D antigen** that is the most important factor in the Rh system of blood groups.)
- Once inside, about one-half of the CO_2 is directly bound to hemoglobin (at a site different from the one that binds oxygen).
- The rest is converted — following the equation above — by the enzyme **carbonic anhydrase** into
  - bicarbonate ions that diffuse back out into the plasma and
  - hydrogen ions (H^+) that bind to the protein portion of the hemoglobin (thus having no effect on pH).

Only about 5% of the CO_2 generated in the tissues dissolves directly in the plasma. (A good thing, too: if all the CO_2 we make were carried this way, the pH of the blood would drop from its normal 7.4 to an instantly-fatal 4.5!)

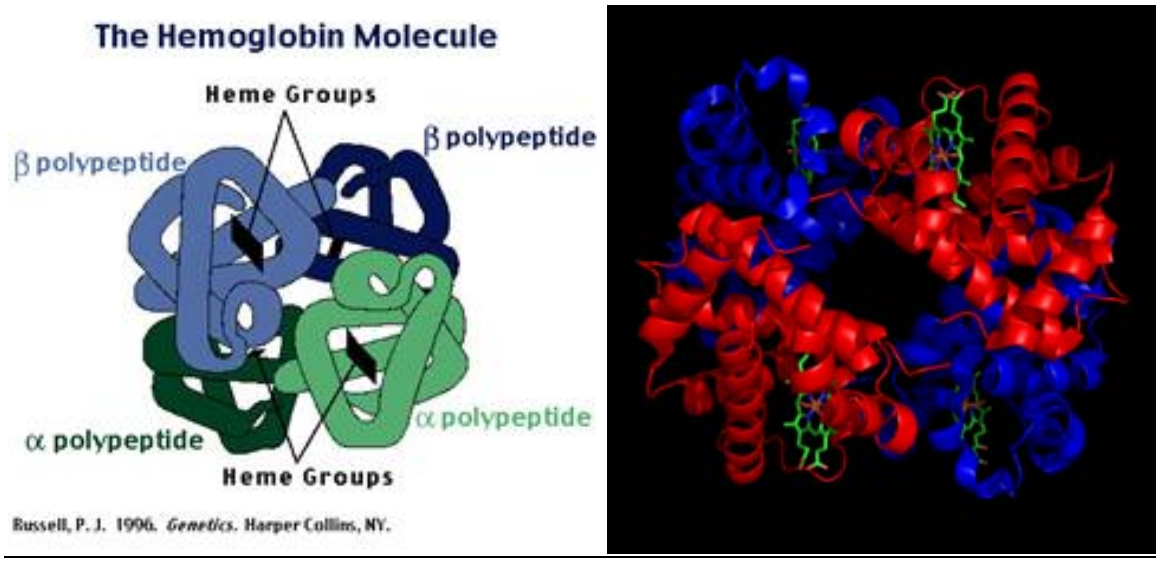
When the red cells reach the lungs, these reactions are reversed and CO_2 is released to the air of the alveoli.

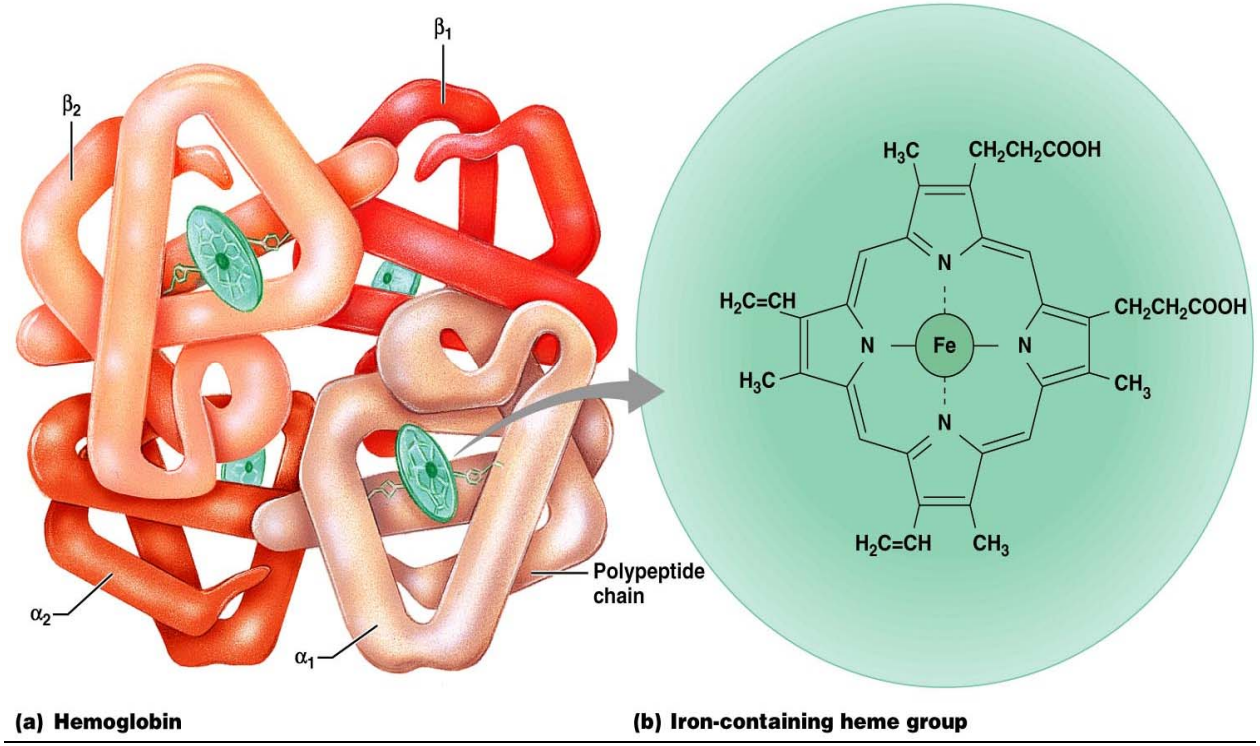
Structure of Hemoglobin

Name of Hemoglobin	Subunit Structure	Time of Expression
Hemoglobin Portland	$\zeta_2\gamma_2$	**Embryonic**
Hemoglobin Gower I	$\zeta_2\varepsilon_2$	**Embryonic**
Hemoglobin Gower II	$\alpha_2\varepsilon_2$	**Embryonic**
Hemoglobin F	$\alpha_2\gamma_2$	**Fetal**
Hemoglobin Barts	γ_4	**Fetal (pathologic Hb secondary to absence of all 4 α globulin genes; fatal in utero)**
Hemoglobin A_2	$\alpha_2\delta_2$	**Minor adult hemoglobin**
Hemoglobin A	**$\alpha_2\beta_2$**	**Major adult hemoglobin**

- **Men: 13.5 to 16.5 g/dl**
- **Women: 12.1 to 15.1 g/dl**
- **Children: 11 to 16 g/dl**
- **Pregnant women: 11 to 12 g/dl**

oxyhemoglobin-- hemoglobin that is carrying oxygen, it is bright red
deoxyhemoglobin-- hemoglobin that is not carrying oxygen, it is purplish-bluish-red


The Hemoglobin Molecule
Heme Groups
β polypeptide
β polypeptide
α polypeptide
α polypeptide
Heme Groups
Russell, P. J. 1996. Genetics. Harper Collins, NY.


β1
β2
α2
α1
Polypeptide chain
H3C
CH2CH2COOH
N
H2C=CH
CH2CH2COOH
N
Fe
N
H3C
CH3
N
H2C=CH
CH3
(a) Hemoglobin
(b) Iron-containing heme group

Protein type metalloprotein, globulin Function oxygen-transport Cofactor(s) heme (4) Subunit

Name Gene Chromosomal

Locus Hb α1 HBA1 Chromosome 16p13.3

Hb α2 HBA2 Chromosome 16p13.3

Hb β HBB Chromosome 11p15.5

Hemoglobin (Hb) is synthesized in a complex series of steps.
The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol.

Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow.

At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

Hemoglobin (Hb)

- Oxyhemoglobin – Hb bound to oxygen
  - Oxygen loading takes place in the lungs
- Deoxyhemoglobin – Hb after oxygen diffuses into tissues (reduced Hb)
- Carbaminohemoglobin – Hb bound to carbon dioxide
  - Carbon dioxide loading takes place in the tissues

Other oxygen-binding proteins

***Myoglobin**: Found in the muscle tissue of many vertebrates, including humans, it gives muscle tissue a distinct red or dark gray color. It is very similar to hemoglobin in structure and sequence, but is not a tetramer; instead, it is a monomer that lacks cooperative binding. It is used to store oxygen rather than transport it.*

***Hemocyanin**: The second most common oxygen-transporting protein found in nature, it is found in the blood of many arthropods and molluscs. Uses copper prosthetic groups instead of iron heme groups and is blue in color when oxygenated.*

***Hemerythrin**: Some marine invertebrates and a few species of annelid use this iron-containing non-heme protein to carry oxygen in their blood. Appears pink/violet when oxygenated, clear when not.*

***Chlorocruorin**: Found in many annelids, it is very similar to erythrocruorin, but the heme group is significantly different in structure. Appears green when*

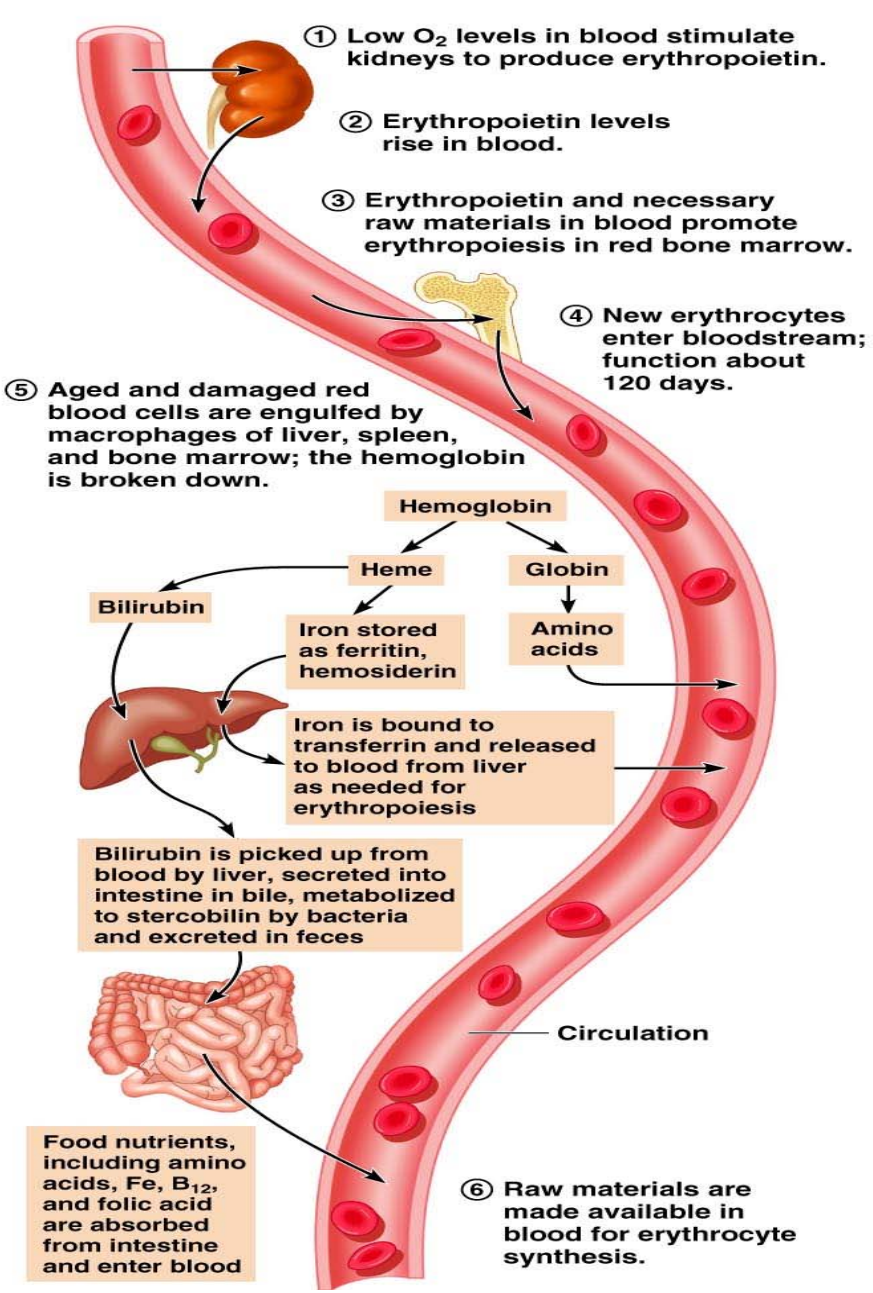
deoxygenated and red when oxygenated.

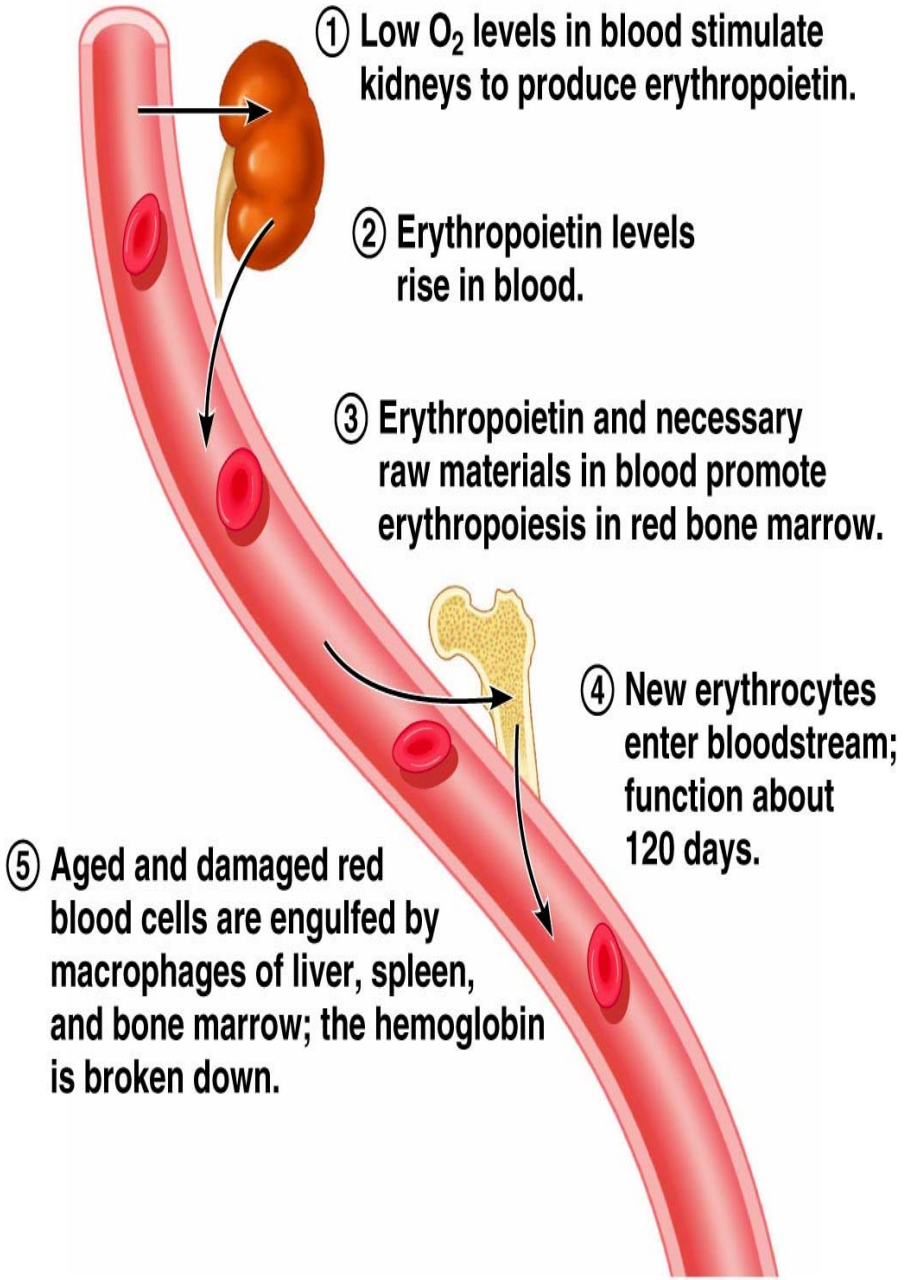
***Vanabins**: Also known as **vanadium chromagens**, they are found in the blood of sea squirts and are hypothesised to use the rare metal vanadium as its oxygen binding prosthetic group.*

***Erythrocruorin**: Found in many annelids, including earthworms, it is a giant free-floating blood protein containing many dozens — possibly hundreds — of iron- and heme-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million daltons.*

***Pinnaglobin**: Only seen in the mollusc Pinna squamosa. Brown manganese-based porphyrin protein.*

***Leghemoglobin**: In leguminous plants, such as alfalfa or soybeans, the nitrogen fixing bacteria in the roots are protected from oxygen by this iron heme containing oxygen-binding protein. The specific enzyme protected is nitrogenase, which is unable to reduce nitrogen gas in the presence of free oxygen.*


① Low O_2 levels in blood stimulate kidneys to produce erythropoietin.
② Erythropoietin levels rise in blood.
③ Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow.
④ New erythrocytes enter bloodstream; function about 120 days.
⑤ Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down.
Hemoglobin
Heme
Globin
Bilirubin
Iron stored as ferritin, hemosiderin
Amino acids
Iron is bound to transferrin and released to blood from liver as needed for erythropoiesis
Bilirubin is picked up from blood by liver, secreted into intestine in bile, metabolized to stercobilin by bacteria and excreted in feces
Circulation
Food nutrients, including amino acids, Fe, B_{12}, and folic acid are absorbed from intestine and enter blood
⑥ Raw materials are made available in blood for erythrocyte synthesis.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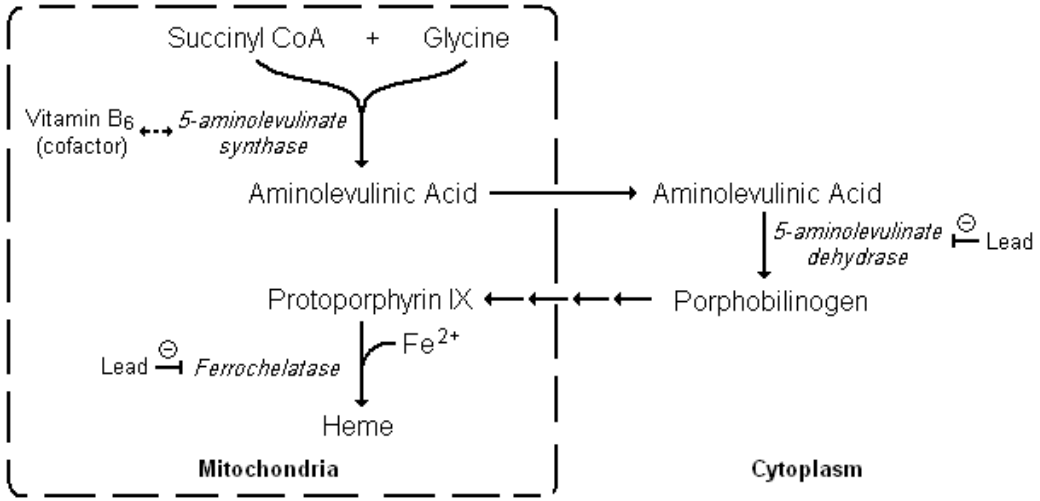
③ Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow.
④ New erythrocytes enter bloodstream; function about 120 days.
⑤ Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down.

Normal Synthesis of Heme

Heme is synthesized from glycine (a non-essential amino acid and succinyl CoA (an intermediate in the citric acid cycle). There are a number of enzymatic steps, some of which occur within the mitochondria, and some within the cytoplasm.



- **Hemoglobin** contains both a protein portion, called globin, and nonprotein heme groups.
- **Globin** consists of four polypeptide chains, each of which contains a heme group.
- **The heme** group is a red pigment that contains a single iron atom surrounded by a ring of nitrogen-containing carbon rings.
- One oxygen atom attaches to the iron of each heme group, allowing a single hemoglobin molecule to carry four oxygen atoms.
- Each erythrocyte contains about 250 million hemoglobin molecules.
- **Oxyhemoglobin** (HbO_2) forms in the lungs when erythrocytes are exposed to oxygen as they pass through the lungs.
- **Deoxyhemoglobin** (Hb) forms when oxygen detaches form the iron and diffuses into surrounding tissues.
- **Carbaminohemoglobin** ($HbCO_2$) forms when CO_2 attaches to amino acids of the globin part of the hemoglobin molecule. About 25 percent of the CO_2 transported from tissues to lungs is in this form.
- **Carbonic anhydrase,** an enzyme in erythrocytes, converts CO_2 and H_2O in the blood plasma to H^+ and $HCO3^-$ About 65% of the CO_2 collected from tissues travels in the blood plasma as HCO_{3-}.

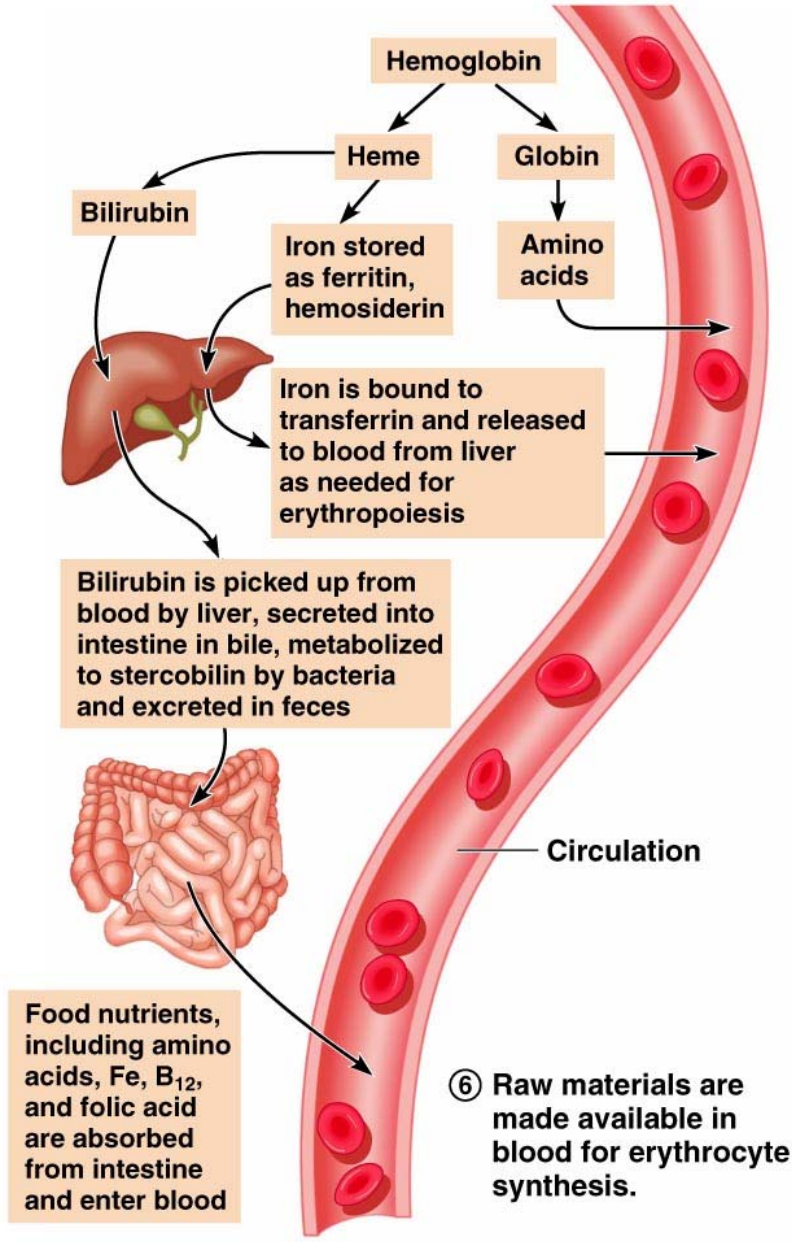
Because they lack cellular organelles and thus physiology to maintain themselves, erythrocytes survive for only about 120 days.

Degenerated erythrocytes are broken down in the spleen and liver by macrophages (phagocytic white blood cells) as follows:

1. The globin and heme parts of the hemoglobin are separated. The globin is reduced to amino acids, which are returned to the blood plasma.
2. Iron is removed from the heme group and bound to the proteins ferritin and hemosiderin, which store the iron for later use (because unbound iron is toxic). Iron is also attached to transferrin, which enters the bloodstream. Transferrin may be picked up by muscles or liver cells, where it may be stored as ferritin or hemosiderin or picked

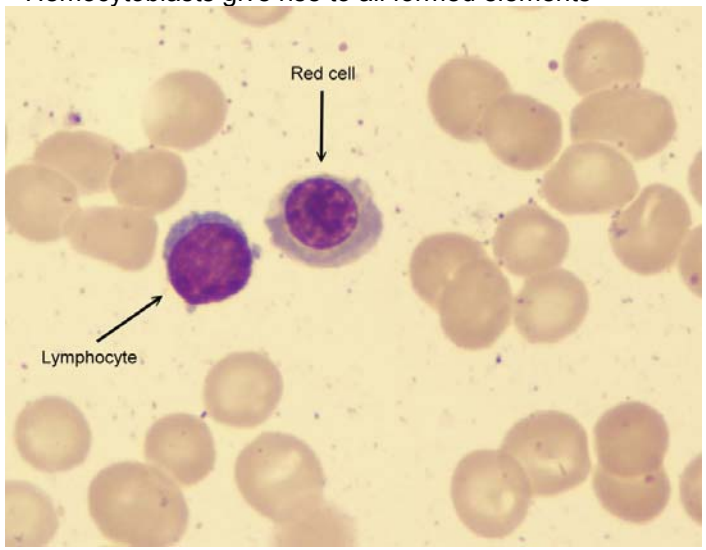
up by bone marrow, where the iron is used to produce new erythrocytes.

