Physiology

Dr. Muna H. AL-Saeed

Haematology

- **Blood:-** is a specialized bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume) and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion.

Vertebrate blood is bright red when its hemoglobin is oxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some mollusks use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen.

Vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have hemocytes as part of their immune system.

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with ***hemo-*** or ***hemato-*** (also spelled ***haemo-*** and ***haemato-***) from the Greek word αἷμα (*haima*) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective

tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

▪ Function of blood:-

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature
- Hydraulic functions

▪ Constituents of blood:-

Blood accounts for 7% of the body weight with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3. The average adult has a blood volume of roughly 5 liters (1.3 gal), which is composed of plasma and several kinds of cells. These blood cells (which are also called *corpuscles* or "**formed elements**") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Whole blood (plasma and cells) exhibits non-Newtonian fluid dynamics; its flow properties are adapted to flow effectively through tiny capillary blood vessels with less resistance than plasma by itself. In addition, if all human hemoglobin were free in the plasma rather than being contained in RBCs, the circulatory fluid would be too viscous for the cardiovascular system to function effectively.

- **Erythrocytes:** Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles in mammals. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%. The combined surface area of all red blood cells of the

human body would be roughly 2,000 times as great as the body's exterior surface.[6]

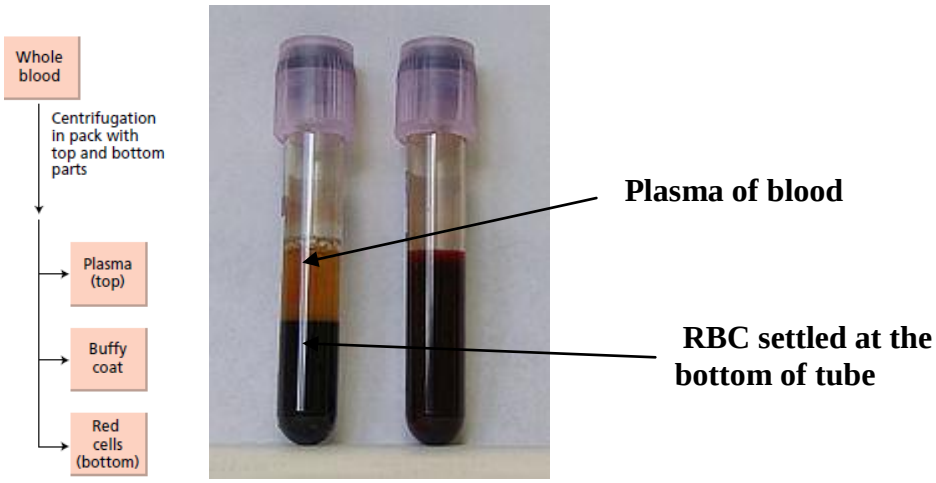
- **Leukocytes:** White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukemia.
- **Thrombocytes:** Also called platelets, thrombocytes are responsible for blood clotting (coagulation). They change fibrinogen into fibrin. This fibrin creates a mesh onto which red blood cells collect and clot, which then stops more blood from leaving the body and also helps to prevent bacteria from entering the body.

- **Plasma:-**

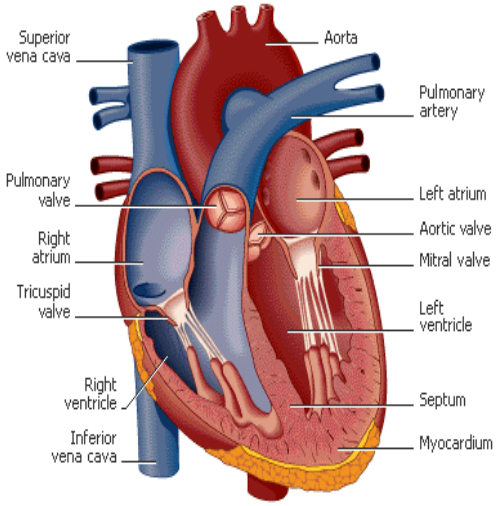
About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid.
Other important components include:

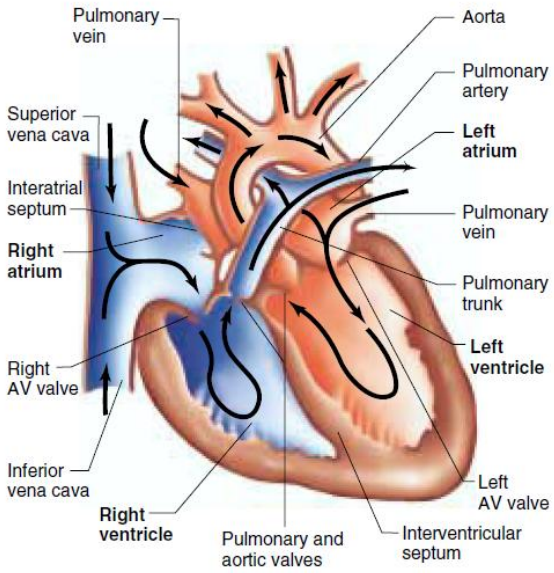
- Serum albumin
- Blood-clotting factors (to facilitate coagulation)
- Immunoglobulins (antibodies)
- lipoprotein particles
- Various other proteins
- Various electrolytes (mainly sodium and chloride)

The term **serum** refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins.



- **Blood** is circulated around the body through blood vessels by the pumping action of the heart. Blood is pumped from the strong left ventricle of the heart through arteries to peripheral tissues and returns to the right atrium of the heart through veins. It then enters the right ventricle and is pumped through the pulmonary artery to the lungs and returns to the left atrium through the pulmonary veins. Blood then enters the left ventricle to be circulated again. Arterial blood carries oxygen from inhaled air to all of the cells of the body, and venous blood carries carbon dioxide, a waste product of metabolism by cells, to the lungs to be exhaled. However, one exception includes pulmonary arteries, which contain the most deoxygenated blood in the body, while the pulmonary veins contain oxygenated blood.
  Additional return flow may be generated by the movement of skeletal muscles, which can compress veins and push blood through the valves in veins toward the right atrium.





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- **Production and degradation of blood cells:-**

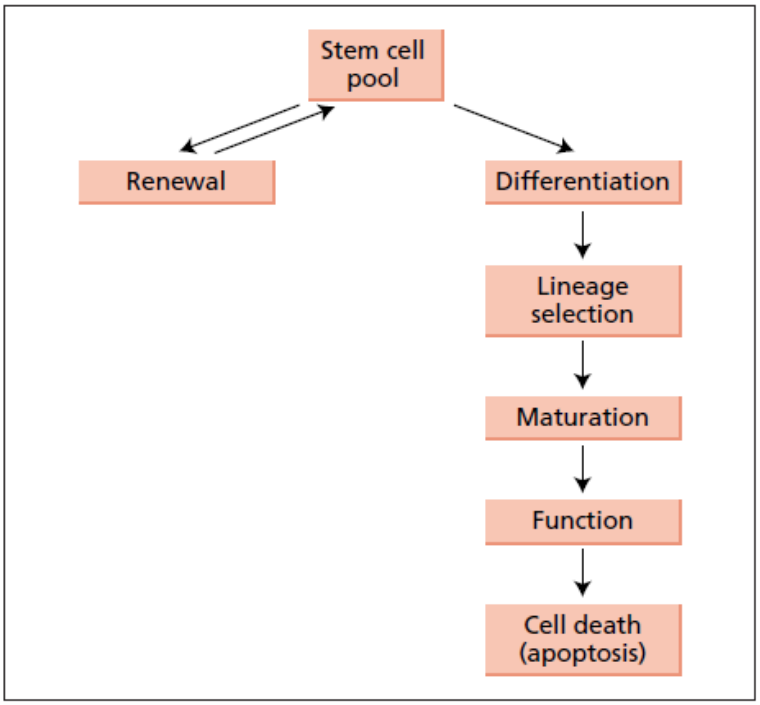
  In vertebrates, the various cells of blood are made in the bone marrow in a process called hematopoiesis, which includes erythropoiesis, the production of red blood cells; and myelopoiesis, the production of white blood cells and platelets.