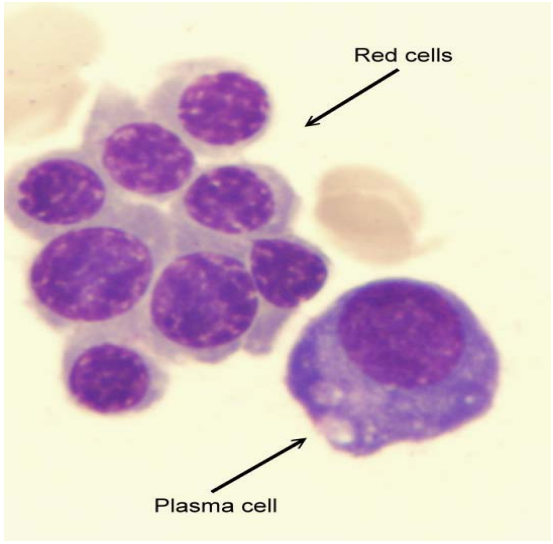
3. The remainder of the heme group is broken down into bilirubin (a yellow-orange pigment), which enters the bloodstream and is picked up by the liver. Liver cells incorporate bilirubin into bile, which enters the small intestine during the digestion of fats. Bilirubin is then converted into urobilinogen by intestinal bacteria. Finally, most urobilinogen is converted to the brown pigment stercobilin, which is eliminated with the feces (and which gives feces its brown color). A small amount of urobilinogen is absorbed into the blood, converted to the yellow pigment urobilin, picked up by the kidneys, and eliminated with the urine (contributing to the yellow color of urine).



Production of Erythrocytes

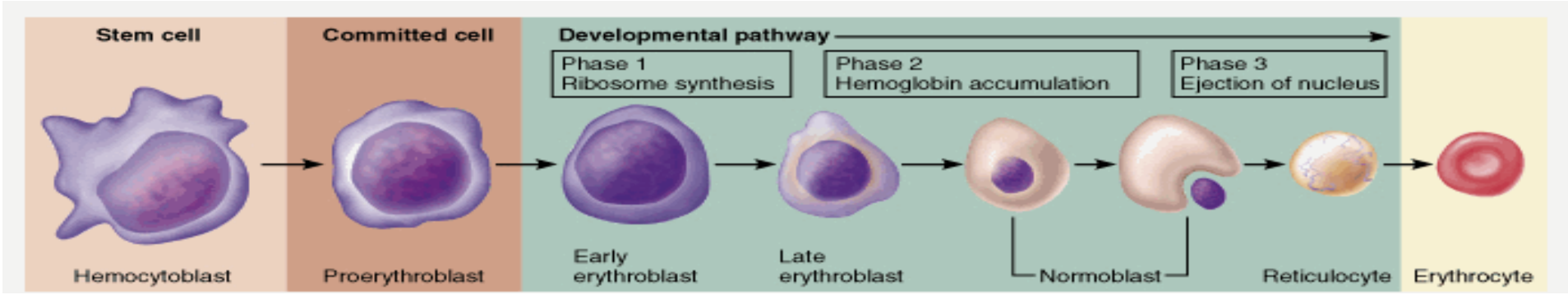
- Hematopoiesis – blood cell formation
- Hematopoiesis occurs in the red bone marrow of the:
  - Axial skeleton and girdles
  - Epiphyses of the humerus and femur
- Hemocytoblasts give rise to all formed elements

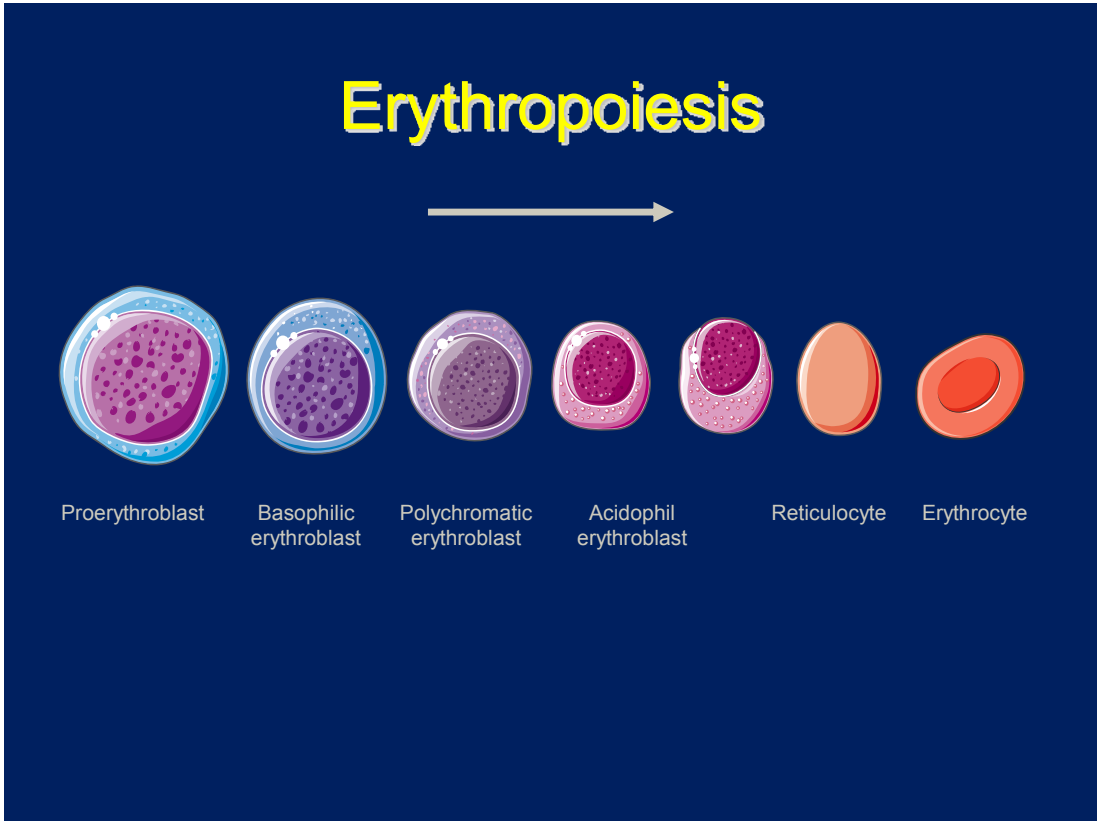




Erythropoiesis

- A hemocytoblast is transformed into a proerythroblast
- Proerythroblasts develop into early erythroblasts
- The developmental pathway consists of three phases
  - 1 – ribosome synthesis in early erythroblasts
  - 2 – Hb accumulation in late erythroblasts and normoblasts
  - 3 – ejection of the nucleus from normoblasts and formation of reticulocytes
- Reticulocytes then become mature erythrocytes





Production of Erythrocytes: Erythropoiesis

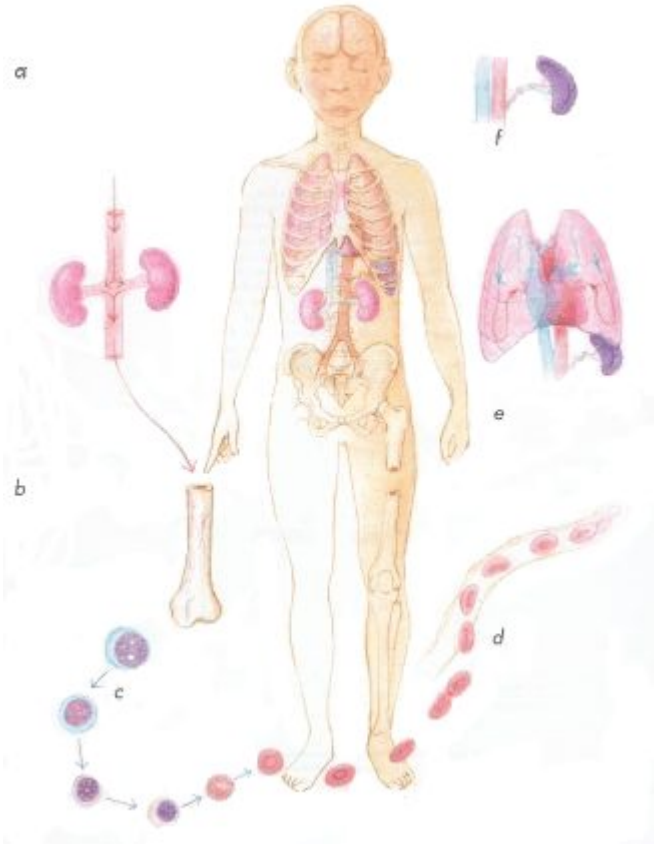
- A hemocytoblast is transformed into a proerythroblast

- Proerythroblasts develop into early erythroblasts

- The developmental pathway consists of three phases
  - 1 – ribosome synthesis in early erythroblasts
  - 2 – Hb accumulation in late erythroblasts and normoblasts
  - 3 – ejection of the nucleus from normoblasts and formation of reticulocytes

- Reticulocytes then become mature erythrocytes

1). Hemocytoblast stem cell 2). Stem cell becomes committed **3).** Early erythroblasts have ribosomes 4**).** Erythroblasts accumulate iron and hemoglobin **5).** Normoblasts eject organelles 6). Released as erythrocyte	▪ In humans, erythropoiesis occurs almost exclusively in the **red bone marrow**. (The yellow bone marrow is primarily composed of fat, but, in response to a greater need for RBC production, the yellow bone marrow can turn to red marrow.) ▪ The red bone marrow of essentially **all** bones produces RBCs from birth to about five years of age. ▪ Between the ages of 5 to 20, the long bones slowly lose their ability to produce RBCs. ▪ Above age 20, most RBCs are produced primarily in the marrow of the vertebrae, the sternum, the ribs, and the pelvis. ▪ The organ responsible for "turning on the faucet" of RBC production is the **kidney** . ▪ The kidneys can detect low levels of oxygen in the blood. ▪ When this happens, the kidneys respond by releasing a hormone called **erythropoietin**, which then travels to the red bone marrow to stimulate the marrow to begin RBC production. ▪ Now, once the erythropoietin stimulates the red bone marrow to begin manufacturing RBCs, a series of events occurs. ▪ In the bone marrow there are many special **stem cells** from which RBCs can be formed. ▪ As these cells mature, they extrude their nucleus as they slowly fill with hemoglobin until they are bright red **reticulocytes** ready to escape the bone marrow and squeeze into the blood capillaries to begin circulating around the body. ▪ In a blood sample, the reticulocytes can be distinguished from RBCs because they still contain some speckles or pieces of their nucleus. ▪ Within a few days, this reticulocyte completely loses all its nuclear material and becomes a full-fledged RBC that is ready to serve the oxygen needs of the body.

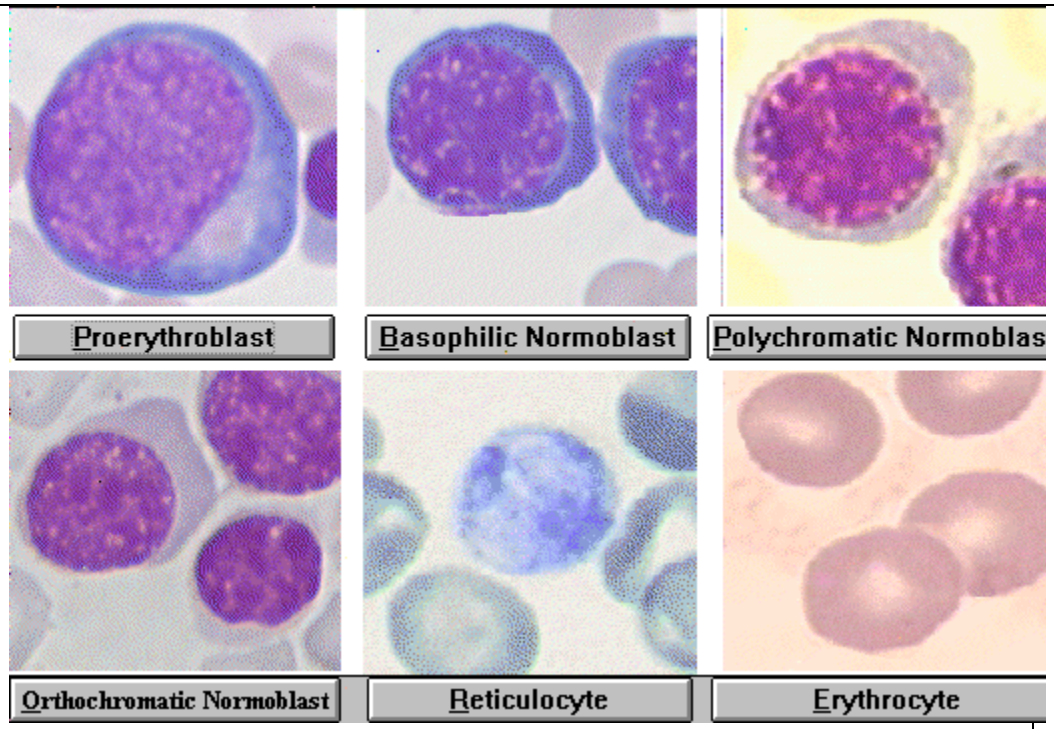


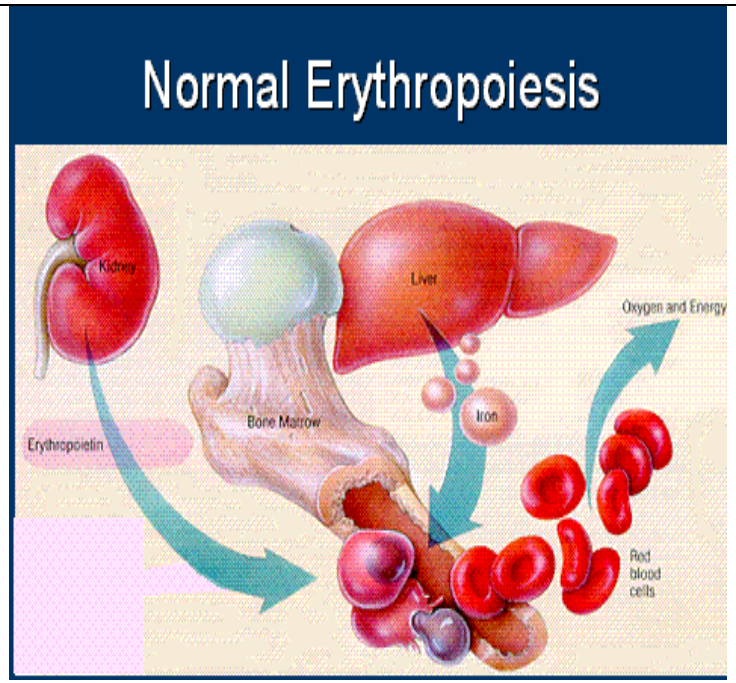
The RBC lifetime is about 120 days.

The life cycle of a red blood cell.

a) **Kidneys** respond to a lower than normal oxygen concentration in the blood by releasing the hormone **erythropoietin**.
b) Erythropoietin travels to the **red bone marrow** and stimulates an increase in the production of **red blood cells (RBCs)**.
c) The red bone marrow manufactures RBCs from **stem cells** that live inside the marrow.
d) RBCs squeeze through blood vessel membranes to enter the circulation.
e) The **heart** and **lungs** work to supply continuous movement and oxygenation of RBCs.
f) Damaged or old RBCs are destroyed primarily by the **spleen**.

- After about three to four months, the RBC has worked so hard that it begins to weaken.
- The membranes of old RBCs become very fragile and the cells may rupture during passage through some tight spots in the circulation. These old and damaged RBCs are "eaten" primarily by the **spleen**, and most of the leftover components (especially the iron from the hemoglobin) are recycled to form new RBCs.
- The production of new RBCs occurs as the need arises.
- A natural need always exists to produce new RBCs to replace the ones that have gotten old, or have been damaged, and have "died." Old RBCs die every day in our bodies and more new ones are also born every day.
- The body can also increase production of RBCs in response to special needs.
- As mentioned previously, new RBCs must be produced when a person enters a high altitude environment. At very high altitudes, where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues, and red cells are produced so rapidly that their number in the blood is considerably increased.
- Therefore, it is obvious that it is not the concentration of RBC's that controls the rate of red cell production, but instead, it is the functional ability of the RBCs to transport oxygen to the tissues **in response to the tissue demand for oxygen** that controls the rate of RBC production. In other words, it's just like the economic concept of "supply and demand." If the supply of oxygen is LESS than what the body demands, the MORE RBCs are produced. If the supply of oxygen is MORE than what the body demands, the FEWER RBCs are produced.



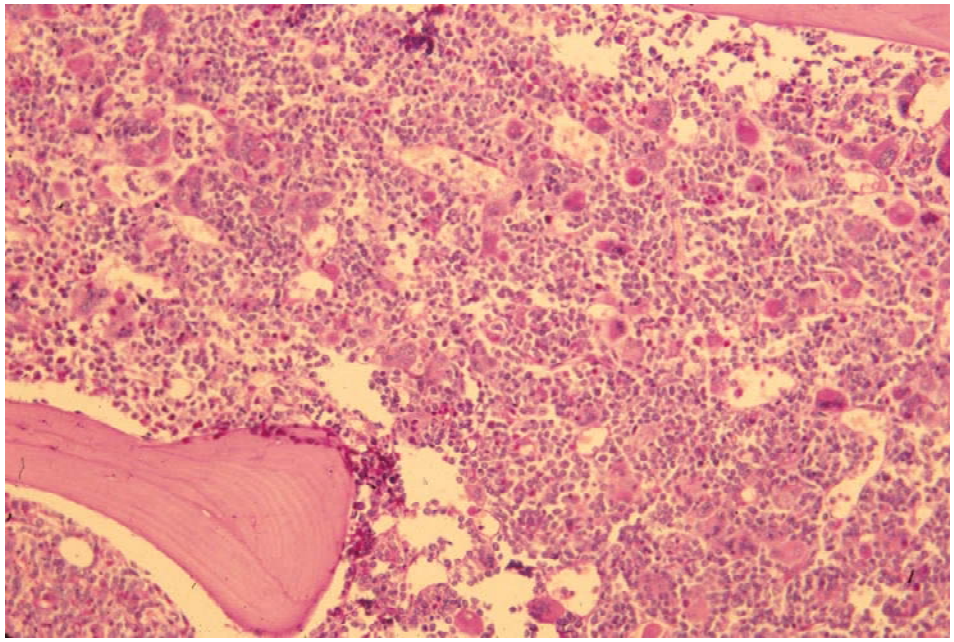


Regulation and Requirements for Erythropoiesis

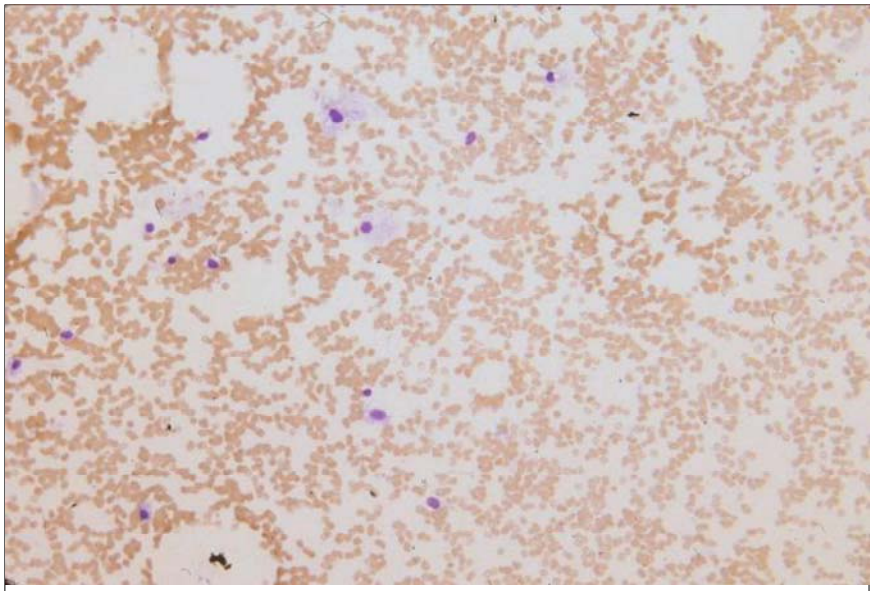
- Circulating erythrocytes – the number remains constant and reflects a balance between RBC production and destruction
  - Too few RBCs leads to tissue hypoxia
  - Too many RBCs causes undesirable blood viscosity
- Erythropoiesis is hormonally controlled and depends on adequate supplies of iron, amino acids, and B vitamins

Hormonal Control of Erythropoiesis

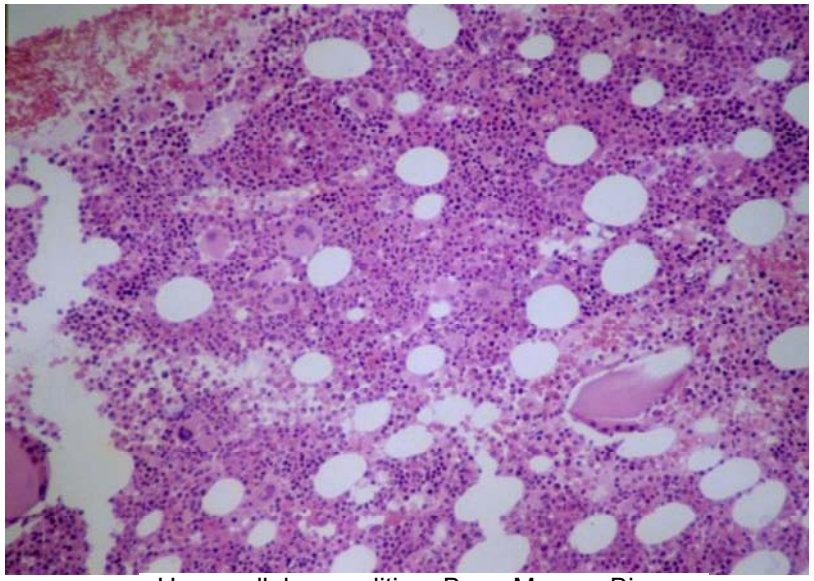
- **Erythropoietin** (EPO) release by the kidneys is triggered by:
  - ***Hypoxia due to decreased RBCs***
  - ***Decreased oxygen availability***
  - ***Increased tissue demand for oxygen***
- Enhanced erythropoiesis increases the:
  - ***RBC count in circulating blood***
  - ***Oxygen carrying ability of the blood***



Hypercellular condition, Bone Marrow Biopsy



Bone Marrow



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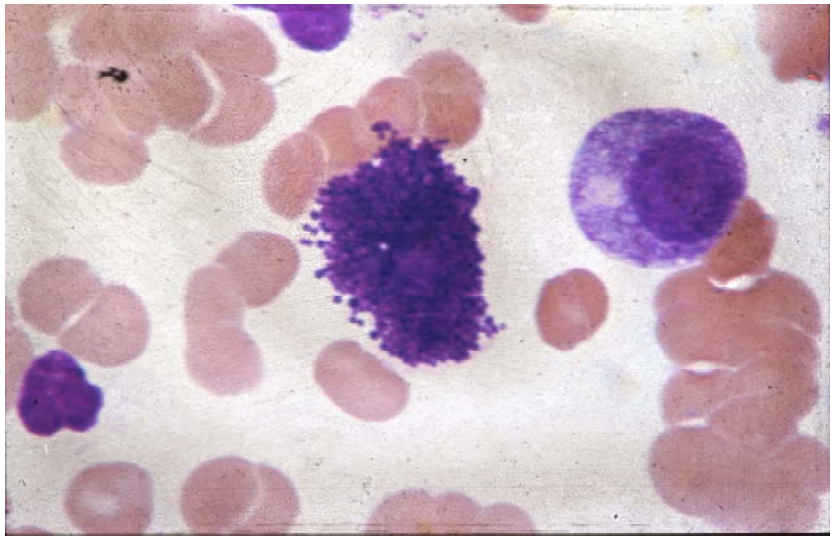


Mast Cell

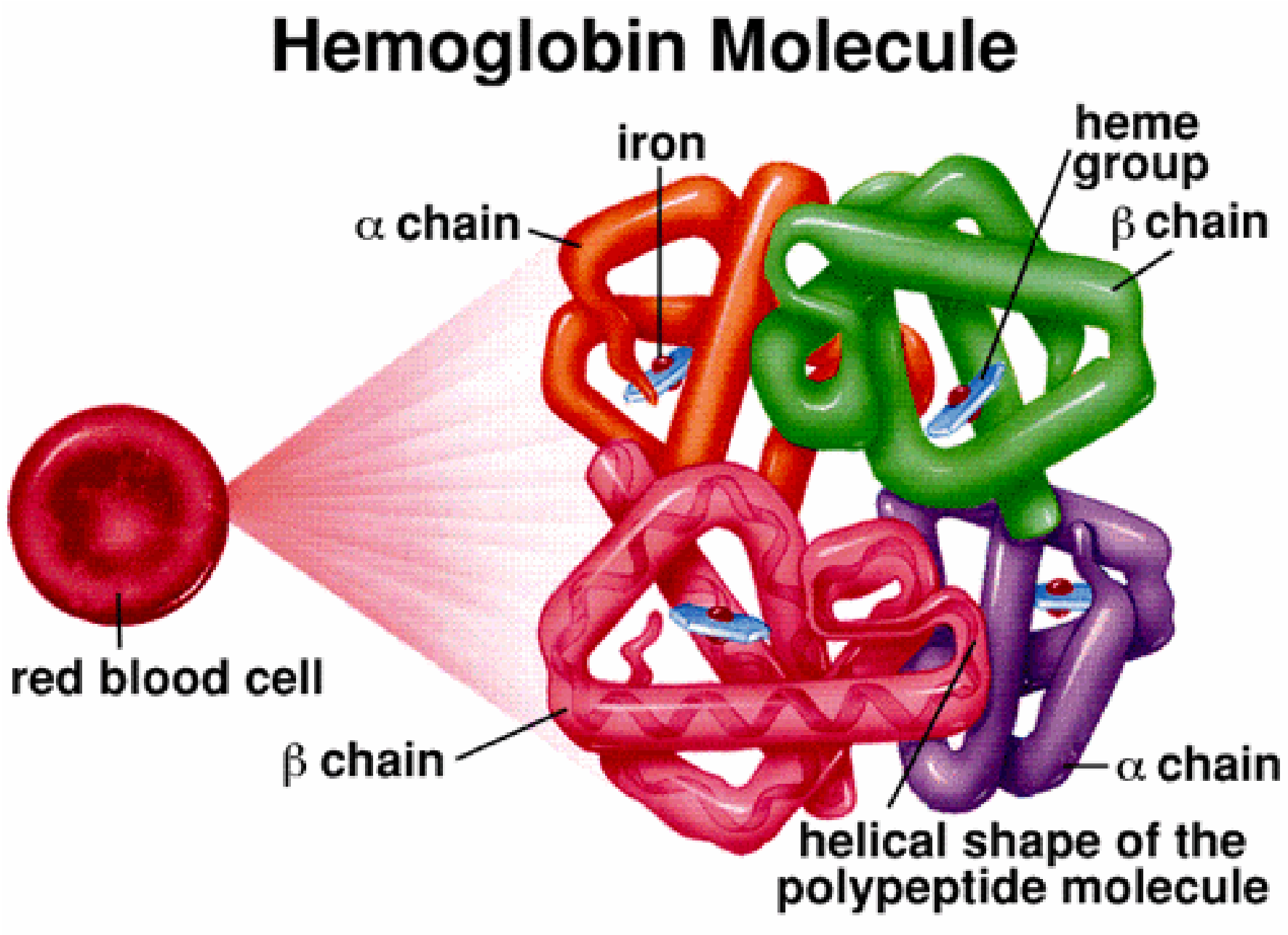
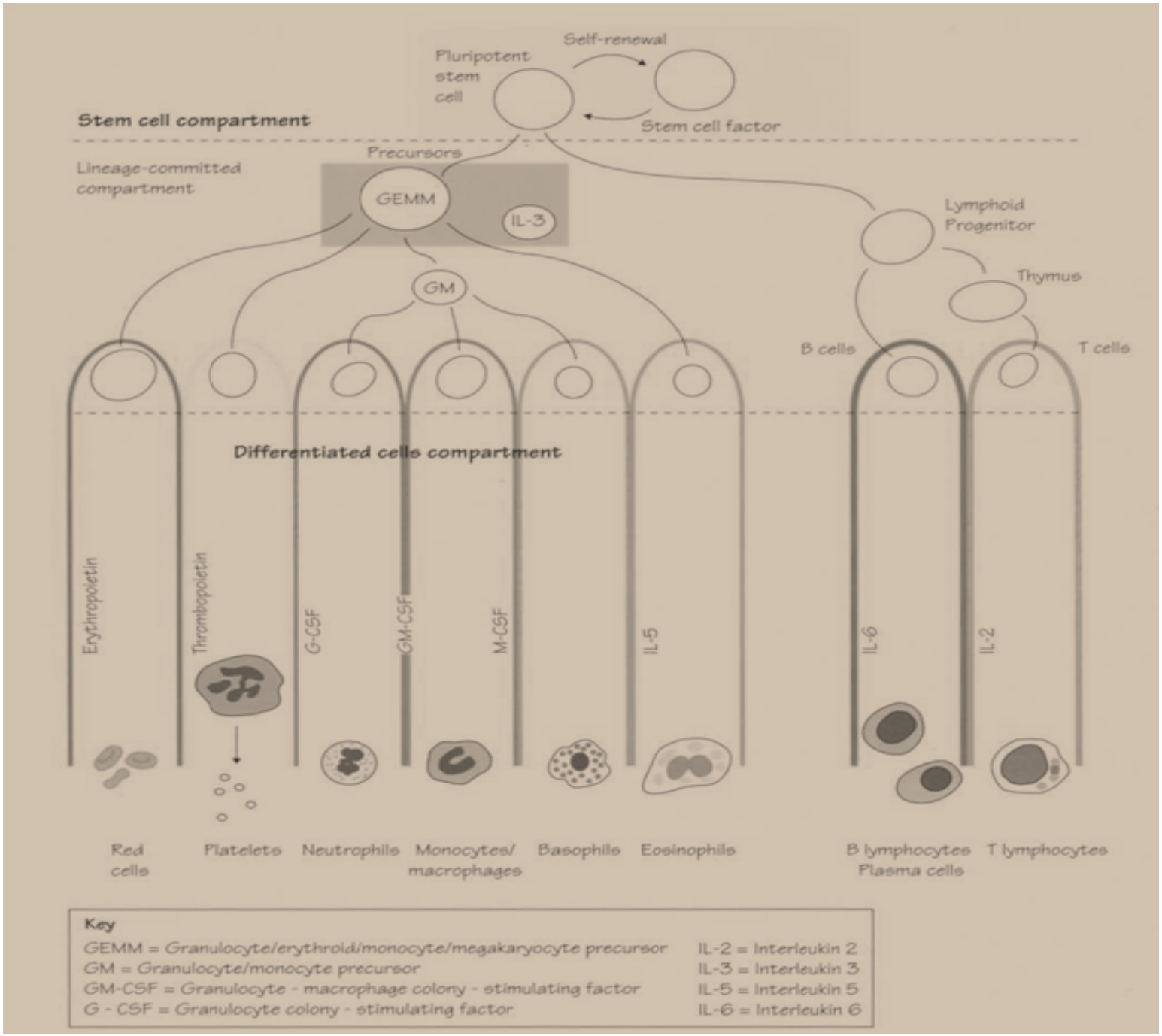

Hemoglobin Molecule
iron
heme group
α chain
β chain
red blood cell
β chain
α chain
helical shape of the polypeptide molecule

Table 1.1 Haemopoietic growth factors.

Act on stromal cells
IL-1, TNF } Stimulate production of GM-CSF, G-CSF, M-CSF, IL-6
Act on pluripotential cells
Stem cell factor
Act on early multipotential cells
IL-3
IL-4
IL-6
GM-CSF
Act on committed progenitor cells*
G-CSF
M-CSF
IL-5 (eosinophil CSF)
Erythropoietin
Thrombopoietin

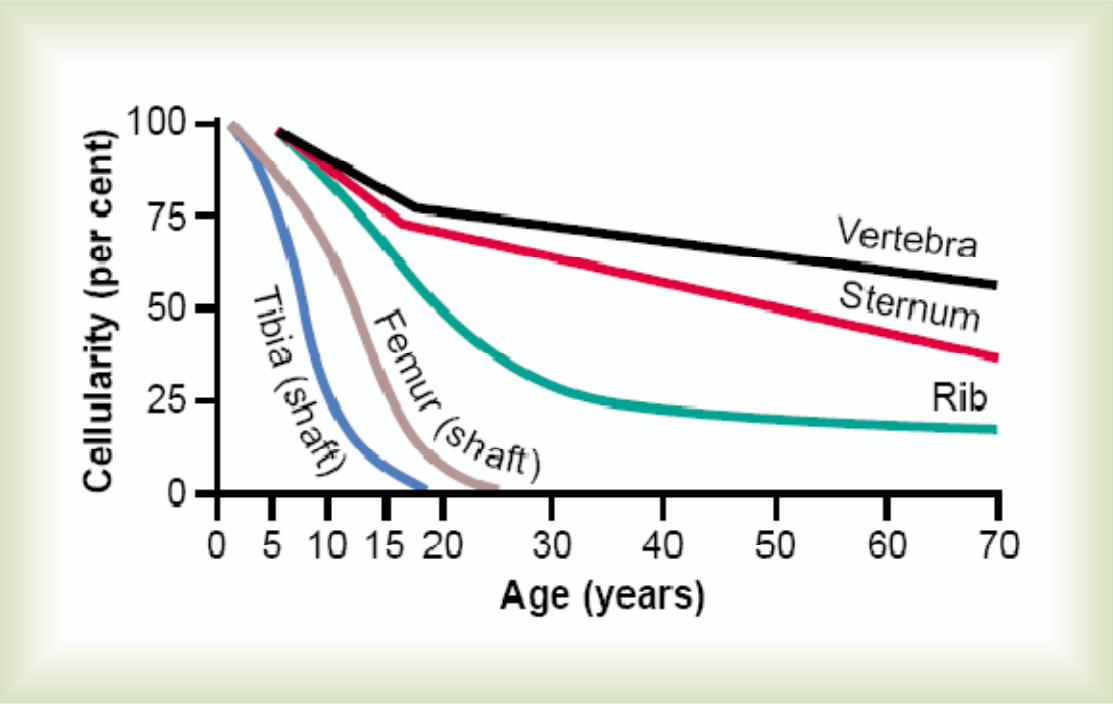
*These growth factors (especially G-CSF and thrombopoietin) also act on earlier cells.
G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL,


Self-renewal
Pluripotent stem cell
Stem cell factor
Stem cell compartment
Precursors
Lineage-committed compartment
GEMM
IL-3
GM
Lymphoid Progenitor
Thymus
B cells
T cells
Differentiated cells compartment
Erythropoietin
Thrombopoietin
G-CSF
GM-CSF
M-CSF
IL-5
IL-6
IL-2
Red cells
Platelets
Neutrophils
Monocytes/ macrophages
Basophils
Eosinophils
B lymphocytes Plasma cells
T lymphocytes
Key
GEMM = Granulocyte/erythroid/monocyte/megakaryocyte precursor
GM = Granulocyte/monocyte precursor
GM-CSF = Granulocyte - macrophage colony - stimulating factor
G - CSF = Granulocyte colony - stimulating factor
IL-2 = Interleukin 2
IL-3 = Interleukin 3
IL-5 = Interleukin 5
IL-6 = Interleukin 6

Erythropoietin Mechanism

Dietary Requirements of Erythropoiesis

- Erythropoiesis requires:
  - Proteins, lipids, and carbohydrates
  - Iron, vitamin B_{12}, and folic acid
- The body stores iron in Hb (65%), the liver, spleen, and bone marrow
- Intracellular iron is stored in protein-iron complexes such as ferritin and hemosiderin
- Circulating iron is loosely bound to the transport protein transferrin



Fate and Destruction of Erythrocytes

- The life span of an erythrocyte is 100–120 days
- Old RBCs become rigid and fragile, and their Hb begins to degenerate
- Dying RBCs are engulfed by macrophages
- Heme and globin are separated and the iron is salvaged for reuse
- Heme is degraded to a yellow pigment called bilirubin
- The liver secretes bilirubin into the intestines as bile
- The intestines metabolize it into urobilinogen
- This degraded pigment leaves the body in feces, in a pigment called stercobilin
- Globin is metabolized into amino acids and is released into the circulation
- Hb released into the blood is captured by haptoglobin and phgocytized

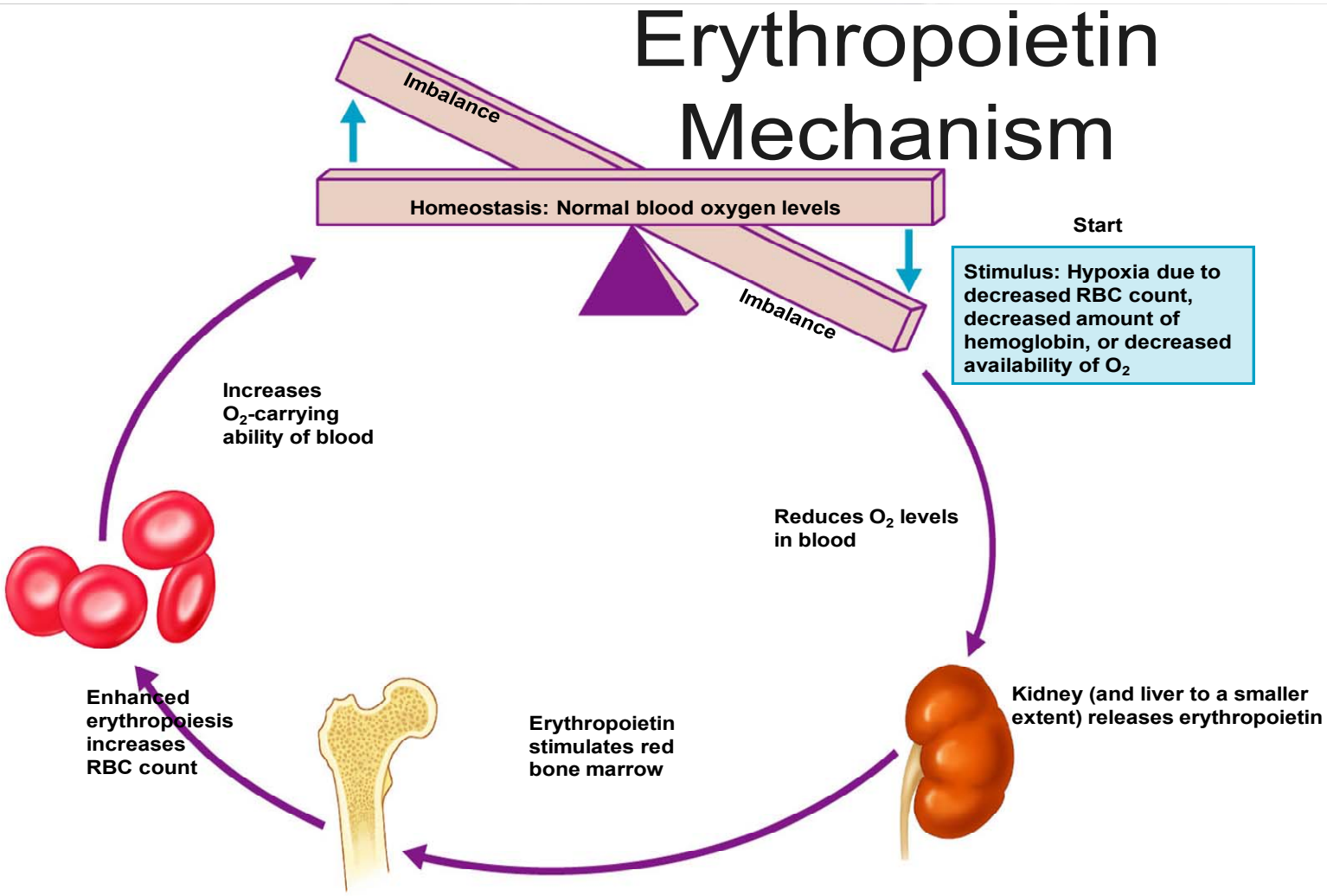


Figure 17.6

When red cell membranes are damaged, hemoglobin and other dissolved contents may escape from the cells, leaving the membranous structures as “ghosts.” This process, called hemolytic, is produced not only by the osmotic effects of water but also by numerous other mechanisms.
These include physical damage to red cells, as when blood is heated, is forced under great pressure through a small needle, or is subjected to freezing and thawing; chemical damage to red cells by agents such as bile salts, detergents, and certain snake venoms; and damage caused by immunologic reactions that may occur when antibodies attach to red cells in the presence of complement.
When such destruction proceeds at a greater than normal rate, hemolytic anemia results.

Most of the heme which is degraded comes from hemoglobin.

Since in the steady state 6-8 grams of hemoglobin are synthesized daily, 6-8 grams must also be degraded.

This gives rise to about 300 milligrams of heme. Heme is not reutilized, so it must be degraded and excreted.

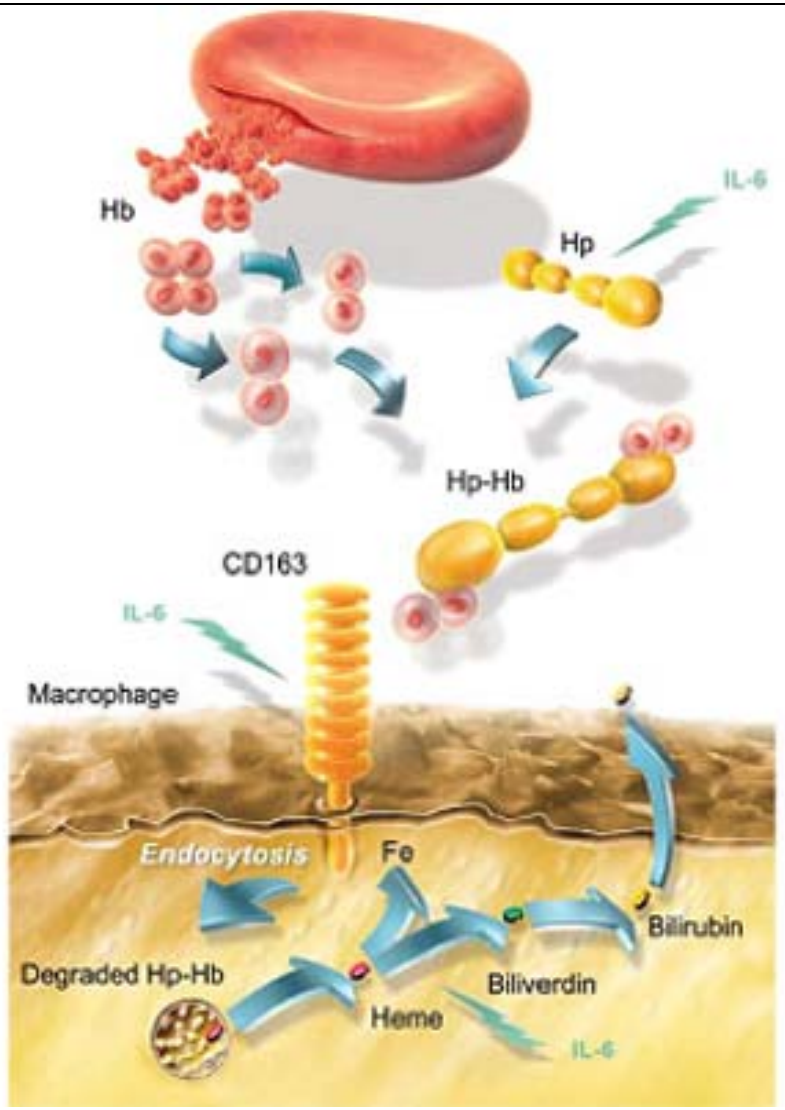
Although heme is not recycled, **its iron is conserved**.

- Normally, senescent and damaged erythrocytes are sequestered by the spleen, which processes them in a manner that preserves their iron content.
- **If hemolysis occurs**, hemoglobin (with its iron) is released into the plasma.
    - Possible causes of hemolysis include
        - erythrocyte fragility
        - thermal burns
        - erythroblastosis fetalis.
    - **In the plasma**, oxyhemoglobin dissociates into alpha-beta dimers, which can escape through the glomerular filtration system of the kidney to appear in the urine.

      To prevent this, there is a plasma protein, haptoglobin, which binds the dimer and

        - delivers it to the reticuloendothelial system for processing
        - activates the heme to prepare it for degradation.

      Any free heme is bound to another plasma protein, hemopexin, which then transports it to the liver for degradation.



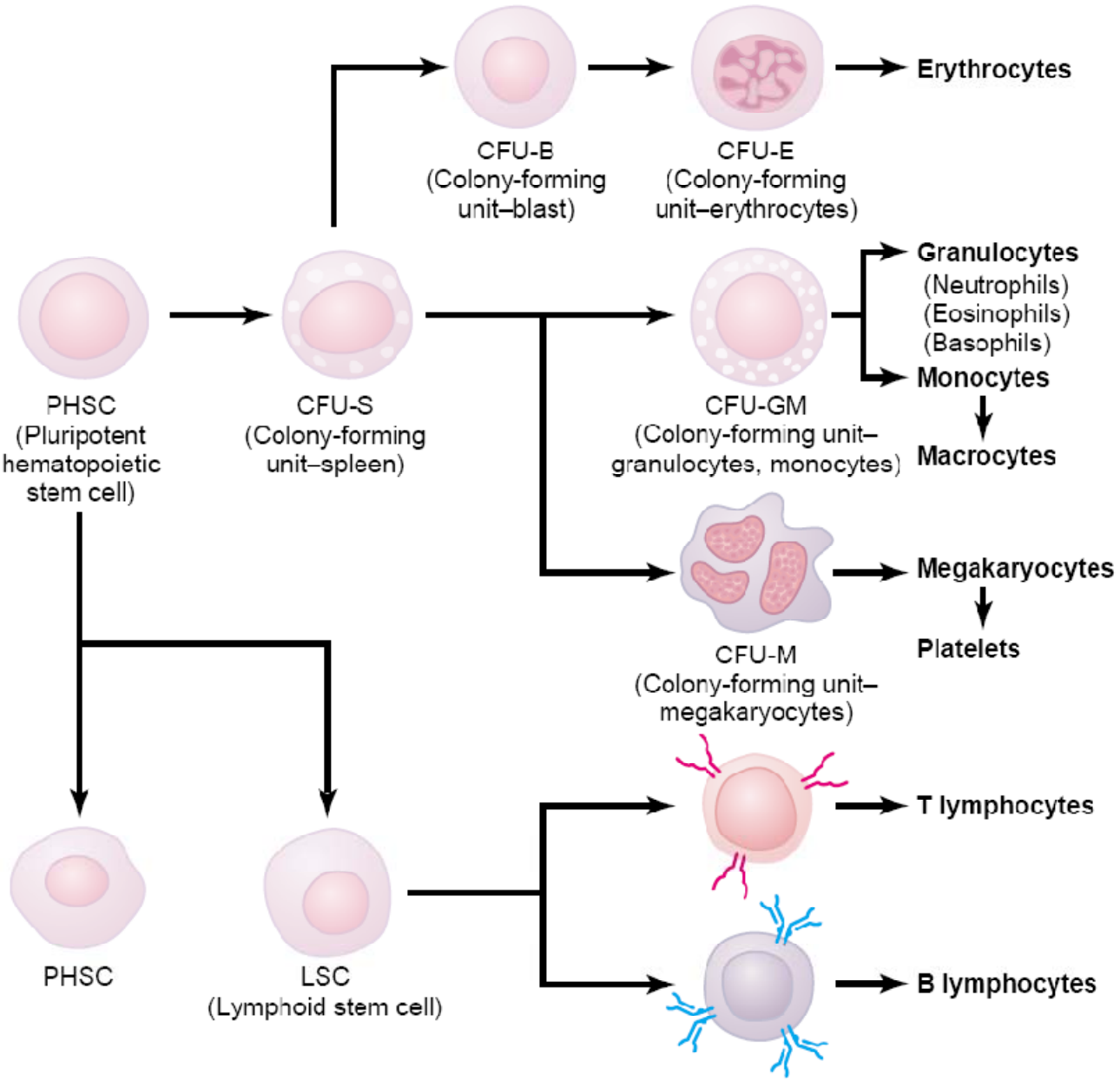


Figure 32–2

Formation of the multiple different blood cells from the original *pluripotent hematopoietic stem cell* (PHSC) in the bone marrow.