- **Stem cells and haemopoiesis:-**

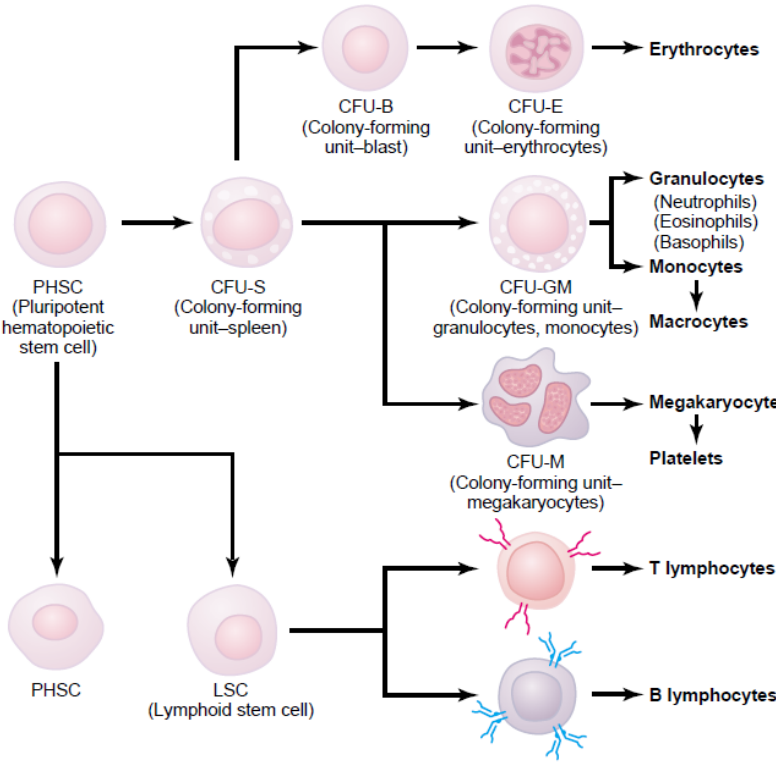
The lifelong production of blood cells occurs in haemopoietic tissue. This involves a very high level of cell turnover, demanded by the need to replace mature circulating blood cells at a rapid rate, and is necessitated by the limited lifespan of the mature cells. Granulocytes survive for only a few hours and erythrocytes for a few months, so that some 10^{13} new cells must be replaced

each day to maintain steady-state blood counts. This is equivalent to an annual number of cells approximating the total body weight, but the total bone marrow of an adult human contains around 10^{12} cells, 10-fold less than daily needs. From these estimates it is clear that the blood cells required for lifelong haemopoiesis cannot be preformed in the body.

The bone marrow, which is the major site of haemopoiesis in adult humans, contains cells that represent the stages in the development of the different types of blood cells.



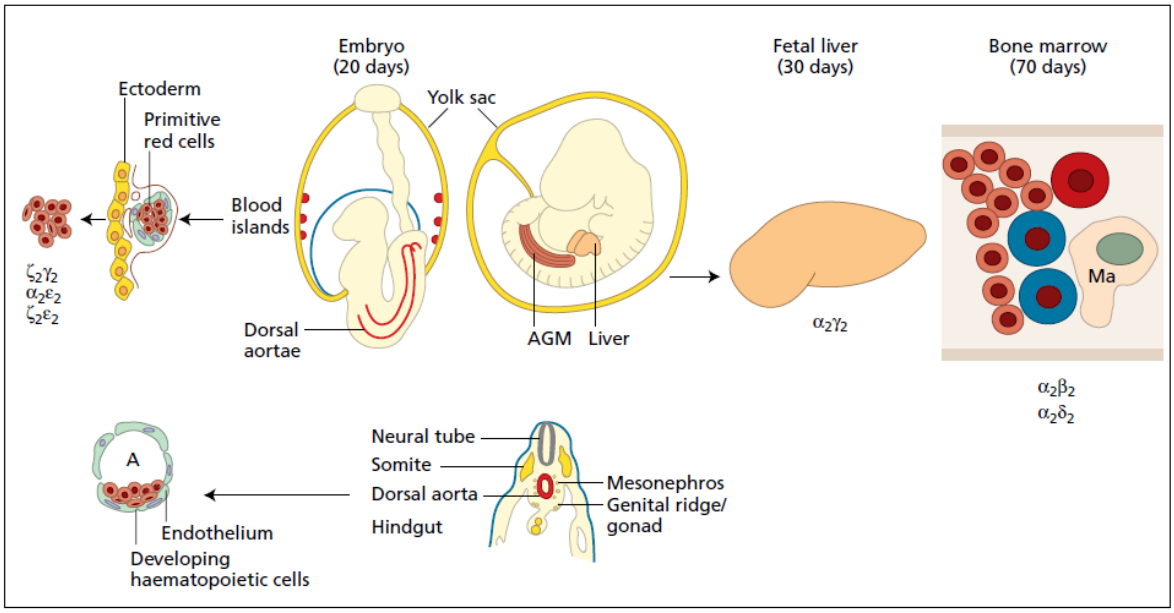
Stages in the haemopoietic cell development.