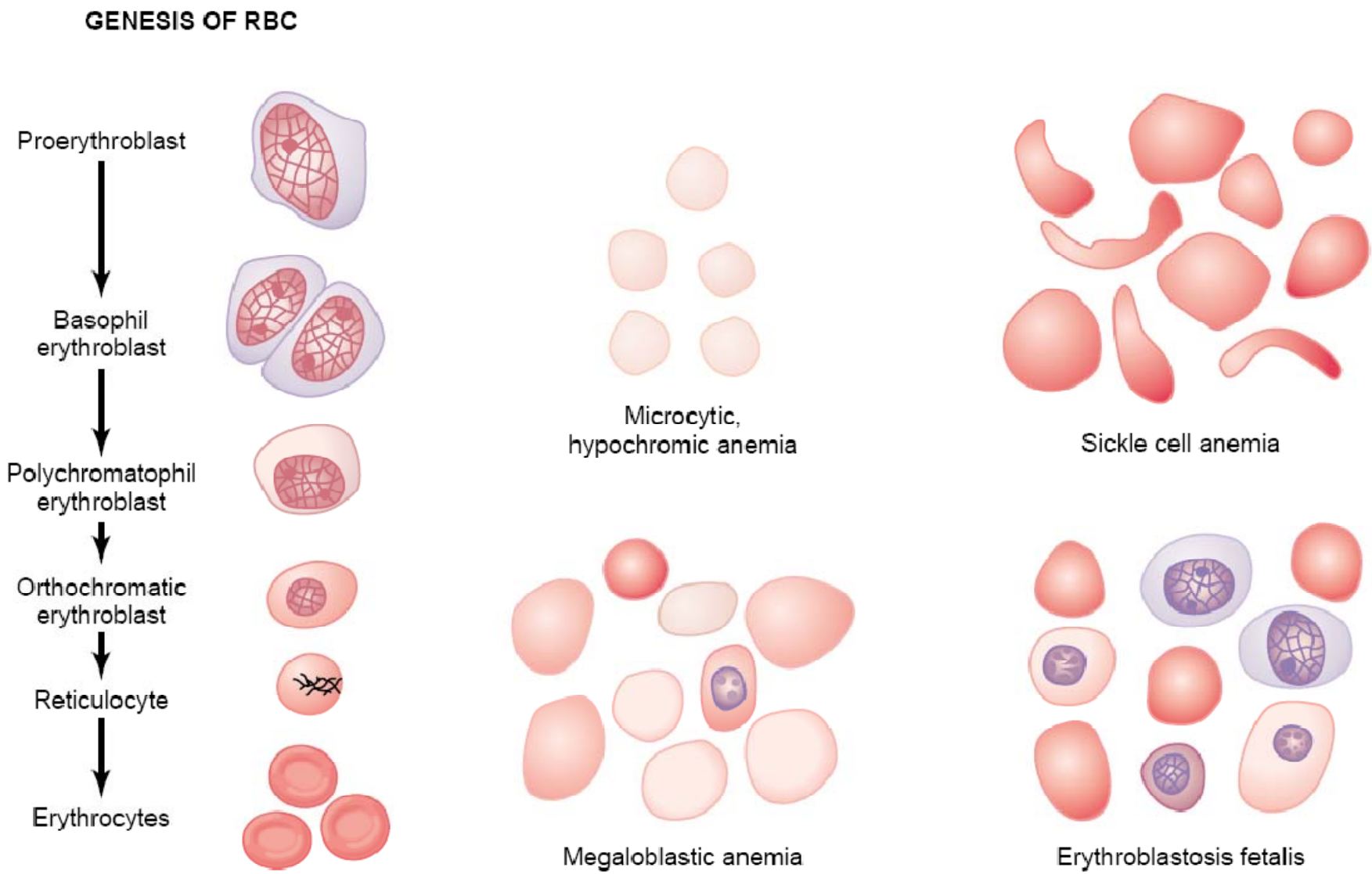
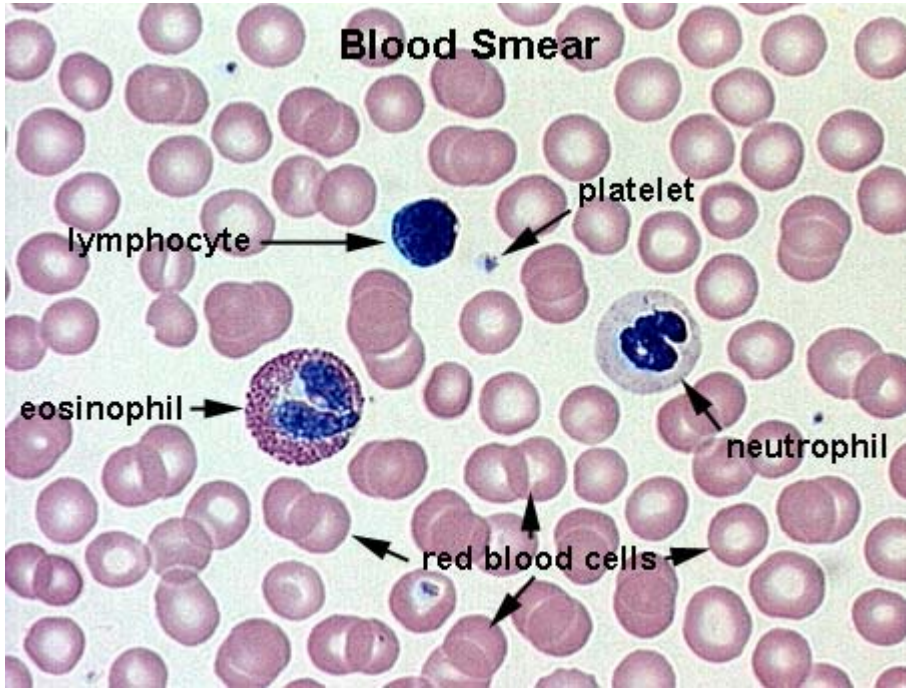
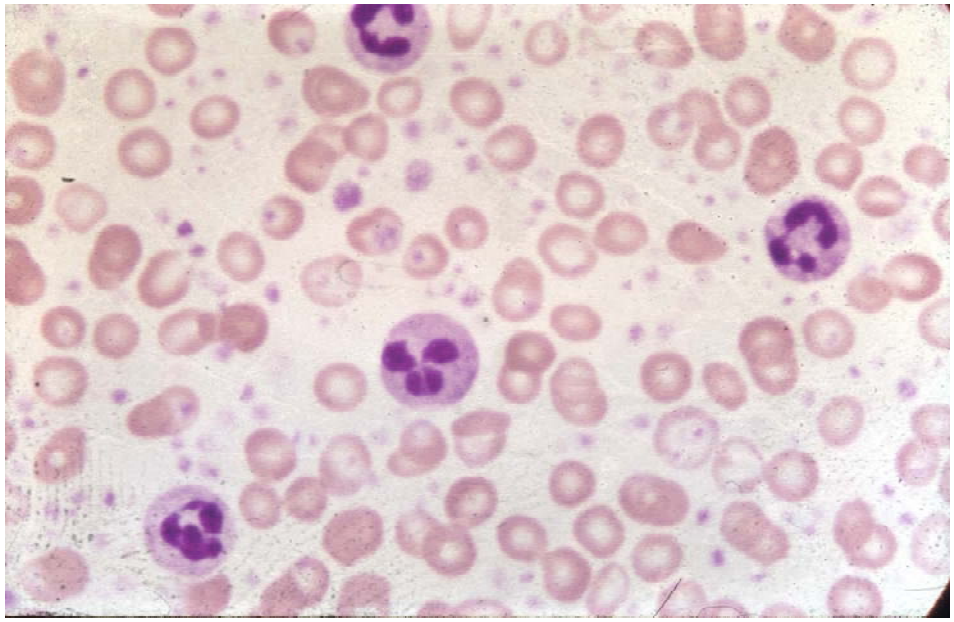


Figure 32–3

Genesis of normal red blood cells (RBCs) and characteristics of RBCs in different types of anemias.





Normal Blood

What happens to the hemoglobin when a red blood cell dies?

Iron is one of those things that we need in our bodies in small amounts. We get iron in our foods (especially meat, since muscle is so highly vascularized), and certainly, we can't ingest straight iron! Since we need iron, but it is not always easy to get anew, our bodies need to preserve as much iron as possible.

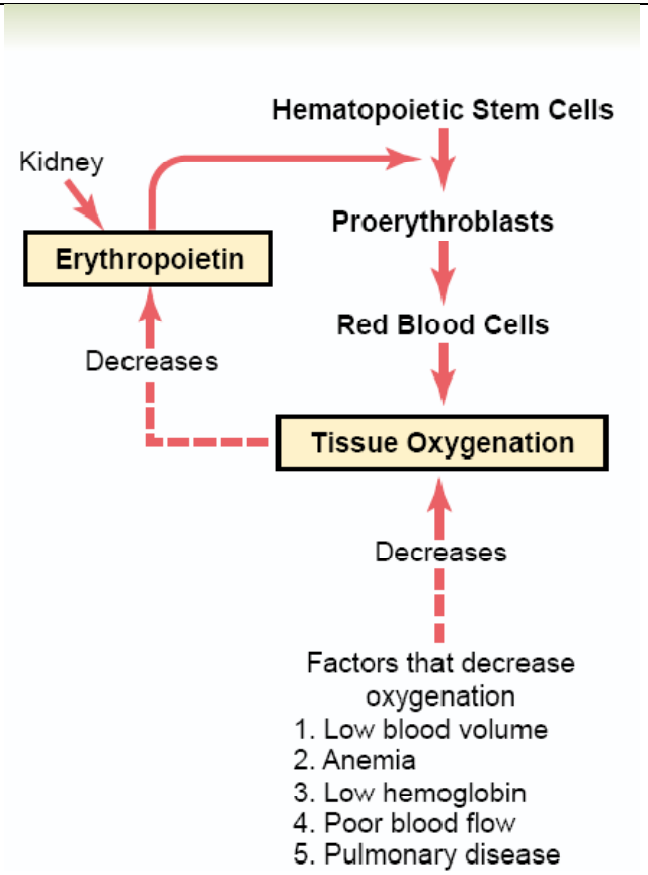
Therefore, when our red blood cells die, we need to extract back out the hemoglobin that they contained. Here's what happens. *the red words are the ones that relate to what happens to the iron atom*

- after RBC death, hemoglobin leaks out (typically into the blood)
- hemoglobin disassembles outside of the RBC into 4 globins plus 4 heme groups
- the globins can be broken down and re-used for its amino acids
- the heme groups unfold and the iron atom leaks out: this produces one biliverdin molecule plus one iron atom for each heme group
- the iron quickly binds to a plasma protein called **transferrin**, which carries the iron through the blood either to bone marrow for more hemoglobin production or to the liver for storage *(it is called transferrin not because it transfers iron, but because ferrin stands for the Latin name for iron, that's why it iron is*

represented by "Fe")

- much of the biliverdin (a greenish molecule, verdi- stands for green) gets changed into **bilirubin**, which is an orangish molecule
- the biliverdin and bilirubin end up being released by the gallbladder into the digestive system within the gallbladder secretion which is called "bile," and they can be broken down for use and excretion in the digestive system

We haven't looked at how our bodies recycle organic material before... so why are we doing it now? First of all, because of the importance of the iron atom being recycled. But also, because if you think about how many red blood cells are in any one person, as I show you in the table below, you'll realize what a huge amount of total body material they make up... we can't just destroy all that, we have to recycle it.



Hemoglobin and Erythrocyte Function

The function of the red cell is to mediate the exchange of respiratory gases, oxygen and carbon dioxide, between the lungs and the tissues.

Of fundamental importance to this process is the oxygen-transport protein, **hemoglobin**.

It is the major constituent of the red cell cytoplasm, accounting for about 90% of the dry weight of the mature cell. In man at rest, about **250 ml** of oxygen are consumed and **200 ml** of carbon dioxide are produced per minute. During exercise these quantities increase tenfold.

If the respiratory gases were carried in physical solution in the plasma, man's activity would be restricted to only **one fiftieth** of that possible in the presence of hemoglobin-containing red cells.

The development of hemoglobin makes possible the transportation of a hundred times as much oxygen as could be carried by the plasma alone.

In order to bind oxygen reversibly, the iron in the heme moiety of hemoglobin must be maintained in the **reduced (ferrous)** state despite exposure to a variety of endogenous and exogenous oxidizing agents.

The red cell maintains several metabolic pathways to prevent the action of these oxidizing agents and to reduce the hemoglobin iron if it becomes oxidized. Under certain circumstances, these mechanisms fail and hemoglobin becomes nonfunctional.

These abnormalities are particularly likely to occur

(1) if the red cell is exposed to certain oxidant drugs or toxins

(2) if the intrinsic protective mechanisms of the cell are defective or

(3) if there are genetic abnormalities of the hemoglobin molecule affecting globin stability or the heme crevice.

Methemoglobin is unable to bind oxygen. It has a distinctive, pH-dependent spectrum, and in concentrations greater than 10% of the total hemoglobin imparts to blood a distinctive, brownish color that does not disappear on vigorous shaking in air.

When methemoglobin is present in vivo in concentrations greater than 1.5 to 2.0 mg/dl, patients appear visibly **cyanotic**.

Methemoglobin combines readily with cyanide to form **cyanmethemoglobin**, a pigment so stable that it is utilized in laboratory procedures for quantifying hemoglobin.

Known mechanisms for preventing or reversing oxidative denaturation of hemoglobin in the erythrocyte include

(1) the **methemoglobin reductases**,

(2) **superoxide dismutase**,

(3) **glutathione peroxidase**, and

(4) **catalase**.

Methemoglobin Reduction

Methemoglobin may be **reduced nonenzymatically** by certain compounds found in erythrocytes, such as **ascorbic acid** and **glutathione**. However, **most of the physiologically important methemoglobin reduction occurs enzymatically**. **Glutathione** is the principal reducing agent in erythrocytes and the essential cofactor in the glutathione peroxidase reaction. Reduced glutathione (GSH) is a tripeptide (glutamyl-cysteinyl-glycine). Two enzymatic reactions are required for the de novo synthesis of glutathione:

1. **glutamic acid + cysteine ----------> glutamyl-cysteine**
2. **glutamyl-cysteine + glycine -------> GSH**

Reaction 1 is catalyzed by glutamyl-cysteine synthetase, and reaction 2, by glutathione synthetase.

Both reactions can take place in normal erythrocytes. The capacity of normal red cells to synthesize glutathione exceeds the rate of turnover by 150-fold.

In the course of reactions protecting hemoglobin from oxidation, **GSH is oxidized**, forming oxidized glutathione (GSSG), which consists of two GSH molecules joined by a disulfide linkage, and mixed disulfides with hemoglobin. GSSG rapidly leaves the erythrocyte.

Thus, if a continuous supply of GSH is to be maintained, there is need for a system to reduce the oxidized forms of glutathione. Such a system is provided by **glutathione reductase**, which **catalyzes the reduction of GSSG by NADPH**, a product of the **pentose phosphate** pathway.

Glutathione reductase also catalyzes the reduction of hemoglobin-glutathione disulfides, yielding GSH and hemoglobin.

ENERGY METABOLISM

Although the mature red cell contains the enzymes required for glycogen metabolism, the balance between synthesis and utilization is such that no significant amount of glycogen accumulates within the cell under normal circumstances.

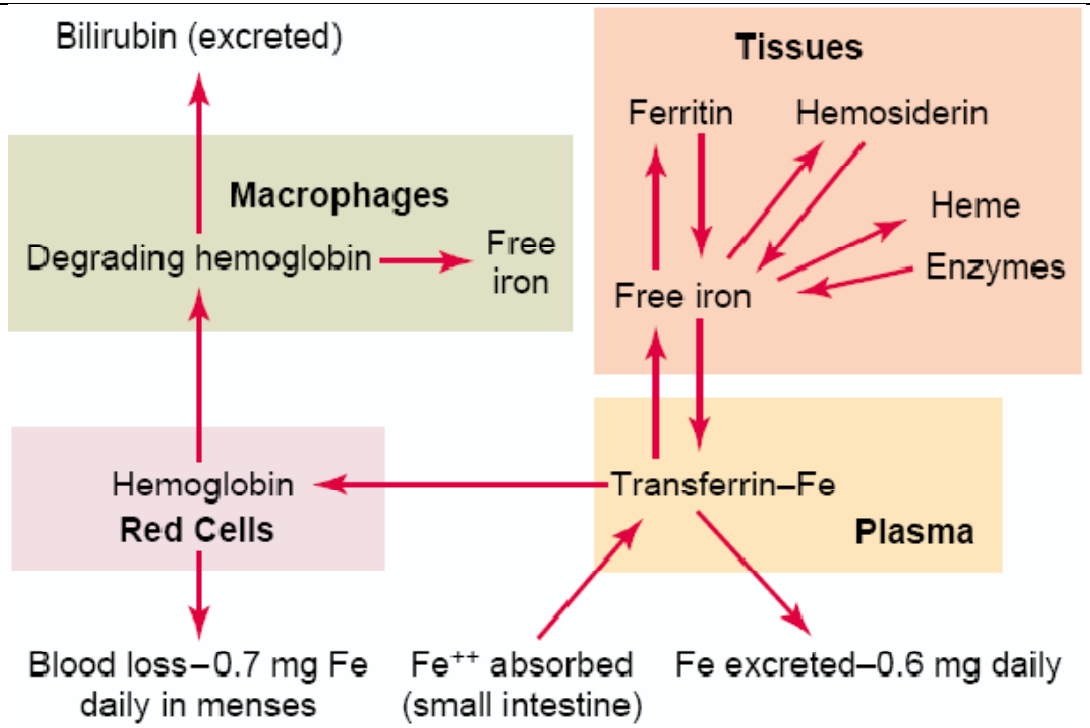
Since there are **no mitochondria** in erythrocytes, these cells must depend on two much less efficient pathways for production of high-energy compounds, the **anaerobic glycolytic (Embden-Meyerhof) pathway**, which is also known as the **hexose monophosphate shunt** or the phosphogluconate pathway.

Under normal circumstances, about **90% of glucose entering the red cell is metabolized by the anaerobic pathway** and 10% by the aerobic pathway.

Three important products are formed by the anaerobic glycolytic pathway: **NADH**, a cofactor in the methemoglobin reductase reaction. **ATP**, the major high-energy phosphate nucleotide that powers the cation pump; and **2,3-DPG**, a regulator of hemoglobin function. For each molecule of glucose that enters the pathway, **two molecules of NADH** are generated.

The **yields of ATP and 2,3-DPG vary** depending on the activity of the Rapoport-Luebering shunt.

The most important product of the pentose phosphate pathway in erythrocytes is reduced nicotinamide-adenine dinucleotide phosphate (**NADPH**). The red cell lacks the reactions to utilize NADPH for energy; instead, **NADPH, by serving as a cofactor in the reduction of oxidized glutathione (GSSG), is a major reducing agent in the cell** and the ultimate source of protection against oxidative attack.



Effect of Erythropoietin in Erythrogenesis.

When an animal or a person is placed in an atmosphere of low oxygen, erythropoietin begins to be formed within minutes to hours, and it reaches maximum production within 24 hours. Yet almost no new red blood cells appear in the circulating blood until about 5 days later.
From this fact, as well as other studies, it has been
determined that the important effect of erythropoietin is to stimulate the production of proerythroblasts from hematopoietic stem cells in the bone marrow.

Maturation of Red Blood Cells—Requirement for Vitamin B12 (Cyanocobalamin) and Folic Acid

- Because of the continuing need to replenish red blood cells, the erythropoietic cells of the bone marrow are among the most rapidly growing and reproducing cells in the entire body.
- Therefore, as would be expected, their maturation and rate of production are affected greatly by a person's nutritional status.
- Especially important for final maturation of the red blood cells are two vitamins, *vitamin B12* and *folic*

Failure of Maturation Caused by Deficiency of Folic Acid (Pteroylglutamic Acid).

Folic acid is a normal constituent of:

- green vegetables,
- some fruits,
- and meats (especially liver).

However, it is easily destroyed during cooking.

In addition,once the proerythroblasts are formed, the erythropoietin causes these cells to pass more rapidly through the different erythroblastic stages than they normally do, further speeding up the production of new red blood cells.

The rapid production of cells continues as long as the person remains in a low oxygen state or until enough red blood cells have been produced to carry adequate amounts of oxygen to the tissues despite the low oxygen; at this time, the rate of erythropoietin production decreases to a level that will maintain the required number of red cells but not an excess.

In the absence of erythropoietin, few red blood cells are formed by the bone marrow. At the other extreme, when large quantities of erythropoietin are formed available, and if there is plenty of iron and other required nutrients available, the rate of red blood cell production can rise to perhaps 10 or more times normal.

Therefore, the erythropoietin mechanismfor controlling red blood cell production is a powerful one.

Maturation Failure Caused by Poor Absorption of Vitamin B12 from the Gastrointestinal Tract—Pernicious Anemia.

A common cause of red blood cell maturation failure is failure to absorb vitamin B12 from the gastrointestinal tract.

This often occurs in the disease *pernicious anemia,* in which the basic abnormality is an *atrophic gastric mucosa* that fails to produce normal gastric secretions.

The parietal cells of the gastric glands secrete a glycoprotein called *intrinsic factor,* which combines with vitamin B12 in food and makes the *acid.*

- Both of these are essential for the synthesis of DNA,because each in a different way is required for the formation of thymidine triphosphate, one of the essential building blocks of DNA. Therefore, lack of either vitamin B12 or folic acid causes abnormal and diminished DNA and, consequently, failure of nuclear maturation and cell division.

- Furthermore, the erythroblastic cells of the bone marrow, in addition to failing to proliferate rapidly, produce mainly larger than normal red cells called *macrocytes,* and the cell itself has a flimsy membrane and is often irregular, large, and oval instead of the usual biconcave disc.

- These poorly formed cells, after entering the circulating blood, are capable of carrying oxygen normally, but their fragility causes them to have a short life, one half to one third normal.

- Therefore, it is said that deficiency of either vitamin B12 or folic acid causes *maturation failure* in the process of erythropoiesis.

Also, people with gastrointestinal absorption abnormalities, such as the frequently occurring small intestinal disease called *sprue,* often have serious difficulty absorbing both folic acid and vitamin B12.

Therefore, in many instances of maturation failure, the cause is deficiency of intestinal absorption of both folic acid and vitamin B12.

B12 available for absorption by the gut. It does this in the following way: (1) Intrinsic factor binds tightly with the vitamin B12. In this bound state, the B12 is protected from digestion by the gastrointestinal secretions. (2) Still in the bound state, intrinsic factor binds to specific receptor sites on the brush border membranes of the mucosal cells in the ileum. (3) Then, vitamin B12 is transported into the blood during the next few hours by the process of pinocytosis, carrying intrinsic factor and the vitamin together through the membrane. Lack of intrinsic factor, therefore, causes diminished availability of vitamin B12 because of faulty absorption of the vitamin. Once vitamin B12 has been absorbed from the gastrointestinal tract, it is first stored in large quantities in the liver, then released slowly as needed by the bone marrow. The minimum amount of vitamin B12 required each day to maintain normal red cell maturation is only 1 to 3 micrograms, and the normal storage in the liver and other body tissues is about 1000 times this amount. Therefore, 3 to 4 years of defective B12 absorption are usually required to cause maturation failure anemia.		

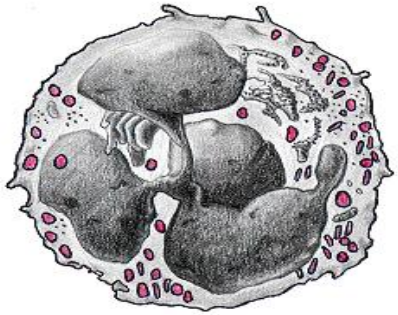


Fig. 8 - Neutrophil

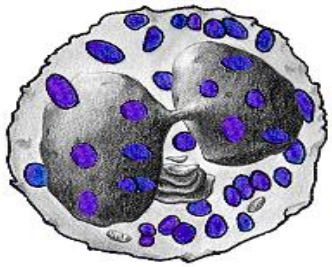
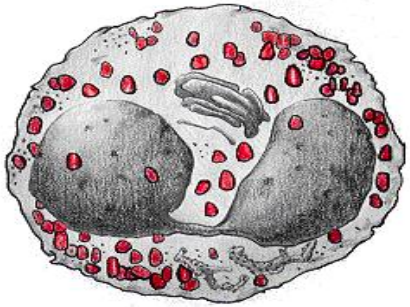


fig. 10 - Basophil



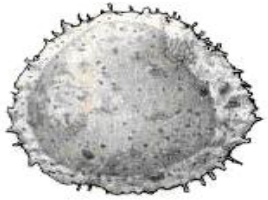


Fig. 11 - Lymphocyte

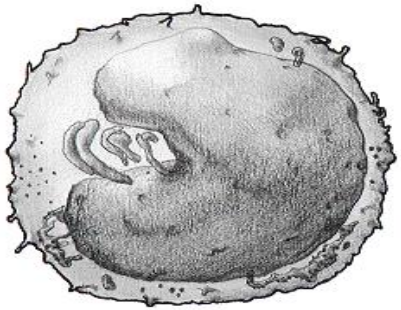


Fig. 12 - Monocyte

WHITE BLOOD CELLS
Granulocytes

Leukocytes can be further subdivided into granular leukocytes, i.e. neutrophils, basophils and eosiniphils, and non-granular leukocytes, i.e. monocytes and lymphocytes.