Erythropoiesis:-

This process includes all steps of haemopoiesis,starting with the initial specification of haemopoietic stem cells (HSCs) from mesoderm during embryogenesis. This continues with the decisions of these cells to undergo self-renewal or differentiation,through the process of lineage specification and proliferation to form committed erythroid progenitors. Finally, erythroblasts undergo terminal differentiation and post-mitotic maturation as they develop into red blood cells.

In a normal adult, the numbers of circulating red blood cells and their precursors remain more or less constant with a balance between the continuous loss of mature cells by senescence andnew red cell production in the marrow.

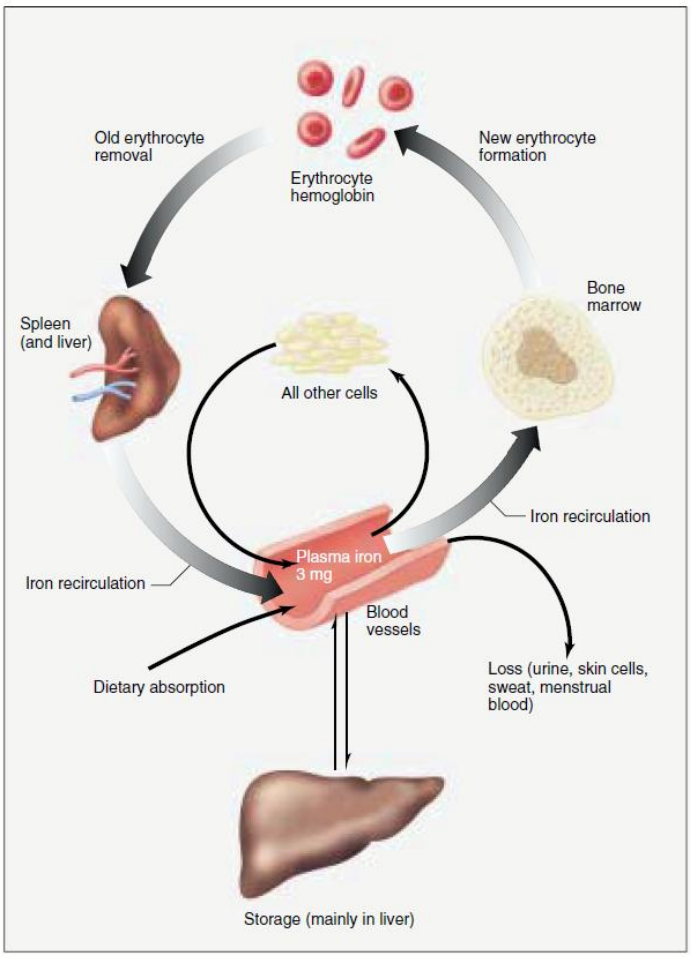


The origin and development of erythropoiesis during embryogenesis

Erythrocytes have the shape of a biconcave disk that is, a disk thicker at the edges than in the middle, like a doughnut with a center depression on each side instead of a hole. This shape and their small size (7 m in diameter) impart to the erythrocytes a high surface-to-volume ratio, so that oxygen and carbon dioxide can diffuse rapidly to and from the interior of the cell. The plasma membrane of erythrocytes contains specific polysaccharides and proteins that differ from person to person, and these confer upon the blood its so-called type, or group. The site of erythrocyte production is the soft interior of bones called **bone marrow,** specifically the "red" bone marrow. With differentiation, the erythrocyte precursors produce hemoglobin but then they ultimately lose their nuclei and organelles—their machinery for protein synthesis. Young erythrocytes in the bone marrow still contain a few ribosomes, which produce a web-like (reticular) appearance when treated with special stains, an appearance that gives these young erythrocytes the name **reticulocyte.** Normally, only

mature erythrocytes, which have lost these ribosomes, leave the bone marrow and enter the general circulation. In the presence of unusually rapid erythrocyte production, however, many reticulocytes do enter the blood, a fact of clinical diagnostic usefulness. Because erythrocytes lack nuclei and organelles, they can neither reproduce themselves nor maintain their normal structure for very long. The average life span of an erythrocyte is approximately 120 days, which means that almost 1 percent of the body's erythrocytes are destroyed and must be replaced every day. This amounts to 250 billion cells per day! Erythrocyte destruction normally occurs in the spleen and the liver. The most of the iron released in the process is conserved. The major breakdown product of hemoglobin is bilirubin, which, as noted above, gives plasma its color.