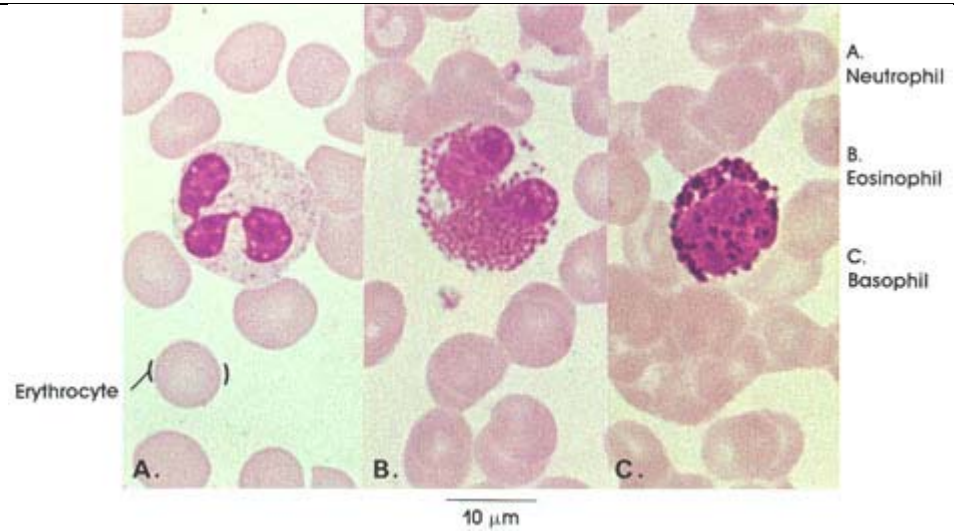
In healthy individuals the relative numbers of circulating leukocyte types are quite stable. A differential leukocyte count would typically produce the following cell frequencies (numbers in parentheses are the range of normal frequencies reported in different texts):

- ~ 60% neutrophils (50% - 70%)
- ~ 3% eosinophils (>0% - 5%)
- ~ 0.5% basophils (>0% - 2%)
- ~ 5% monocytes (1% - 9%)
- ~ 30% lymphocytes (20% - 40%)

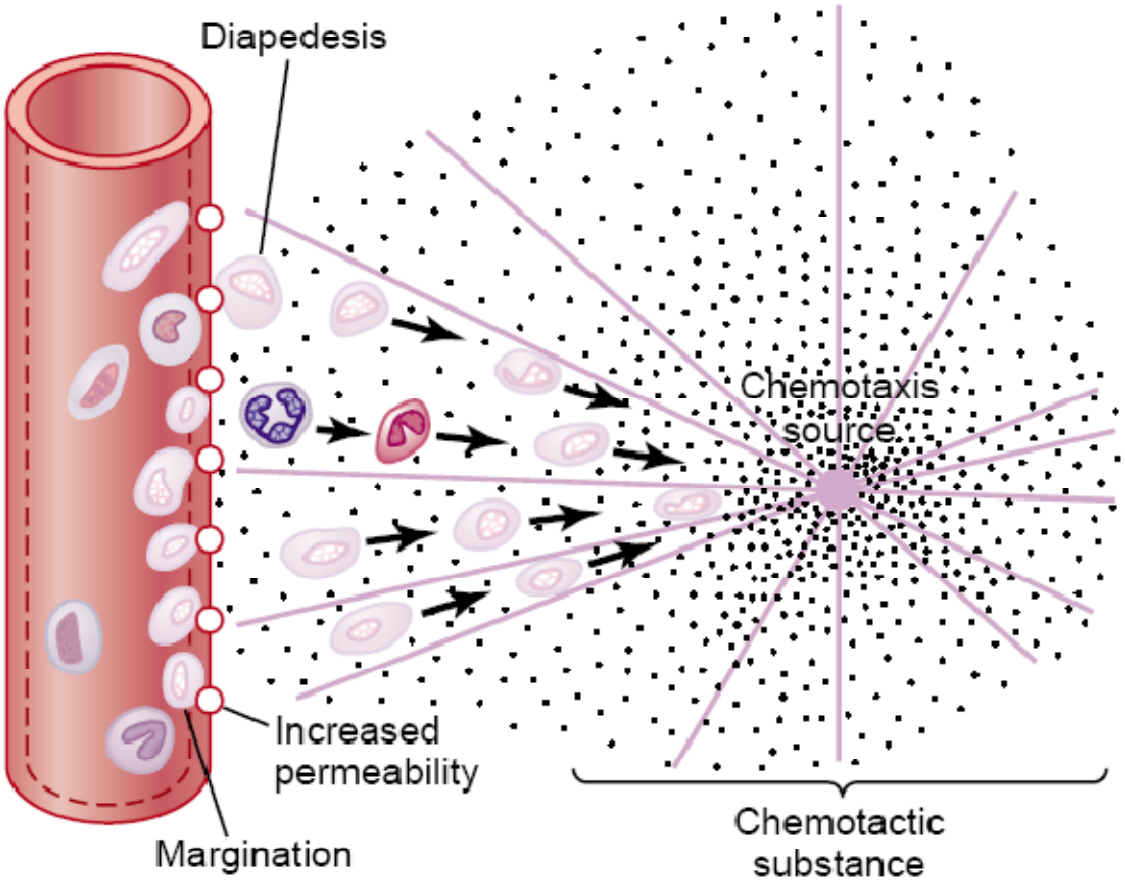
Changes in their relative numbers indicate that something abnormal is happening in the organism.

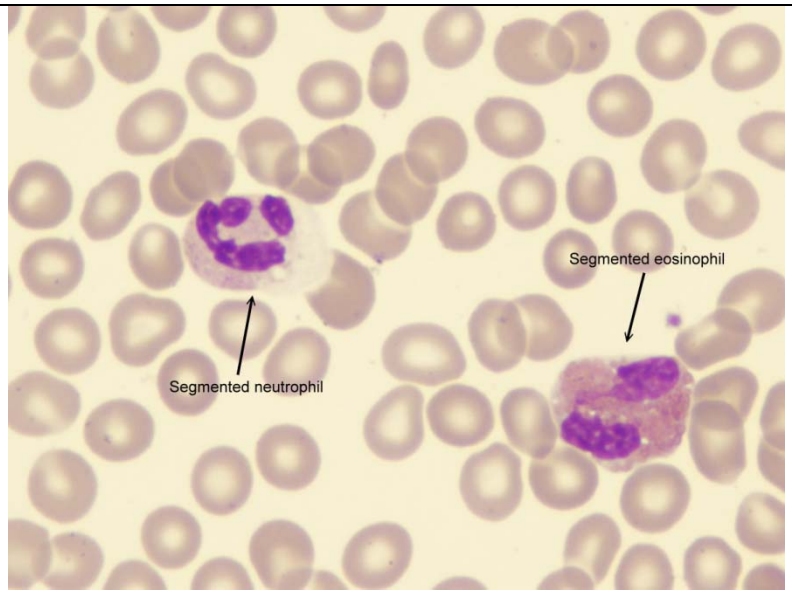
A larger than usual number of neutrophils (neutrophilia) would indicate e.g. an acute or chronic infection.

The number of basophils and eosinophils may increase (eosinophilia or basophilia) as a consequence of e.g. allergic disorders.



Human, air-dried blood smear, Wright's stain, 1416 x.
http://www.anatomyatlases.org/MicroscopicAnatomy/Section04/Plate0452.shtml


Diapedesis
Chemotaxis source
Increased permeability
Margination
Chemotactic substance



http://www.healthsystem.virginia.edu/internet/hematology/HessIDB/Cell-Comparisons.cfm

Migration of WBCs

- Unlike RBCs leukocytes can penetrate blood vessel wall without injury of it. This process is sometimes called the *leukodiapedesis*.
- Leukocyte migration is important in both hematopoiesis and reactions of immunological defence.
- Positive chemotaxis is a process where cell moves towards higher content of certain chemotactic substance (e.g. Some factors of the complement system).

Leukocyte migration is driven by chemokines and adhesion molecules

Chemokines

- **A big family of proteins which contain 67-127 amino acids. Chemically CXC and CC subfamilies are differentiated. There are several types of chemokine receptors.**
- **Functionally are there inflammatory chemokines, homeostatic chemokines and dual-function chemokines.**
- **The inflammatory chemokines control recruitment of WBCs into inflammation, tissue injury and tumors, e.g. CX3CL1 or fractalkine.**
- **The homeostatic chemokines control migration of cells through hematopoiesis, e.g. CXCL12 (SDF-1).**

- **Chemokines act over G-protein coupled surface receptors after what several important intracellular become activated, among them are phospholipase C (PLCbeeta), phosphoinositid 3-kinases (PtdIns3-Ks), and tyrosin kinases of C-Src family.**
- **The PtdIns3-Ks are crucially important to switch on cellular contractile machinery.**

In hematopoiesis different hematopoietic cells have different sensitivity to chemokines and as a final result only mature cells can reach circulation.

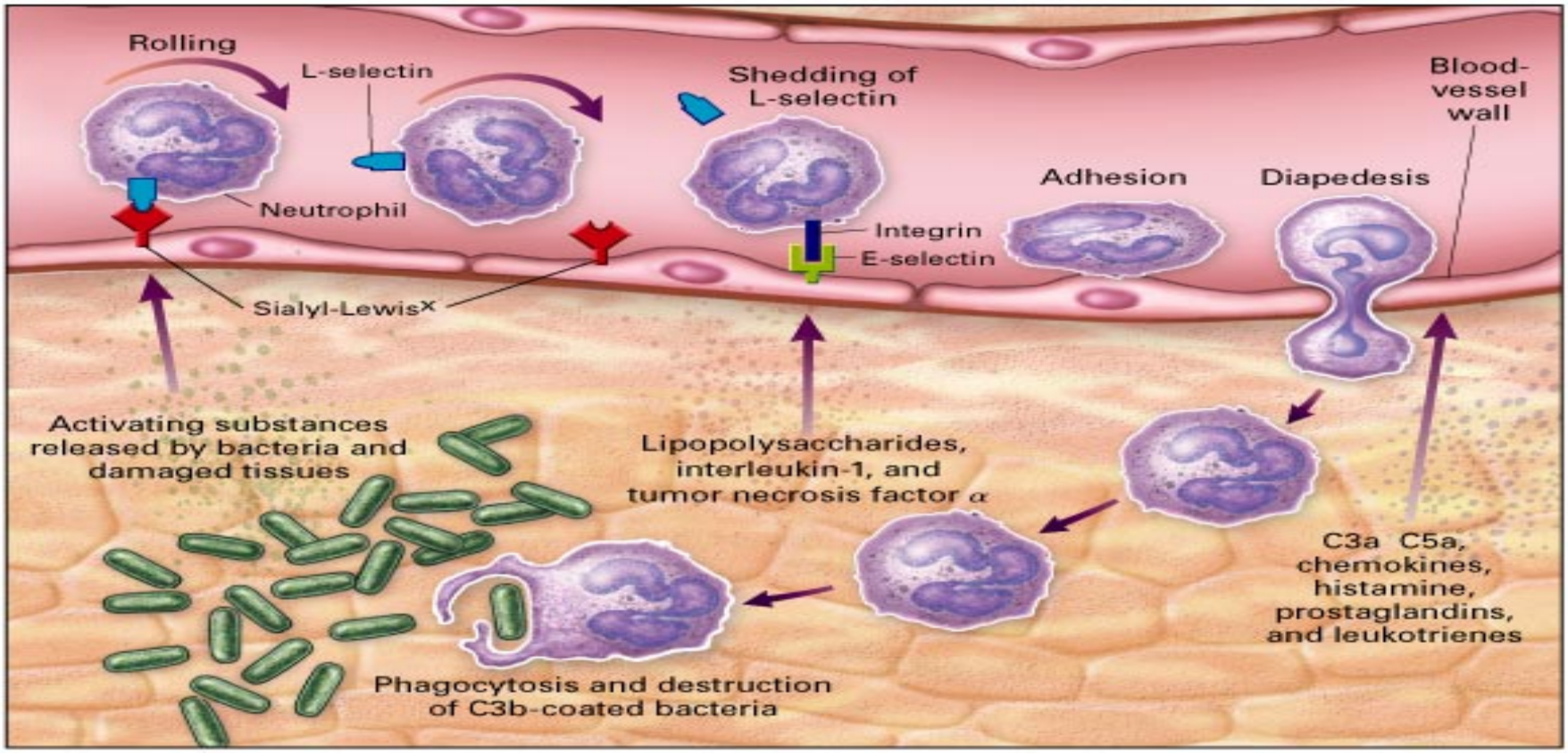
Neutrophil: Compare sizes of the neutrophil and the erythrocyte.

Lobulated nucleus, individual lobes connected by thin bridges.

Cell type-specific cytoplasmic granules are small.

Neutrophils constitute 40 to 75 per cent of the total white blood cell count.

The number of neutrophils increases in inflammation, and they act as the first line of defense against invading pyogenic organisms.



Eosinophil: Nucleus bilobed. Cell type-specific cytoplasmic granules are large and uniform in size and stain intensely red with acid dyes. They constitute 1 to 3 per cent of total white count and increase in number in allergic states and in parasitic infections.

Basophil: The nucleus is large but less lobulated than other white blood cells. Cell type-specific cytoplasmic granules are large and variable in size and have a strong affinity for basic dyes. They constitute 0.5 to 1 per cent of white count and are believed to synthesize the heparin and histamine found in circulating blood.

Leukocytes (WBCs)

- Leukocytes, the only blood components that are complete cells:
  - Are less numerous than RBCs
  - Make up 1% of the total blood volume
  - Can leave capillaries via diapedesis
  - Move through tissue spaces
- Leukocytosis – WBC count over 11,000 / mm^3
  - Normal response to bacterial or viral invasion

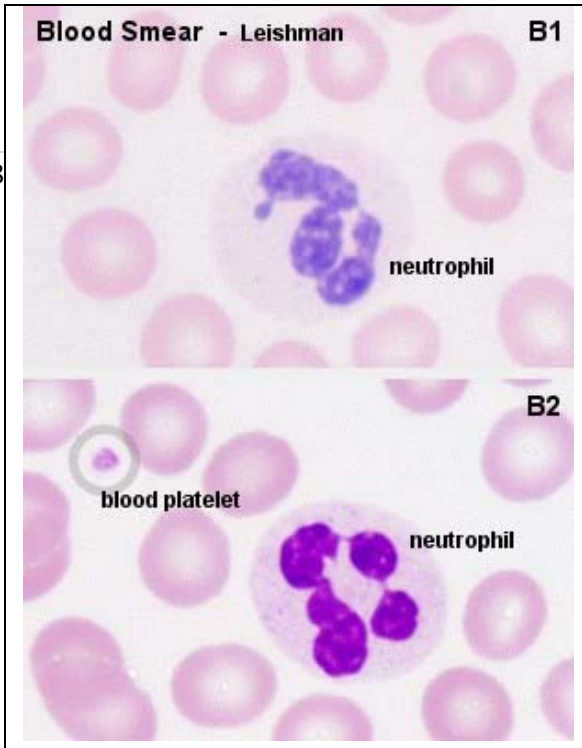
Granulocytes

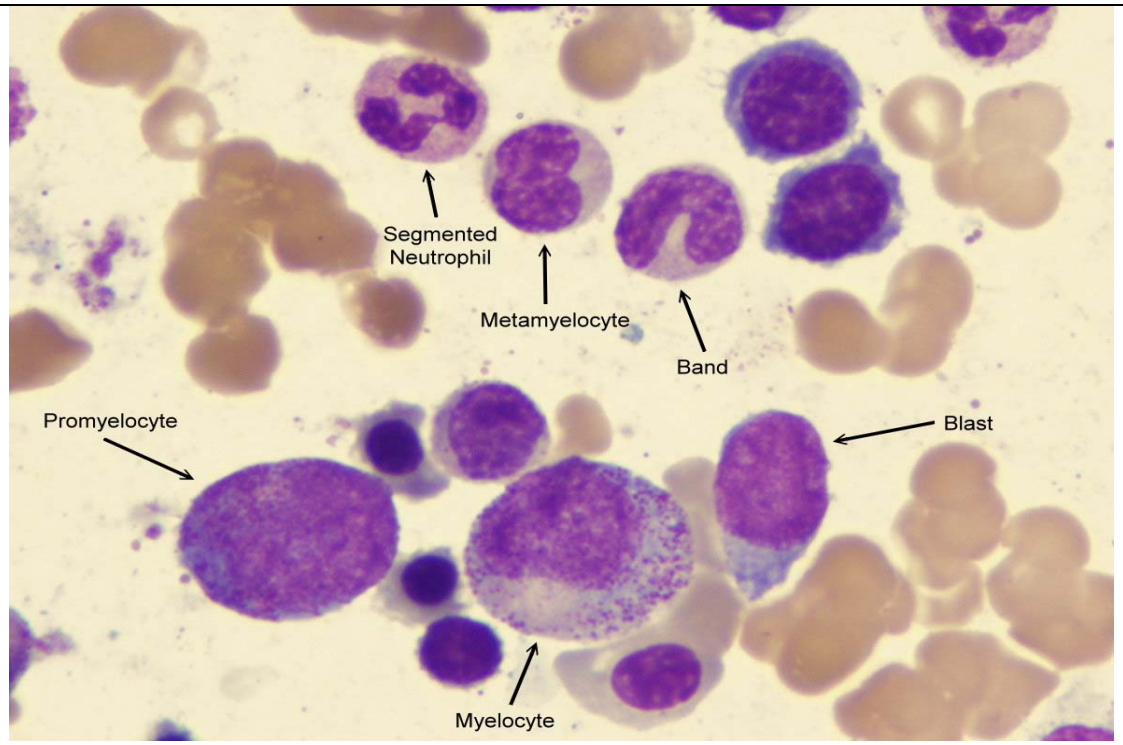
- Granulocytes – neutrophils, eosinophils, and basophils
  - Contain cytoplasmic granules that stain specifically (acidic, basic, or both) with Wright's stain
  - Are larger and usually shorter-lived than RBCs
  - Have lobed nuclei
  - Are all phagocytic cells

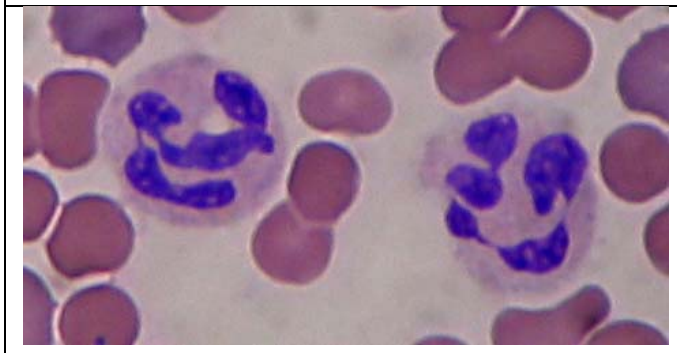
  - Granular leukocytes are all approximately the same size - about 12-15 μm in diameter.
  - Their nuclei form lobes, and nucleoli cannot be seen. The number of nuclear lobes varies according to cell type. All granulocytes are motile.
  - The term granulocytes refers to the presence of granules in the cytoplasm of these cells.
  - The granules correspond to secretory vesicles and lysosomes.
  - Specific granules are the granules which are only found in one particular type of granulocytes.

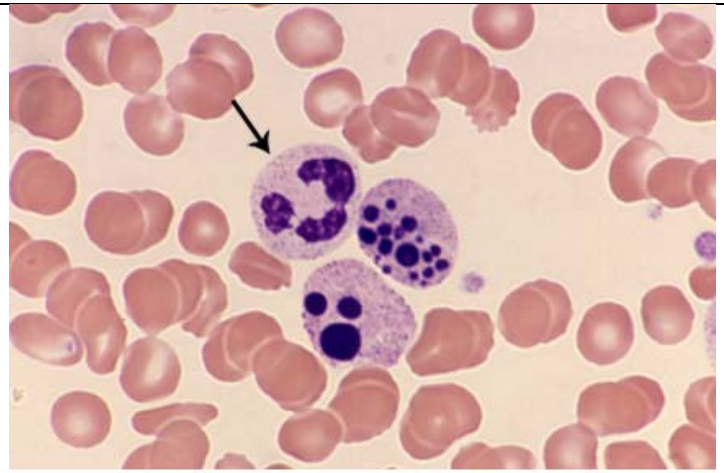
Neutrophils [Neutrophil granulocytes (or neutrophils)]

- Neutrophils have two types of granules that:
  - Take up both acidic and basic dyes
  - Give the cytoplasm a lilac color
  - Contain peroxidases, hydrolytic enzymes, and defensins (antibiotic-like proteins)
- Neutrophils are our body's bacteria slayers









Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:
Lichtman's Atlas of Hematology: http://www.accessmedicine.com

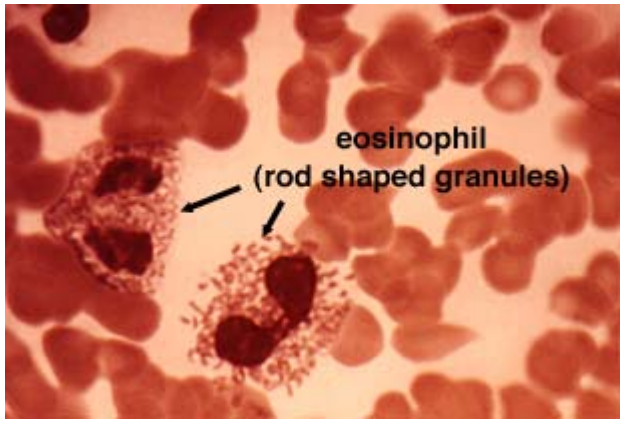
- have a very characteristic nucleus.
- It is divided into 3-5 lobes which are connected by thin strands of chromatin.
- The number of lobes increases with cell age.
- Up to 7 lobes can be found in very old neutrophils (hypersegmented cells).
- Neutrophils (like all other granulocytes, monocytes and lymphocytes) contain all the organelles that make up a typical cell.
- In addition to the usual complement of organelles, they also contain two types of granules.
- Primary granules (or A granules) contain lysosomal enzymes and are likely to be primary lysosomes, although they are larger (0.4 μm) than the "ordinary" primary lysosome.
- Secondary granules (or B granules), the specific granules of the neutrophils, contain enzymes with strong bactericidal actions.
- The specific granules of neutrophils stain only weakly if they are at all visible - they are "neutral", hence the term neutrophil.

Functions

- Neutrophils play a central role in inflammatory processes.
- Large numbers invade sites of infection in response to factors (e.g. cytokines) released by cells which reside at an infection site.
- Neutrophils are the first wave of cells invading infection sires.
- Receptors in their plama membrane allow them to recognise foreign bodies, e.g. bacteria, and tissue debris, which they begin to phagocytose and destroy.
- The phagocytotic activity of neurophils is further stimulated if invading microorganisms are "tagged" with antibodies (or opsonised).
- Neutrophils cannot replenish their store of granules.
- The cells die once their supply of granules has been exhausted.
- Dead neutrophils and tissue debris are the major components of pus.
- Their lifespan is only about one week.
- Lost neutrophils are quickly replenished from a reserve population in the bone marrow.
- Because they are younger, their nuclei have fewer lobes than the "average" neutrophil.
- A high proportion of neutrophils, with few nuclear lobes indicates a recent surge in their release from the bone marrow.

Eosinophils

- Eosinophils account for 1–4% of WBCs
  - Have red-staining, bilobed nuclei connected via a broad band of nuclear material
  - Have red to crimson (acidophilic) large, coarse, lysosome-like granules
  - Lead the body’s counterattack against parasitic worms
  - Lessen the severity of allergies by phagocytizing immune complexes



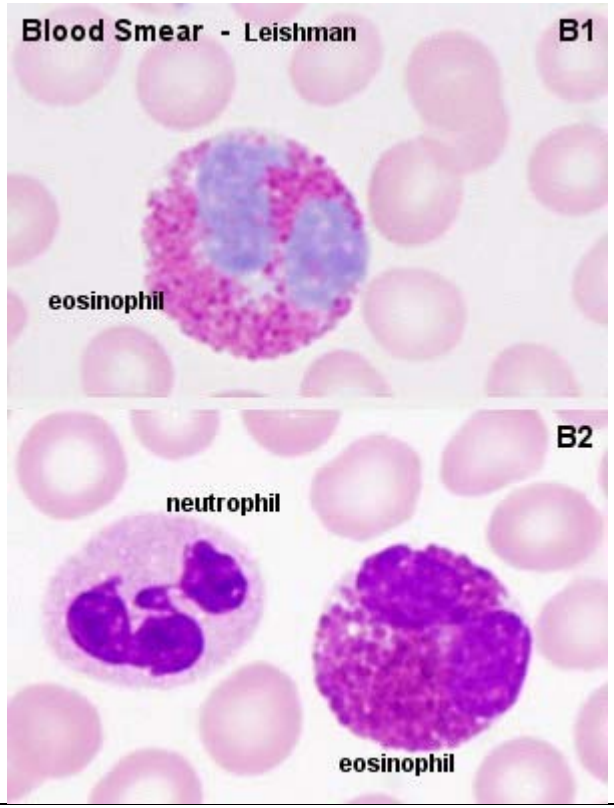
Their nucleus usually has only two lobes.

- Almost all of the cytoplasm appears filled with the specific granules of the eosinophils.
- As the term "eosinophil" indicates, these granules are not neutral but stain red or pink when eosin or a similar dye is used in the staining process.
- Aside from the usual complement of organelles eosinophils contain some large rounded vesicles (up to 1 μm) in their cytoplasm .
- These granules correspond to the eosinophilic grains that we see in the light microscope.
- The specific granules contain, in addition to enzymes that otherwise are found in lysosomes, an electron-dense, proteinaceous crystal.
- This crystal is composed of major basic protein (MBP).

Functions

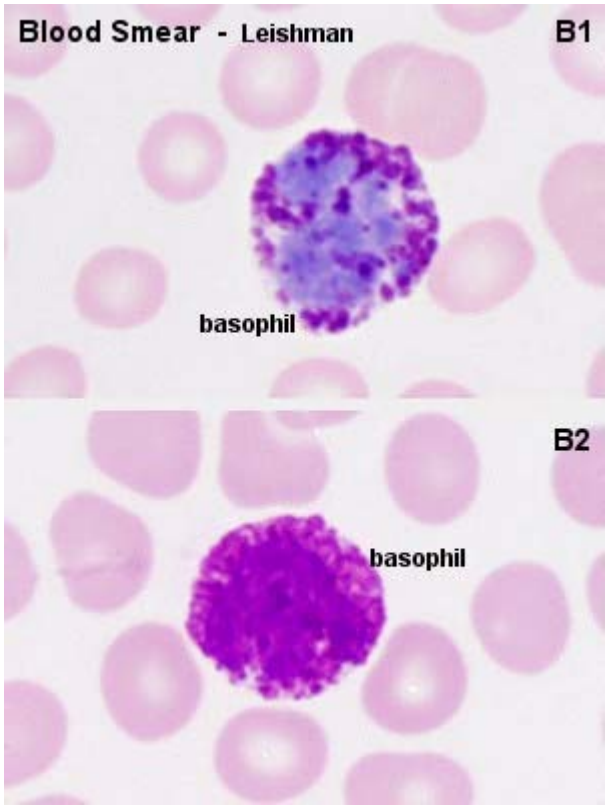
- The presence of antibody-antigen complexes stimulates the immune system.
- Eosinophils phagocytose these complexes and this may prevent the immune system from "overreacting".
- Their granules also contain the enzymes histaminase and arylsufatase.
- These enzymes break down histamine and leukotrienes, which again may dampen the effects of their release by basophils or mast cells. MBP, which can also function as a cytotoxin, and its release by eosinophils may be involved in the response of the body against parasitic infections, which are accompanied by an increase in the number of eosinophils.

- Eosinophils and basophils are the only cell types present in normal blood which initially may be difficult to distinguish - in particular in darker smears.
- If you see them side by side in your drawing the difference between them should become apparent.
- Chances are 6:1 that the you find an eosinophil before you find a basophil.
- The two lobes of the nucleus of eosinophils are usually well-defined and of about equal size. The nucleus is embedded in a cytoplasm crowded with granules, which seem to form a solid mass in the cell.
- The 2-3 nuclear lobes of basophils are not as well defined as those of eosinophils, granules are not as numerous as in eosinophils, and pretty much all of them can be identified "as individuals" rather than the dense mass they form in eosinophils.



Basophils

- Account for 0.5% of WBCs and:
  - Have U- or S-shaped nuclei with two or three conspicuous constrictions
  - Are functionally similar to mast cells
  - Have large, purplish-black (basophilic) granules that contain histamine
    - Histamine – inflammatory chemical that acts as a vasodilator and attracts other WBCs (antihistamines counter this effect)



Basophilic granulocytes have a 2 or 3 lobed nucleus.

- The lobes are usually not as well defined as in neutrophilic granulocytes and the nucleus may appear S-shaped.
- The specific granules of basophils are stained deeply bluish or reddish-violet.
- Their colour corresponds closely to the colour of the nucleus which sometimes is difficult to see amongst or behind the granules.
- The granules are not as numerous as those in eosinophils.
- The specific granules of basophils (about 0.5 µm) appear quite dark in EM pictures.
- They contain heparin, histamine lysosomal enzymes and leukotrienes (the later correspond to the slow-reacting substance of anaphylaxis or SRS-A).

Functions

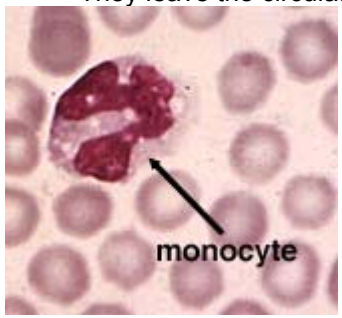
Heparin and histamine are vasoactive substances.

- They dilate the blood vessels, make vessel walls more permeable and prevent blood coagulation.
- As a consequence, they facilitate the access of other lymphocytes and of plasma-borne substances of importance for the immune response (e.g. antibodies) to e.g. a site of infection.
- The release of the contents of the granules of basophils is receptor-mediated.

- Antibodies produced by plasma cells (activated B-lymphocytes) bind to Fc-receptors on the plasma membrane of basophils.
- If these antibodies come into contact with their antigens, they induce the release of the contents of the basophil granules.

Non-granular leukocytes Agranulocytes

- Agranulocytes – lymphocytes and monocytes:
  - Lack visible cytoplasmic granules
  - Are similar structurally, but are functionally distinct and unrelated cell types
  - Have spherical (lymphocytes) or kidney-shaped (monocytes) nuclei
- Monocytes
-
- Monocytes account for 4–8% of leukocytes
  - They are the largest leukocytes
  - They have abundant pale-blue cytoplasms
  - They have purple-staining, U- or kidney-shaped nuclei
  - They leave the circulation, enter tissue, and differentiate into macrophages



- These cells can be slightly larger than granulocytes (about 12-18 μm in diameter).
- Their cytoplasm stains usually somewhat stronger than that of granulocytes, but it does not contain any structures which would be visible in the light microscope using most traditional stains (a few very fine bluish gains may be visible in some monocytes).
- The "textbook" monocyte has a C-shaped nucleus.
- Monocytes contain granules (visible in the EM) which in appearance and content correspond to the primary granules of neutrophils, i.e. the granules correspond to lysosomes.

Functions

- Once monocytes enter the connective tissue they differentiate into macrophages.

- At sites of infection macrophages are the dominant cell type after the death of the invading neutrophils.
- The phagocytose microorganisms, tissue debris and the dead neutrophils. Monocytes also give rise to osteoclasts, which are able to dissolve bone.
- They are of importance in bone remodelling.

Lymphocytes

- Account for 25% or more of WBCs and:
  - Have large, dark-purple, circular nuclei with a thin rim of blue cytoplasm
  - Are found mostly enmeshed in lymphoid tissue (some circulate in the blood)
- There are two types of lymphocytes: T cells and B cells
  - T cells function in the immune response
  - B cells give rise to plasma cells, which produce antibodies

- These cells are very variable in size.
- The smallest may be smaller than erythrocytes (down to ~5 µm in diameter) while the largest may reach the size of large granulocytes (up to 15 µm in diameter).
- How much cytoplasm is discernible depends very much on the size of the lymphocyte.
- In small ones, which are the majority of lymphocytes in the blood, the nucleus may appear to fill the entire cell. Large lymphocytes have a wider rim of cytoplasm which surrounds the nucleus.
- Both the nucleus and the cytoplasm stain blue (and darker than most other cell types in the blood).
- The typical lymphocyte only contains the usual complement of cellular organelles.
- The appearance of lymphocytes may change drastically when they are activated (see below).

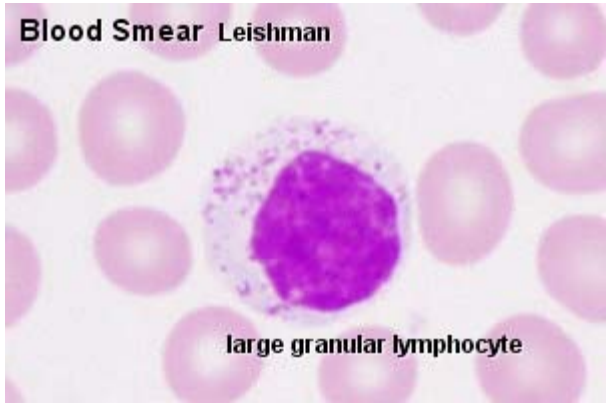
Functions

- Most lymphocytes in the blood stream belong to either the group of B-lymphocytes (~5%) or the group of T-lymphocytes (~90%).
- Unless they become activated, the two groups can not easily be distinguished using routine light or electron microscopy.

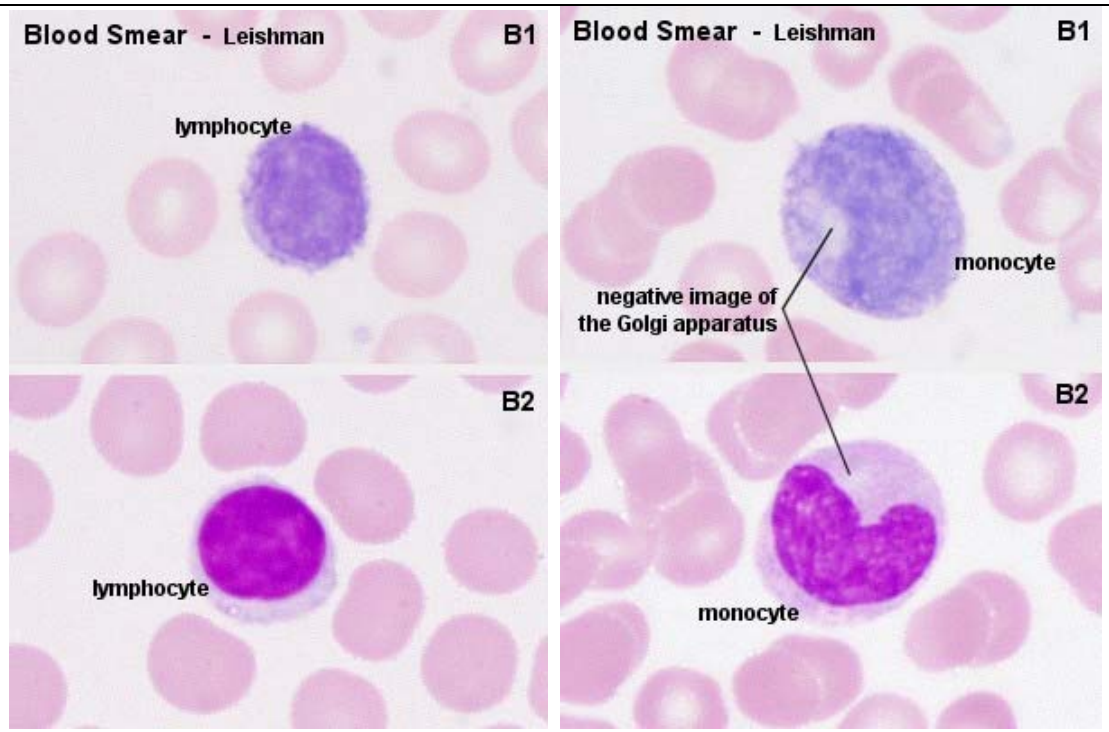
- Upon exposure to antigens by antigen-presenting cells (e.g. macrophages) and T-helper cells (one special group of T-lymphocytes) B-lymphocytes differentiate into antibody producing plasma cells.
- The amount of cytoplasm increases and RER fills a large portion of the cytoplama of plama cells .
- T-lymphocytes represent the "cellular arm" of the immune response (cytotoxic T cells) and may attack foreign cells, cancer cells and cells infected by e.g. a virus.

T-lymphocytes and B-lymphocytes form the vast majority of lymphocytes in the blood stream, but they do not add up to 100%, and they usually are small lymphocytes. The much less frequent medium-sized or large lymphocytes may represent e.g.

- natural killer (Nk-) cells which belong to the group of large granular lymphocytes, or
- hemopoietic stem cells of which a few will be circulating in the blood stream.



- ***Monocytes and lymphocytes definitely look much prettier in darker stained smears (B2) than in lighter ones (B1) - mainly because of a clearer distinction between cytoplasm and nucleus.***
- ***The cell is very likely to be a lymphocyte if the nucleus is round and surrounded by a narrow rim of cytoplasm. The C-shaped nucleus of a textbook monocyte may not be easy to find.***
- ***The nuclei will vary from a peanut- to a "fat" S-shape in smears. Whatever the shape of the nucleus, it is usually not lobed, and it is bound, at least on its concave side, by a wide rim of non-granular cytoplasm. Note also the light area of monocyte cytoplasm which is often visible close to the concave surface of the nucleus.***
- ***The Golgi apparatus is located in the area.***
- ***The Golgi apparatus does not stain as well as the remainder of the cytoplasm and leaves a light "impression" - the phenomenon is also called a "negative image".***



- http://www.lab.anhb.uwa.edu.au/mb140/corepages/blood/blood.htm#labrbc

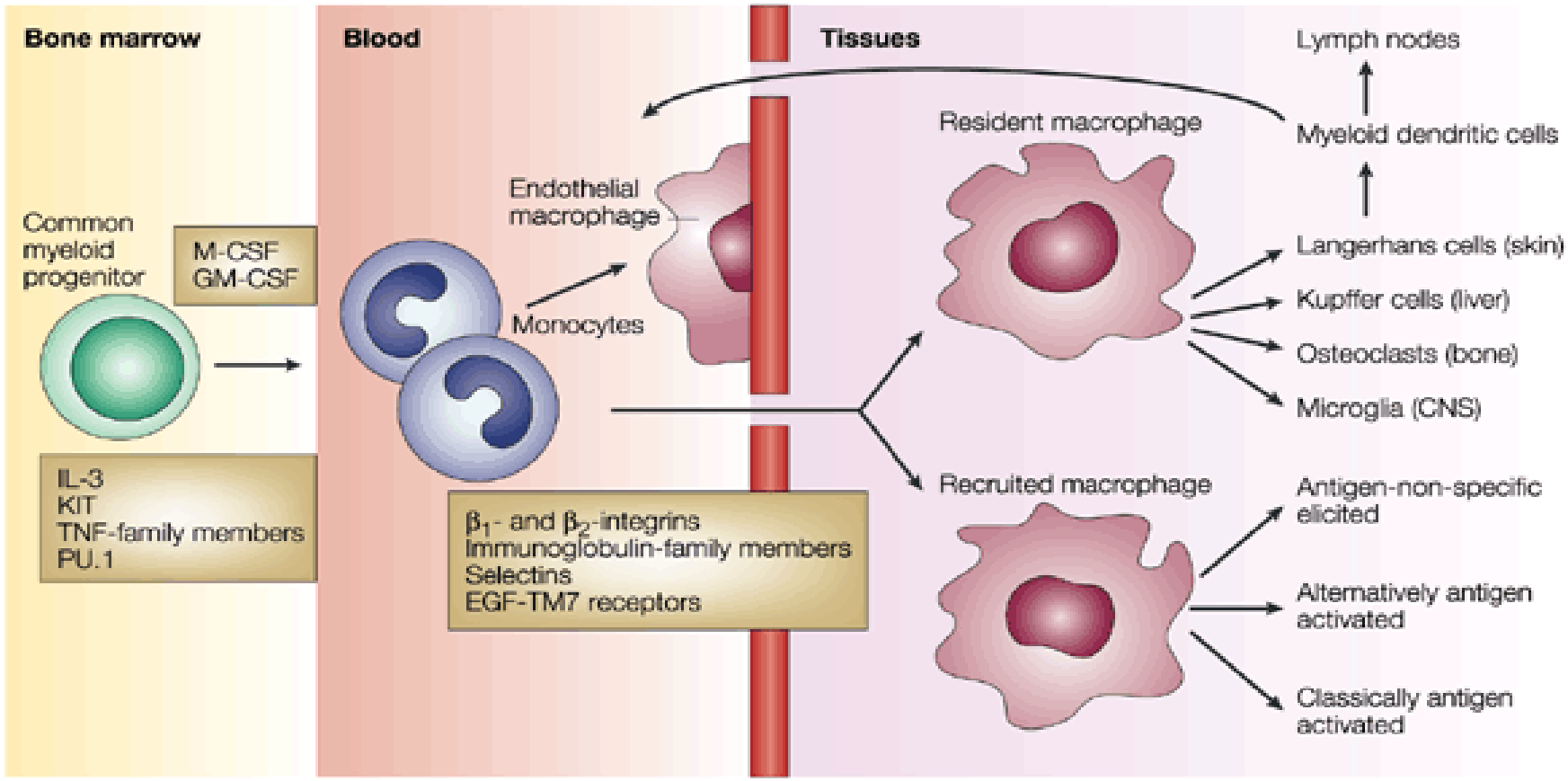
Macrophages

- Are highly mobile and actively phagocytic
- Activate lymphocytes to mount an immune response

Normal macrophages **include macrophages located in tissues that include:**

- **connective tissue –histiocytes**
- **liver sinusoids – Kupffer's cells**
- **lung – alveolar macrophages**
- **lymph nodes – free and fixed macrophages**
- **spleen – free and fixed macrophages**
- **bone marrow – fixed macrophages**
- **serous fluids –pleural and peritoneal macrophages**
- **skin – histiocytes, Langerhans's cell**


An activated macrophage phagocytosing bacteria upon contact
Photo: courtesy of Dennis Kunkel


Bone marrow
Blood
Tissues
Lymph nodes
Myeloid dendritic cells
Resident macrophage
Endothelial macrophage
Common myeloid progenitor
M-CSF
GM-CSF
Monocytes
Langerhans cells (skin)
Kupffer cells (liver)
Osteoclasts (bone)
Microglia (CNS)
IL-3
KIT
TNF-family members
PU.1
β1- and β2-integrins
Immunoglobulin-family members
Selectins
EGF-TM7 receptors
Recruited macrophage
Antigen-non-specific elicited
Alternatively antigen activated
Classically antigen activated

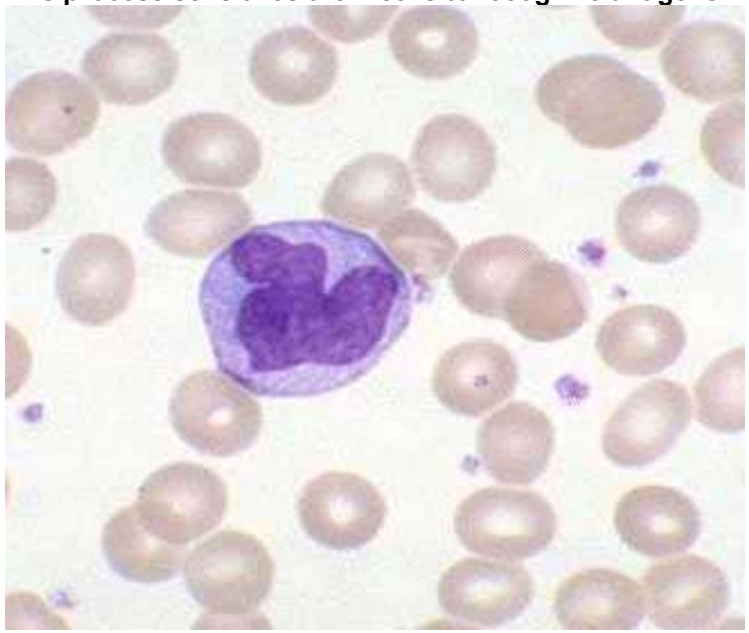
Macrophages trigger acquired immunity **by capturing foreign (exogenous) antigens, which they ingest in cellular lysosomes.**

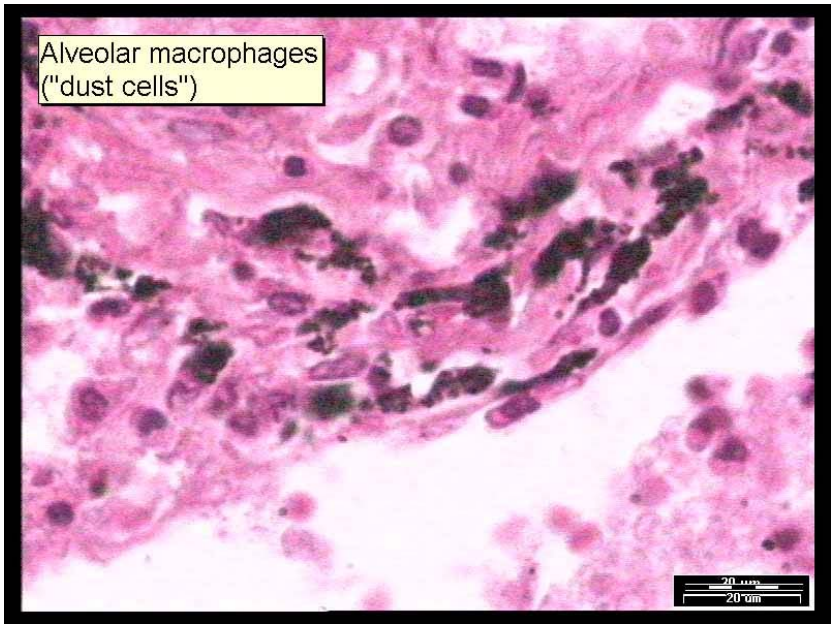
Post-hydrolysis, the fragmented antigens are displayed on the cell surface together with macrophage proteins (APC).

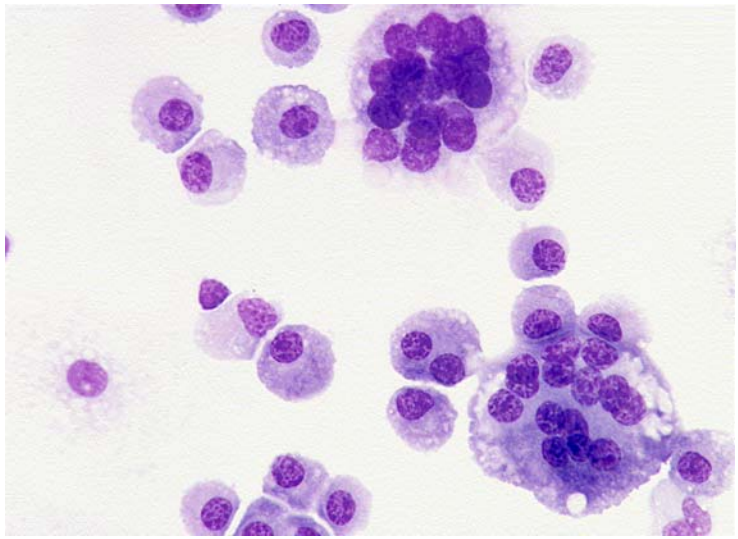
A number of C-type lectins are specifically expressed on macrophages and dendritic cells.

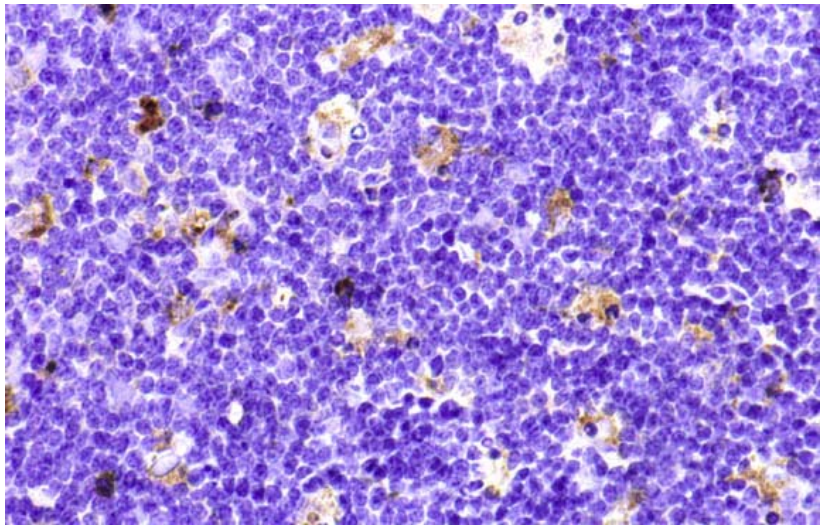
Coated with fragments of foreign antigens, macrophages migrate to secondary lymphoid organs, where they present the antigens to T lymphocytes.

This process sensitizes the T cells to recognize antigens.

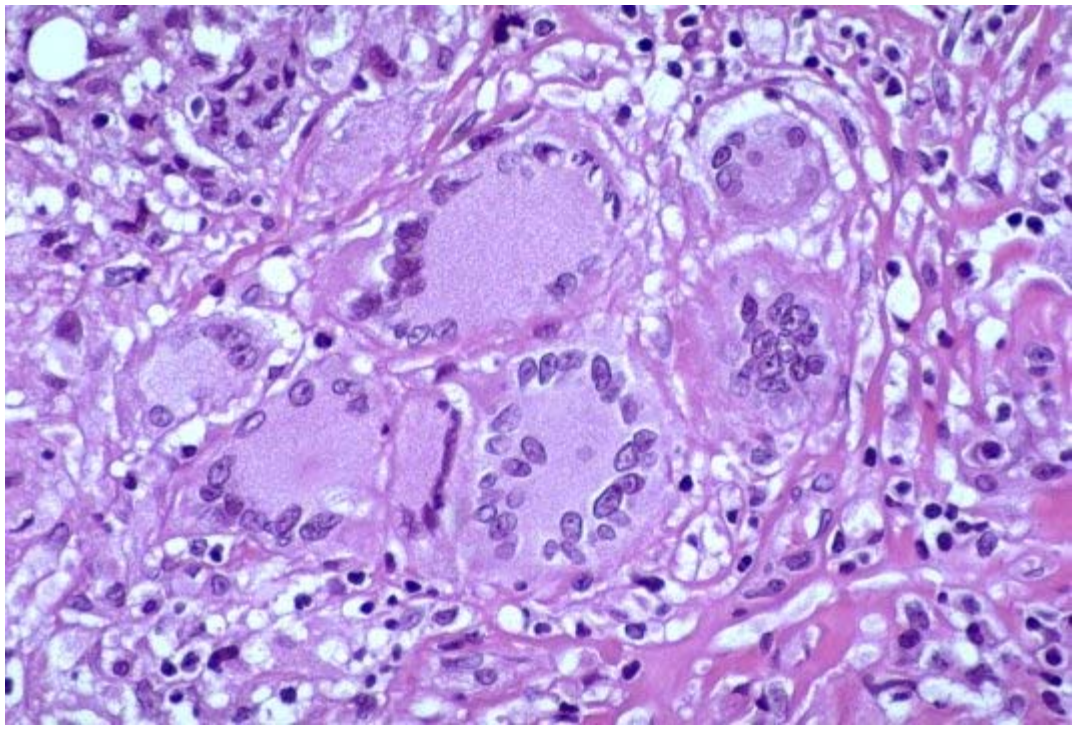
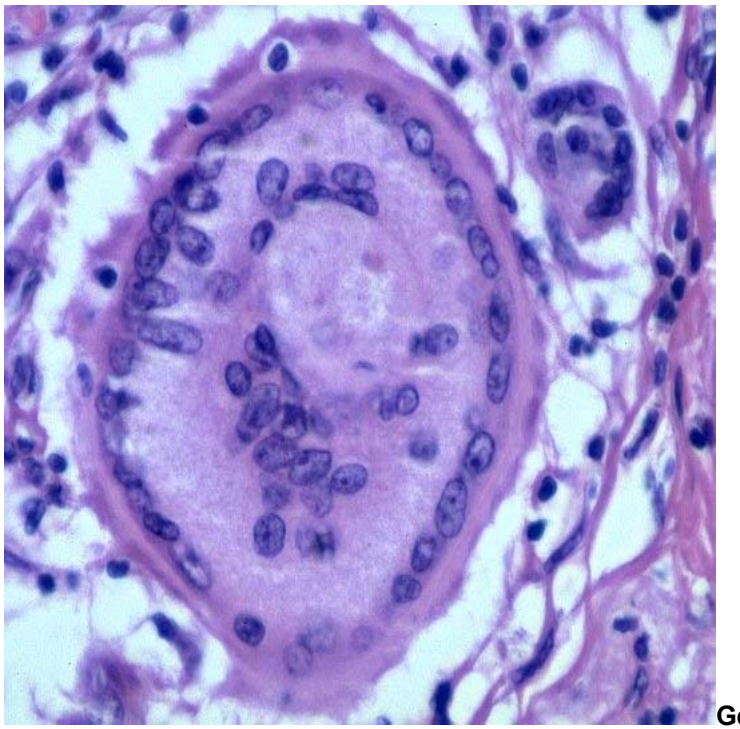








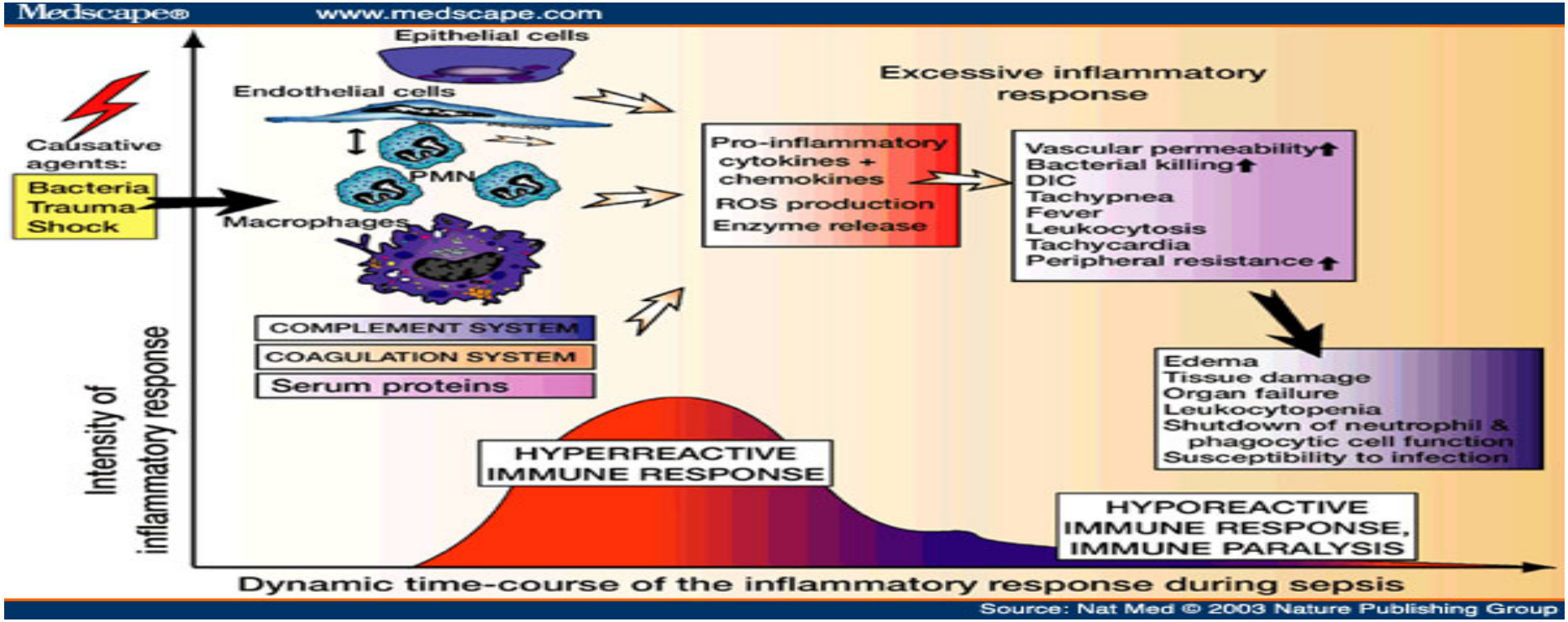
- **Macrophages are ubiquitously distributed mononuclear phagocytes responsible for numerous homeostatic, immunological, and inflammatory processes.**
- **Their wide tissue distribution enables them to provide an immediate defence against foreign elements *prior* to leukocyte immigration.**
- **Macrophages participate in both specific immunity via antigen presentation and in IL-1 production and nonspecific immunity against bacterial, viral, fungal, and neoplastic pathogens, so macrophages display a range of functional and morphological phenotypes.**
- **The** life-span **of macrophages ranges from 6 to 16 days.**
- **Under normal, steady-state conditions, tissue macrophages are renewed by local proliferation of progenitor cells rather than by monocyte influx into tissue, though invagination of monocytes does occur.[http://cellular-immunity.blogspot.com/2007/12/macrophages.html]**

Geant cells [http://granuloma.homestead.com/giant_cell_S98-40211-03.jpg]

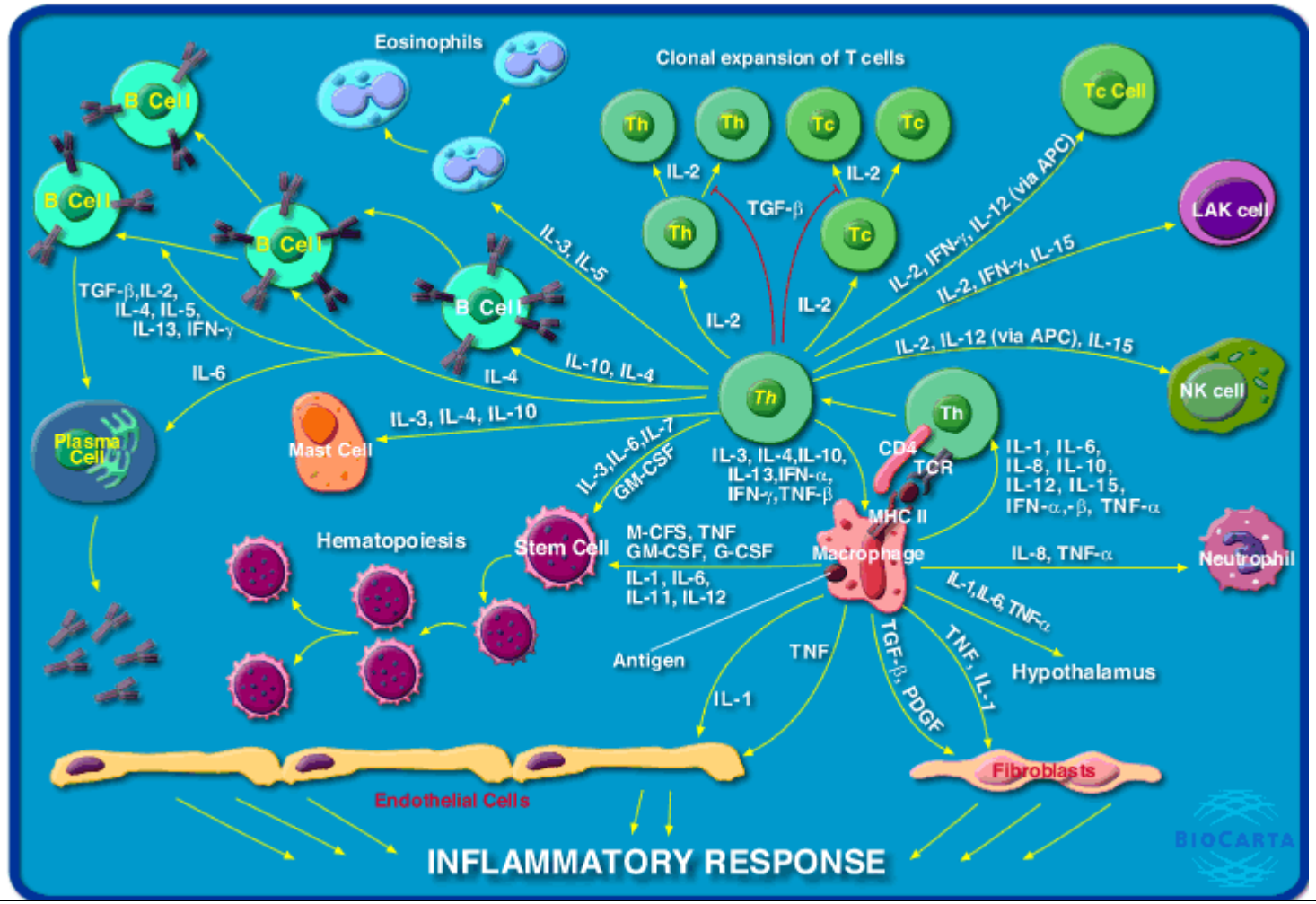
Production of Leukocytes

- Leukopoiesis is stimulated by interleukins and colony-stimulating factors (CSFs)
  - Interleukins are numbered (e.g., IL-1, IL-2), whereas CSFs are named for the WBCs they stimulate (e.g., granulocyte-CSF stimulates granulocytes)
- Macrophages and T cells are the most important sources of cytokines
- Many hematopoietic hormones are used clinically to stimulate bone marrow



Formation of Leukocytes

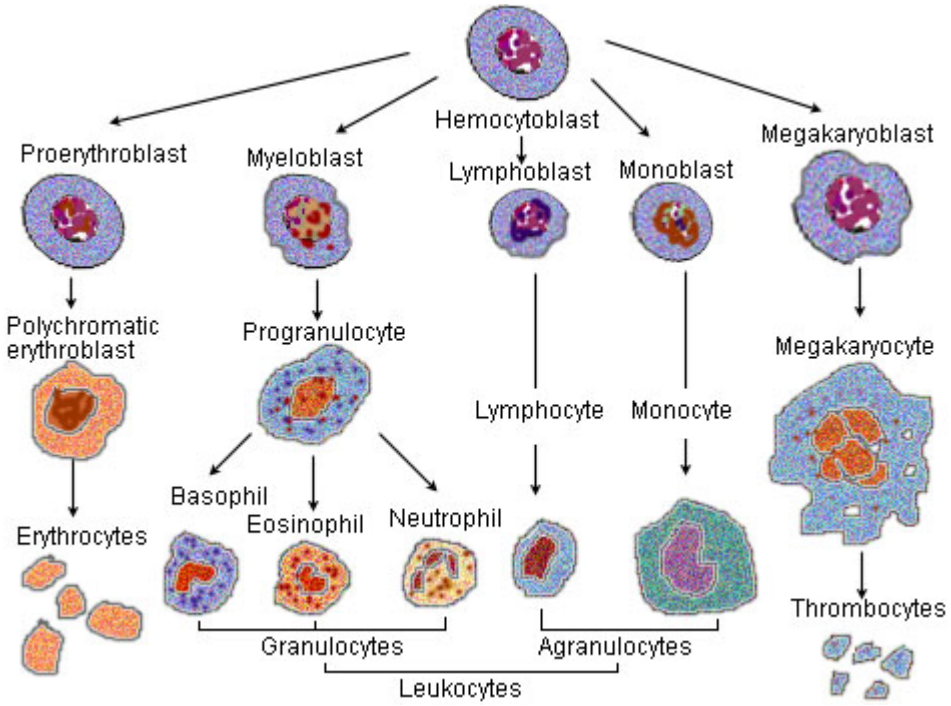
- All leukocytes originate from hemocytoblasts
- Hemocytoblasts differentiate into myeloid stem cells and lymphoid stem cells
- Myeloid stem cells become myeloblasts or monoblasts
- Lymphoid stem cells become lymphoblasts
- Myeloblasts develop into eosinophils, neutrophils, and basophils
- Monoblasts develop into monocytes
- Lymphoblasts develop into lymphocytes

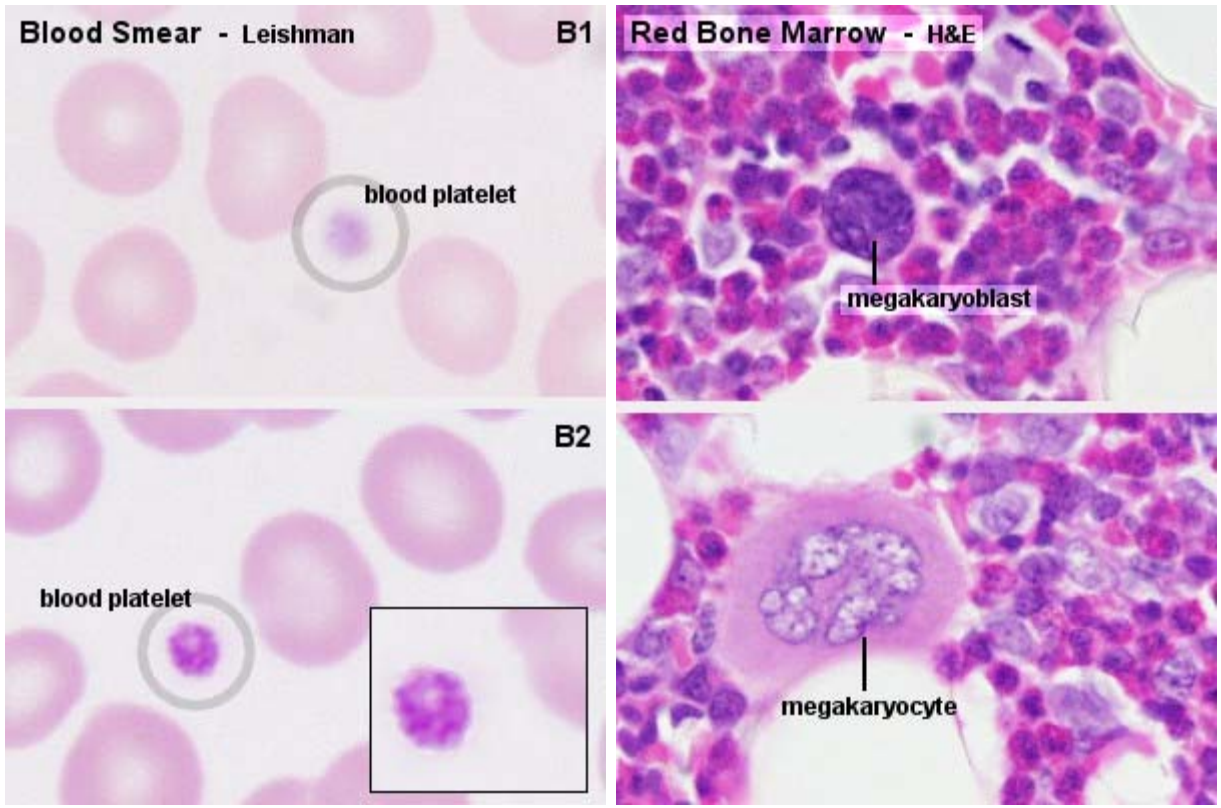


Blood Platelets (or thrombocytes)

- **Platelets**, or **thrombocytes**, are small cytoplasmic bodies derived from cells.
- They circulate in the blood of mammals and are involved in hemostasis leading to the formation of blood clots.
- Like red blood cells, platelets have no nucleus.
- If the number of platelets is too low, excessive bleeding can occur; however, if the number of platelets is too high, blood clots can form (thrombosis) which block blood vessels, and may cause a stroke and/or a heart attack.
- An abnormality or disease of the platelets is called a **thrombocytopathy** which could be either a low number (thrombocytopenia), a decrease in function (**thrombasthenia**) or an increase in number (**thrombocytosis**)
- Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes.
- The physiological range for platelets is 150-400 x 10^9 per litre.

- Around 1 x 10^{11} platelets are produced each day by an average adult.
- The lifespan of circulating platelets is 7-10 days.
- This process is regulated by thrombopoietin, a hormone usually produced by the liver and kidney.
- Each megakaryocyte produces between 5,000 and 10,000 platelets.
- Old platelets are destroyed by the spleen and by Kupffer cells in the liver.





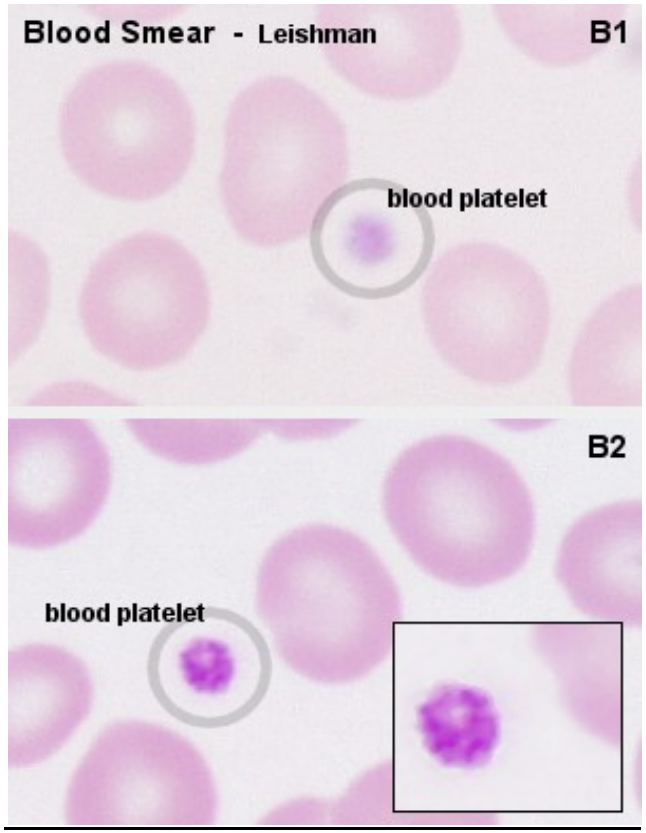
- Platelets are fragments of megakaryocytes with a blue-staining outer region and a purple granular center
- Their granules contain serotonin, Ca^{2+}, enzymes, ADP, and platelet-derived growth factor (PDGF)
- Platelets function in the clotting mechanism by forming a temporary plug that helps seal breaks in blood vessels
- Platelets not involved in clotting are kept inactive by NO and prostacyclin

- Blood platelets do not contain a nucleus.
- Unlike erythrocytes, which also lack a nucleus, the blood platelets of mammals have never been nucleated cells. Instead, blood platelets are fragments of the cytoplasm of very large thrombocyte precursor cells, megakaryocytes.
- Like other cells involved in the formation in blood cells, megakaryocytes are found in the bone marrow.
- Platelets are about 3 µm long but appear somewhat smaller in the microscope.
- This is because their cytoplasm is divided into two zones: and outer hyalomere, which hardly stains, and an inner granulomere, which contains bluish staining granules.
- These granules are usually not individually visible with the highest magnification on your microscope, and the granulomere appears more or less homogeneously blue.
- In addition to different types of vesicles (i.e. the granules), mitochondria, ribosomes, lysosomes and a little ER are present in the thrombocyte granulomere.

- Different types of vesicles contain either serotonin (electron-dense delta granules; few) or compounds important for blood coagulation (alpha granules - they also contain platelet-derived growth factor (PDGF) which may play a role in the repair of damaged tissue).
- The hyalomere contains cytoskeletal fibres, which include actin and myosin.

Cytoplasm includes active proteins such as:

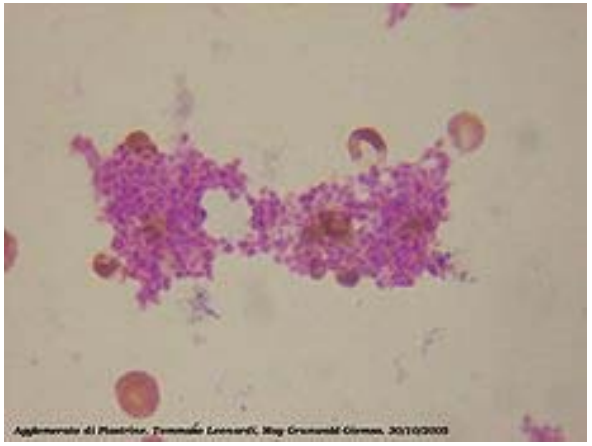
- **Actin.**
- **Myosin.**
- **Thrombesthenin.**



Functions

- Platelets assist in hemostasis, the arrest of bleeding.

- Serotonin is a potent vasoconstrictor.
- The release of serotonin from thrombocytes, which adhere to the walls of a damaged vessels, is sufficient to close even small arteries.
- Platelets, which come into contact with collagenous fibers in the walls of the vessel (which are not usually exposed to the blood stream), swell, become "sticky" and activate other platelets to undergo the same transformation.
- This cascade of events results in the formation of a platelet plug (or platelet thrombus). Finally, activating substances are released from the damaged vessel walls and from the platelets.
- These substances mediate the conversion of the plasma protein prothrombin into thrombin.
- Thrombin catalyzes the conversion of fibrinogen into fibrin, which polymerizes into fibrils and forms a fibrous net in the arising blood clot.
- Platelets captured in the fibrin net contract leading to clot retraction, which further assists in hemostasis.
- Blood coagulation is a fairly complex process, which involves a large number of other proteins and messenger substances.
- Deficiencies in any one of them, either inherited or acquired, will lead to an impairment of hemostasis.[see hemostasis coagulation lecture]



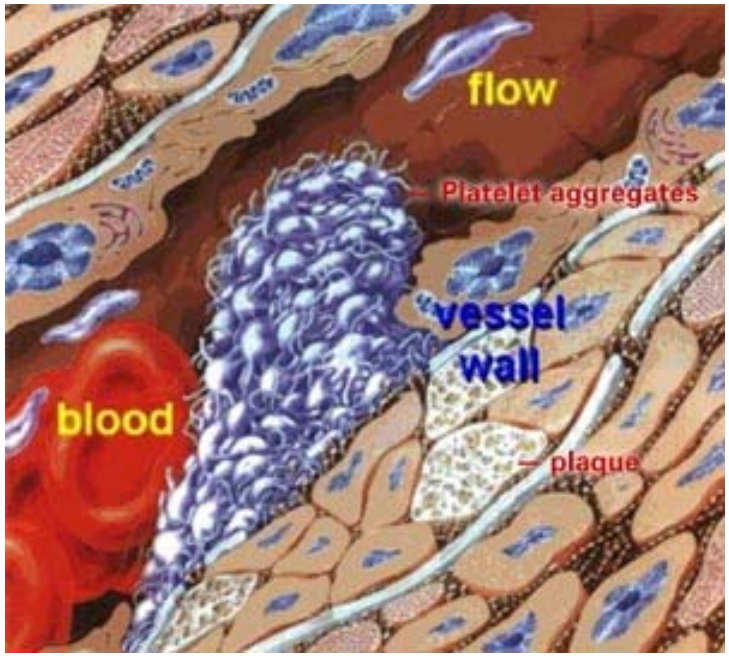
Thrombus formation

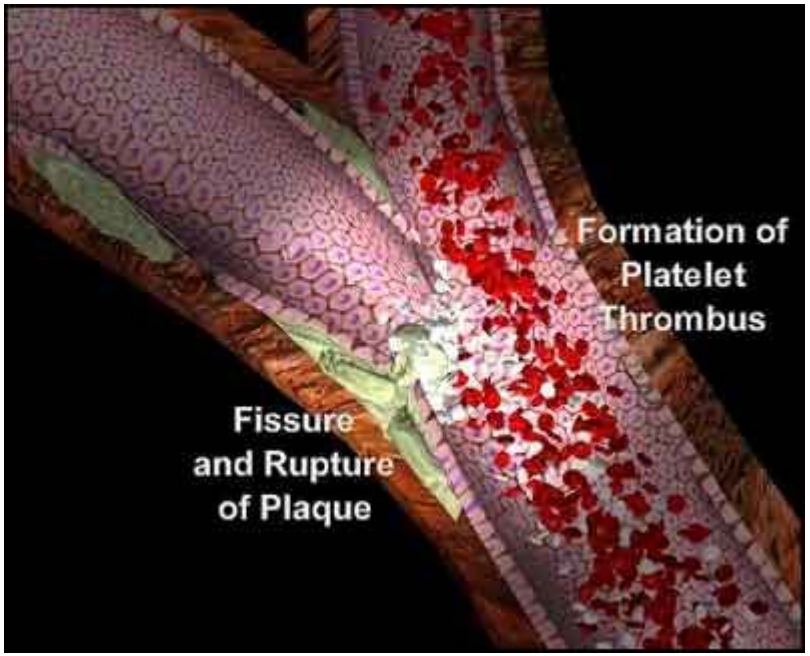
The function of platelets is the maintenance of hemostasis. Primarily, this is achieved by the formation of thrombi, when damage to the endothelium of blood vessels occurs. Conversely, thrombus formation must be inhibited at times when there is no damage to the endothelium.

Activation

- The inner surface of blood vessels is lined with a thin layer of endothelial cells, that in normal hemostasis, acts to inhibit platelet activation with the production of endothelial-ADPase, noradrenaline, and PGI_2. Endothelial-ADPase clears away ADP, a platelet activator, from platelet surface receptors.
- Endothelial cells produce a protein called von Willebrand's factor, a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen does not pass into the bloodstream; however vWF is secreted constitutively into the plasma by the endothelial cells that produce it, or otherwise is stored within the endothelial cell or in platelets.

- When endothelial damage occurs, platelets come into contact with exposed collagen and vWF, and the inhibitors the endothelium normally secretes are reduced.
- The inner surface of blood vessels is lined with a thin layer of endothelial cells. Under this is a layer of collagen. When the endothelial layer is injured, the collagen is exposed.
- When the platelets contact collagen, they are activated. They are also activated by thrombin (primarily through PAR-1), ADP receptors (P2Y1 and P2Y12) expressed on platelets. They can also be activated by a negatively charged surface, such as glass.
- Platelet activation further results in the scramblase-mediated transport of negatively charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase and prothrombinase complexes.





Shape Change

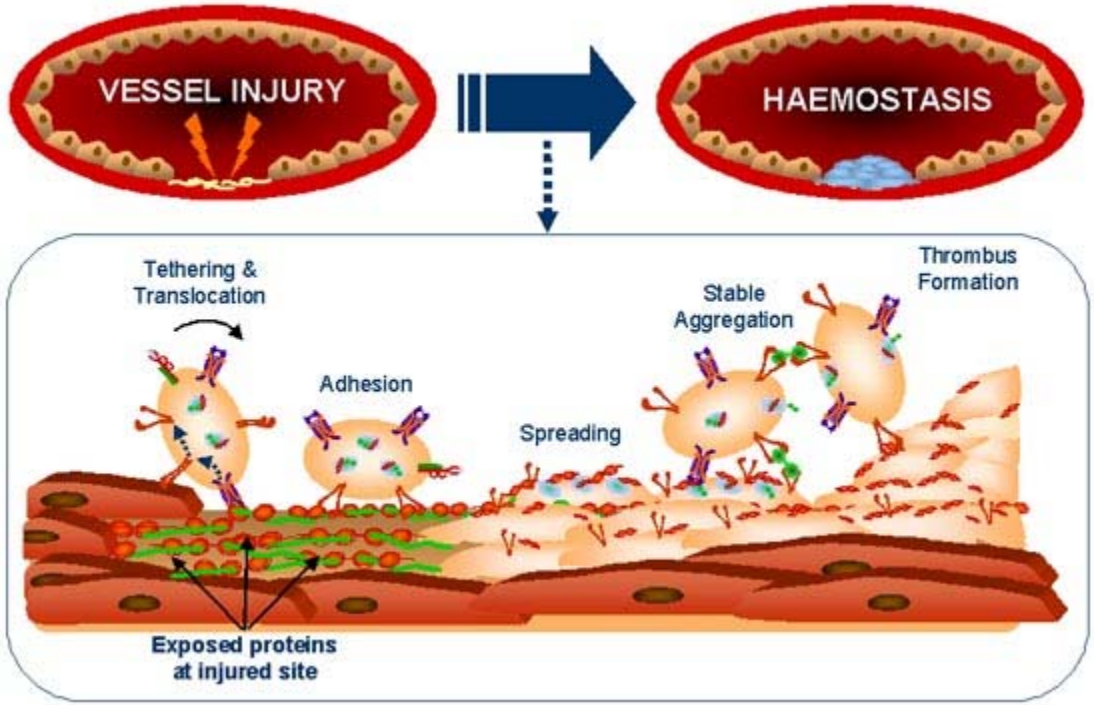
- Activated platelets change in shape to become more spherical, and pseudopods form on their surface.

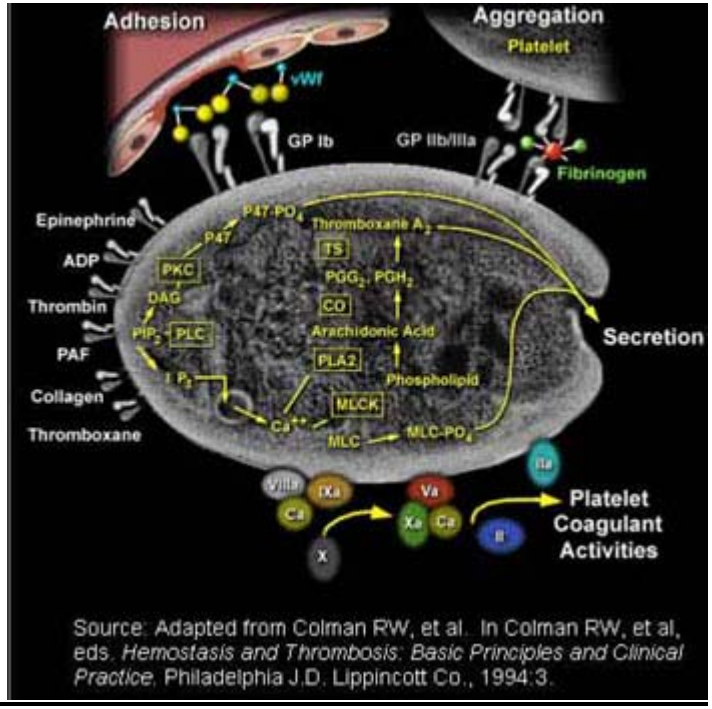
Granule Secretion

- Platelets contain alpha and dense granules.
- Activated platelets excrete the contents of these granules into their canalicular systems and into surrounding blood.

There are two types of granules:

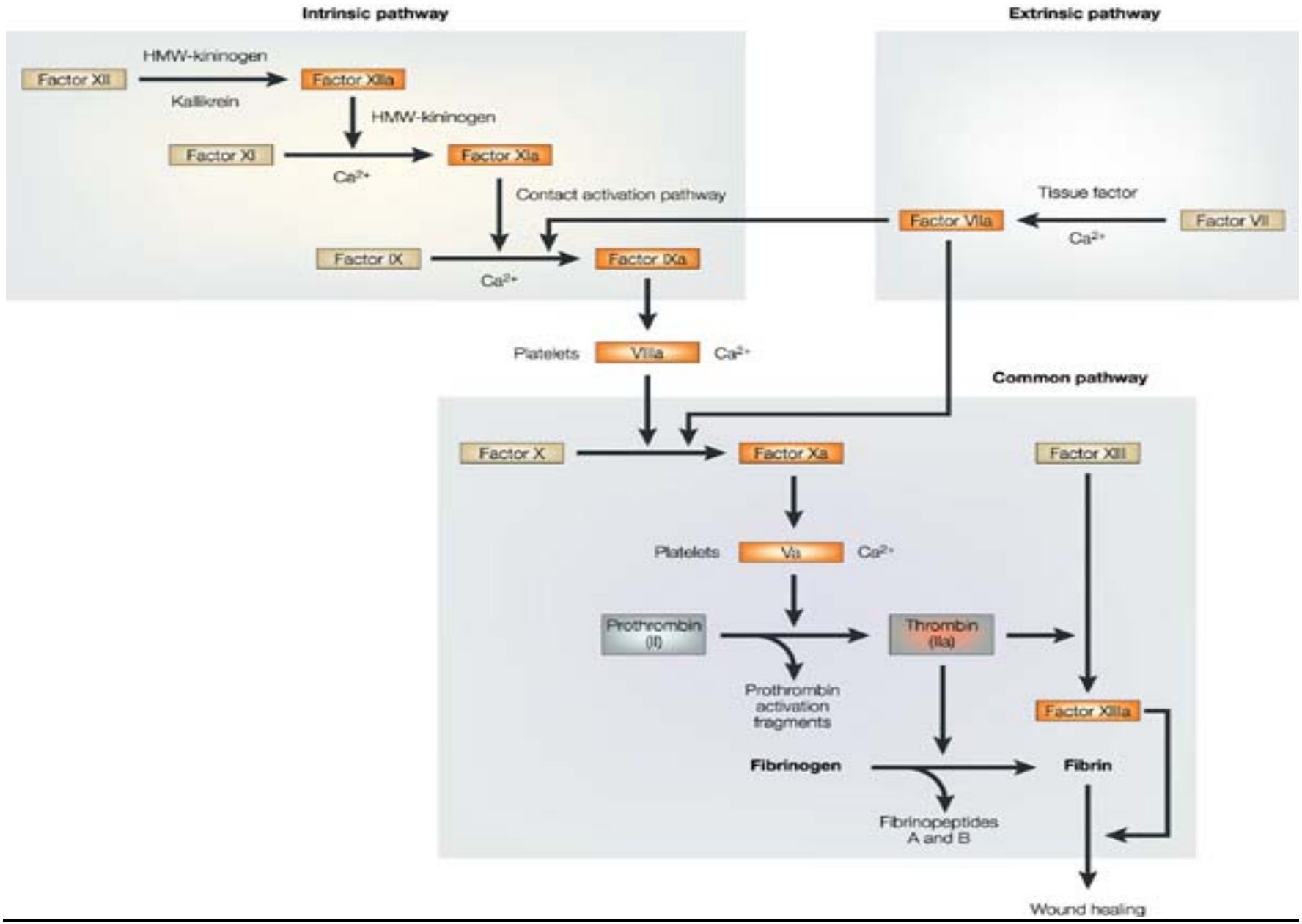
- dense granules (containing ADP or ATP, calcium and serotonin)
- α-granules (containing platelet factor 4, PDGF, fibronectin, B-thromboglobulin, vWF, fibrinogen, and coagulation factors V and XIII).





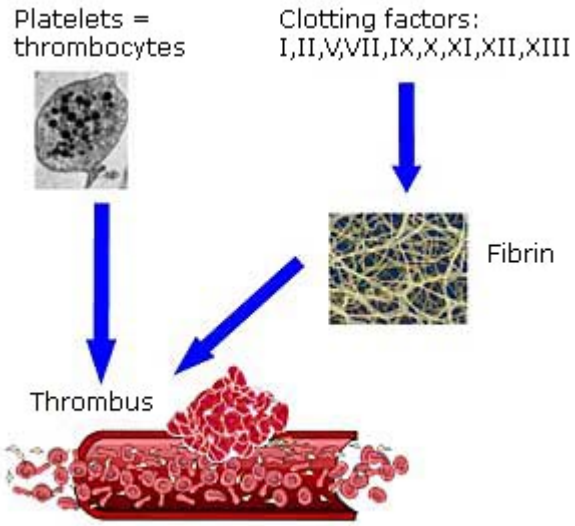
Thromboxane A2 Synthesisis

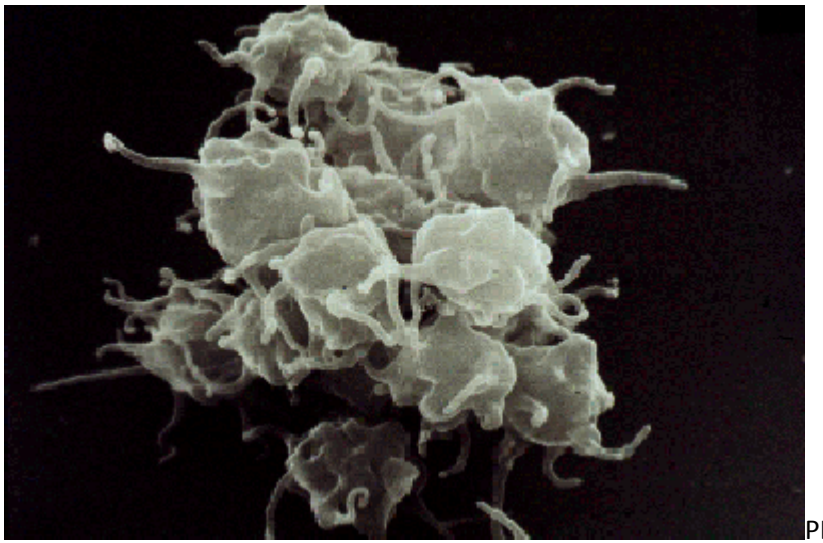
Platelet activation initiates the arachidonic acid pathway to produce TXA_2. TXA_2 is involved in activating other platelets.



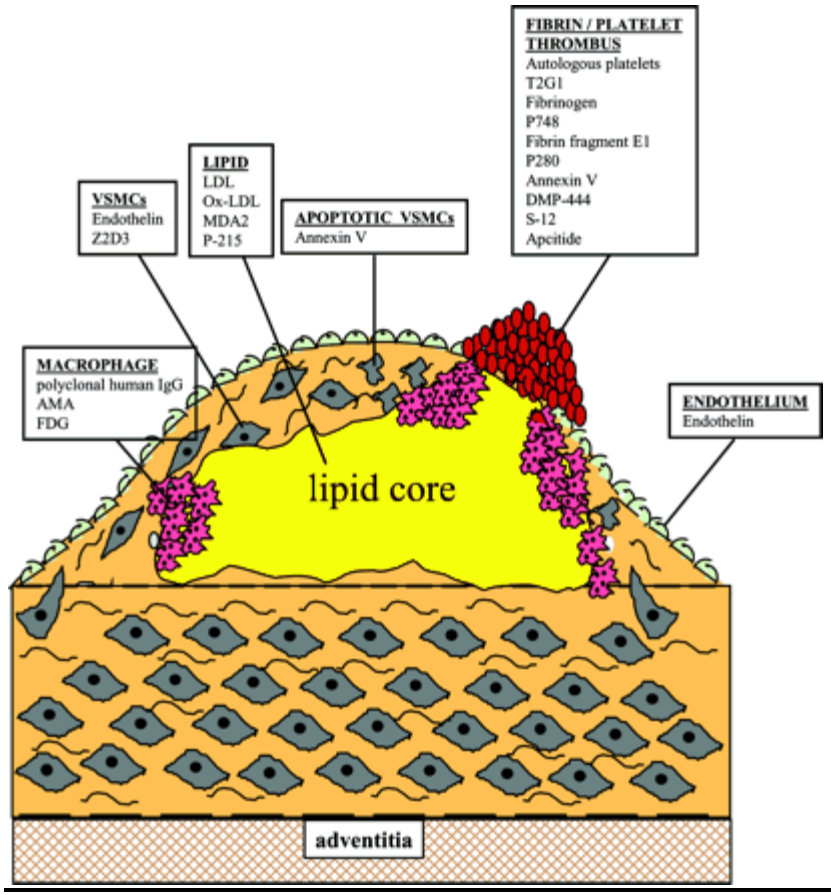
Adhesion and aggregation

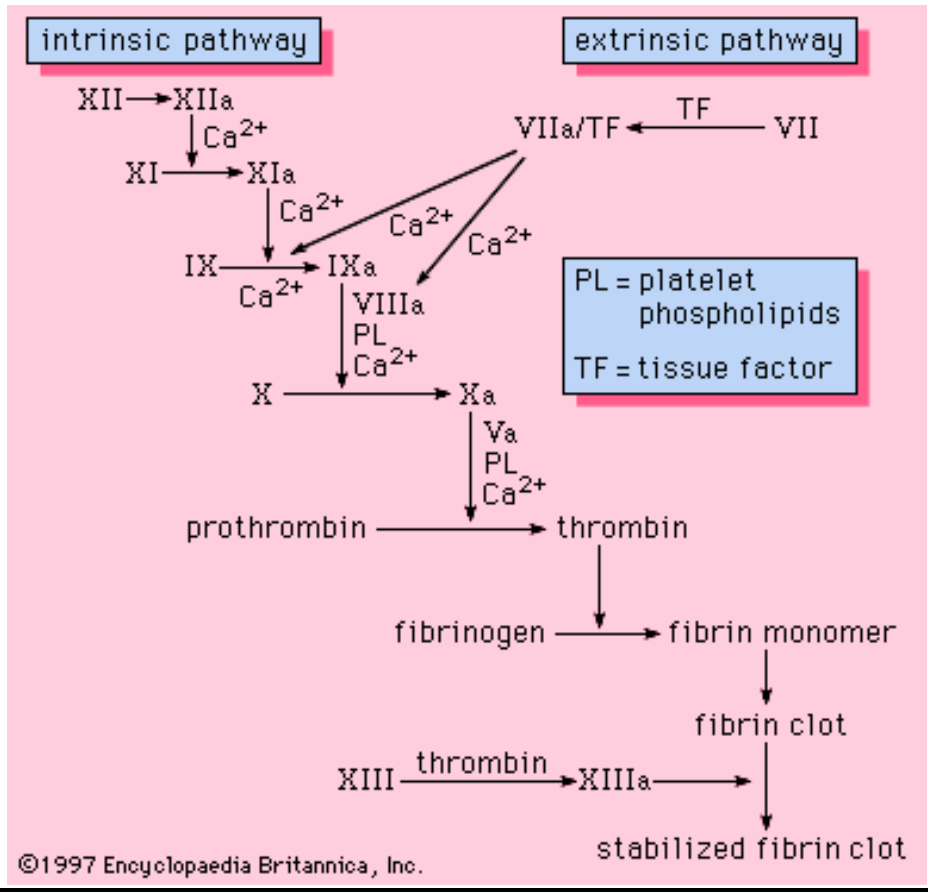
- Platelets aggregate, or clump together, using fibrinogen of vWF as a connecting agent.
- The most abundant platelet aggregation receptor is glycoprotein (GP) IIb/IIIa; this is a calcium-dependent receptor for
- :
  - fibrinogen,
  - fibronectin,
  - vitronectin,
  - thrombospondin
  - and von Willebrand factor (vWF).
  - Other receptors include GPIb-V-IX complex (vWF) and GPVI (collagen).




PLATELETS ACTIVATED

- Activated platelets will adhere, via glycoprotein (GP) Ia, to the collagen that is exposed by endothelial damage
- Aggregation and adhesion act together to form the platelet plug.
- The high concentration of myosin and actin filaments in platelets are stimulated to contract during aggregation, further reinforcing the plug.
- Platelet aggregation is stimulated by ADP, thromboxane and α2 receptor-activation, but inhibited by other inflammatory products like PGI2 and PGD2. Platelet aggregation is enhanced by exogenous administration of anabolic steroids.In a double blind study in young males, testosterone cypionate increased human platelet TXA_2 receptor density and the aggregation responses.
- This is consistent with clinical observations that young adults (both athletes and non-athletes) who abuse steroids, there is increased incidence of premature myocardial infarction, pulmonary embolism and thrombotic stroke





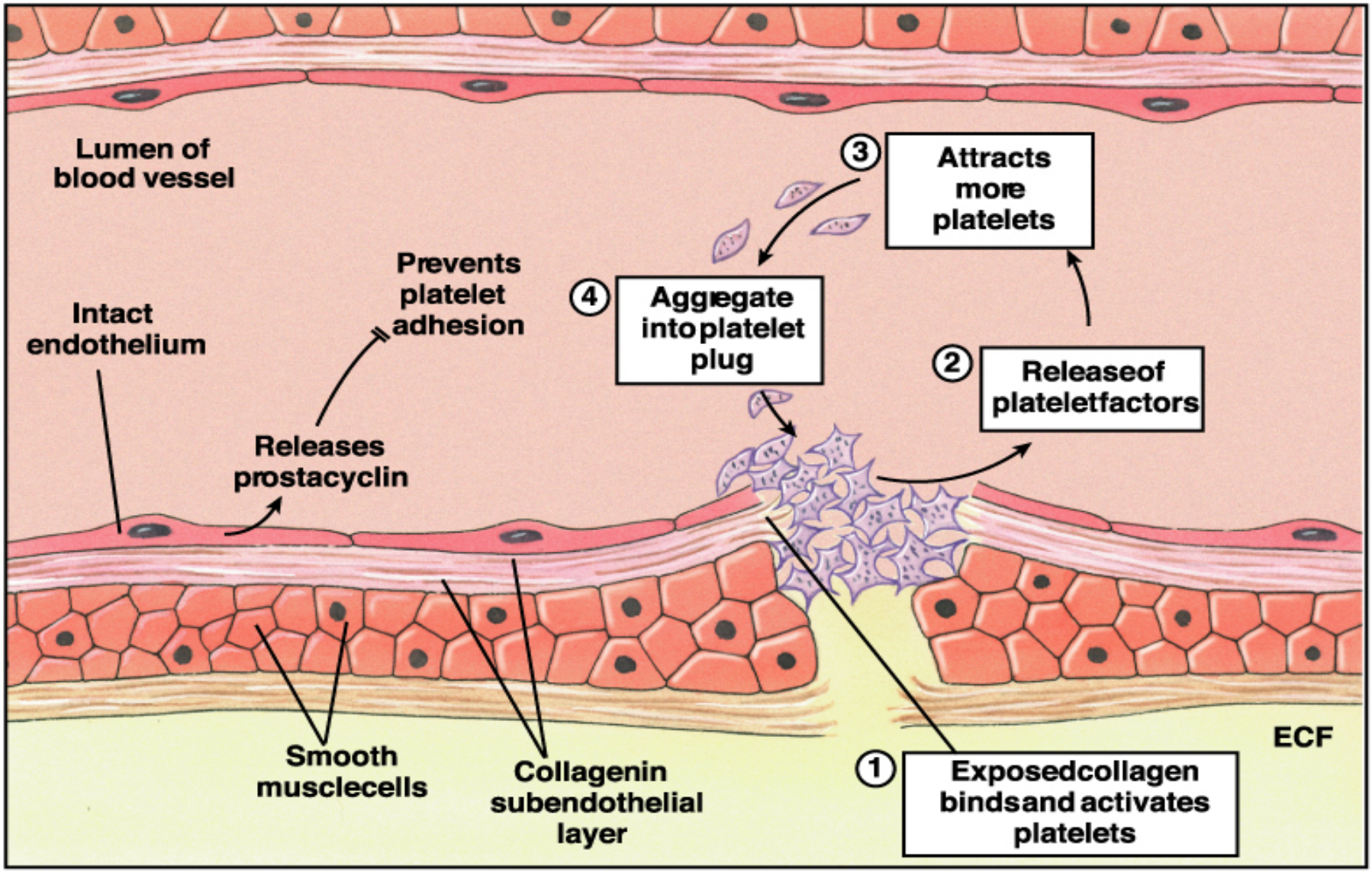
Other functions

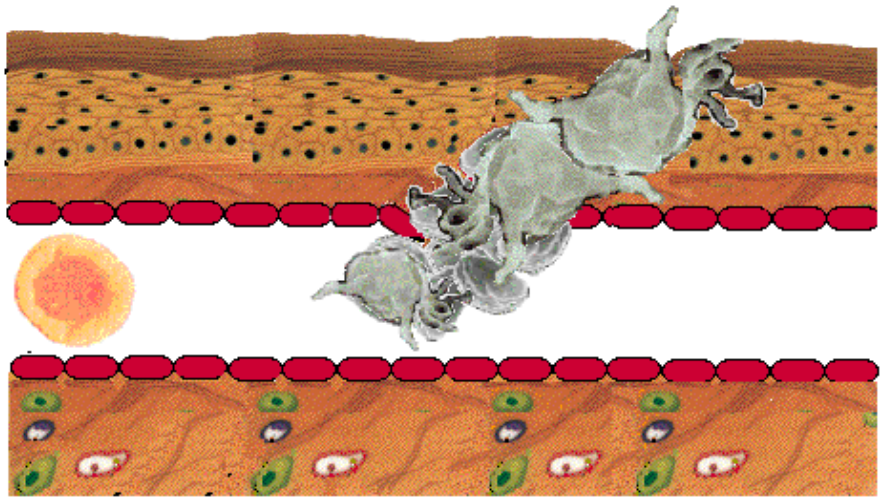
- Clot retraction
- Pro-coagulation
- Cytokine signalling
- Phagocytosis

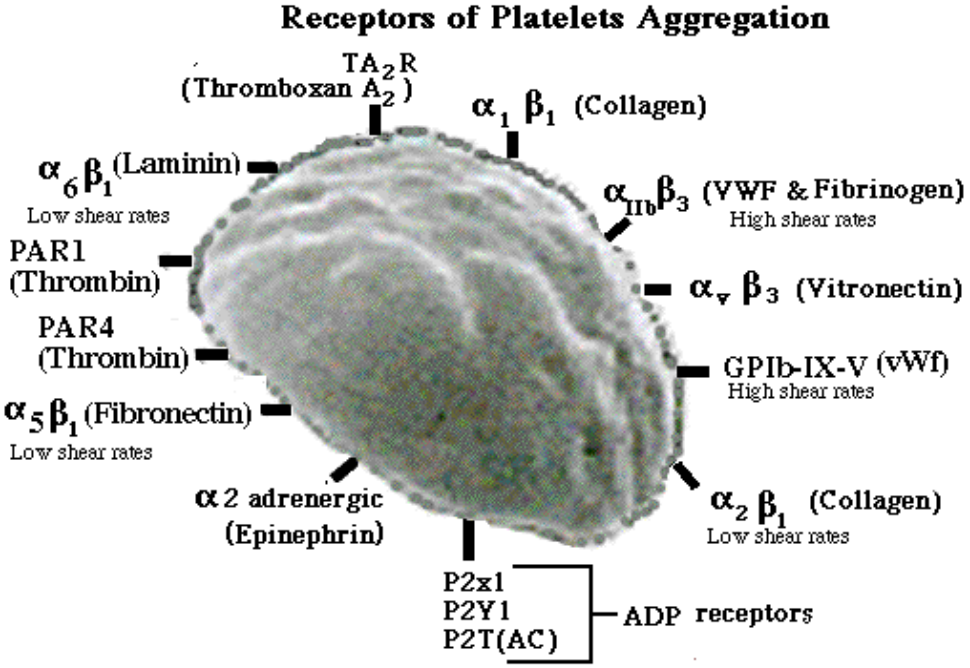
Cytokine signalling

Besides being the chief cellular effector of hemostasis, platelets are rapidly deployed to sites of injury or infection and potentially modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines and other inflammatory mediators

It also secretes e.g. platelet-derived growth factor (PDGF).


Lumen of blood vessel
3
Attracts more platelets
Prevents platelet adhesion
4
Aggregate into platelet plug
Intact endothelium
2
Releaseof plateletfactors
Releases prostacyclin
ECF
Smooth musclecells
Collagenin subendothelial layer
1
Exposedcollagen bindsand activates platelets




Receptors of Platelets Aggregation
TA2R
(Thromboxan A2)
α1 β1 (Collagen)
α6 β1 (Laminin)
Low shear rates
αIIb β3 (VWF & Fibrinogen)
High shear rates
PAR1
(Thrombin)
αv β3 (Vitronectin)
PAR4
(Thrombin)
GPIb-IX-V (vWf)
High shear rates
α5 β1 (Fibronectin)
Low shear rates
α2 adrenergic
(Epinephrin)
α2 β1 (Collagen)
Low shear rates
P2x1
P2Y1
P2T(AC)
ADP receptors

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