The production of erythrocytes requires the usual nutrients needed to synthesize any cell: amino acids, lipids, and carbohydrates. In addition, both iron and certain growth factors, including the vitamins folic acid and vitamin B12, are essential.



- **Regulation of Erythrocyte Production:-**

In a normal person, the total volume of circulating erythrocytes remains remarkably constant because of reflexes that regulate the bone marrow's production of these cells. The direct control of erythrocyte production

(erythropoiesis) is exerted primarily by a hormone called **erythropoietin,** which is secreted into the blood mainly by a particular group of hormone-secreting connective-tissue cells in the kidneys (the liver also secretes this hormone, but to a much lesser extent). Erythropoietin acts on the bone marrow to stimulate the proliferation of erythrocyte progenitor cells and their differentiation into mature erythrocytes. Erythropoietin is normally secreted in relatively small amounts, which stimulate the bone marrow to produce erythrocytes at a rate adequate to replace the usual loss. The erythropoietin secretion rate is increased markedly above basal values when there is a decreased oxygen delivery to the kidneys. Situations in which this occurs include insufficient pumping ofblood by the heart, lung disease, anemia (a decrease in number of erythrocytes or in hemoglobin concentration), and exposure to high altitude. As a result of the increase in erythropoietin secretion, plasma erythropoietin concentration, erythrocyte production, and the oxygen-carrying capacity of the blood all increase; therefore, oxygen delivery to the tissues returns toward normal.Testosterone, the male sex hormone, also stimulates the release of erythropoietin. This accounts, at least in part, for the higher hemoglobin concentration in men than in women.

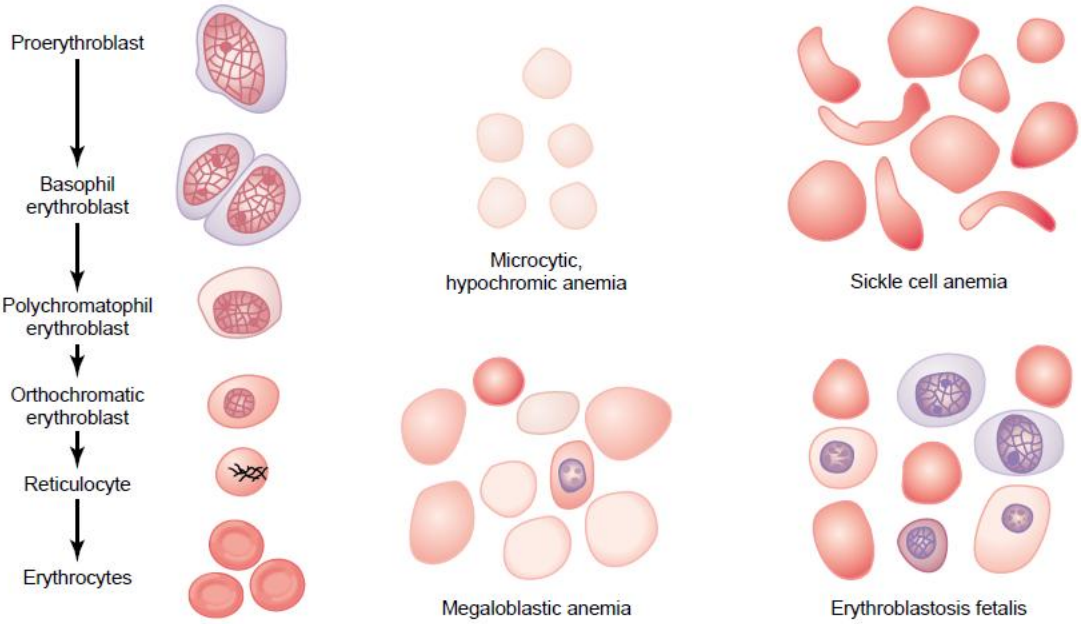
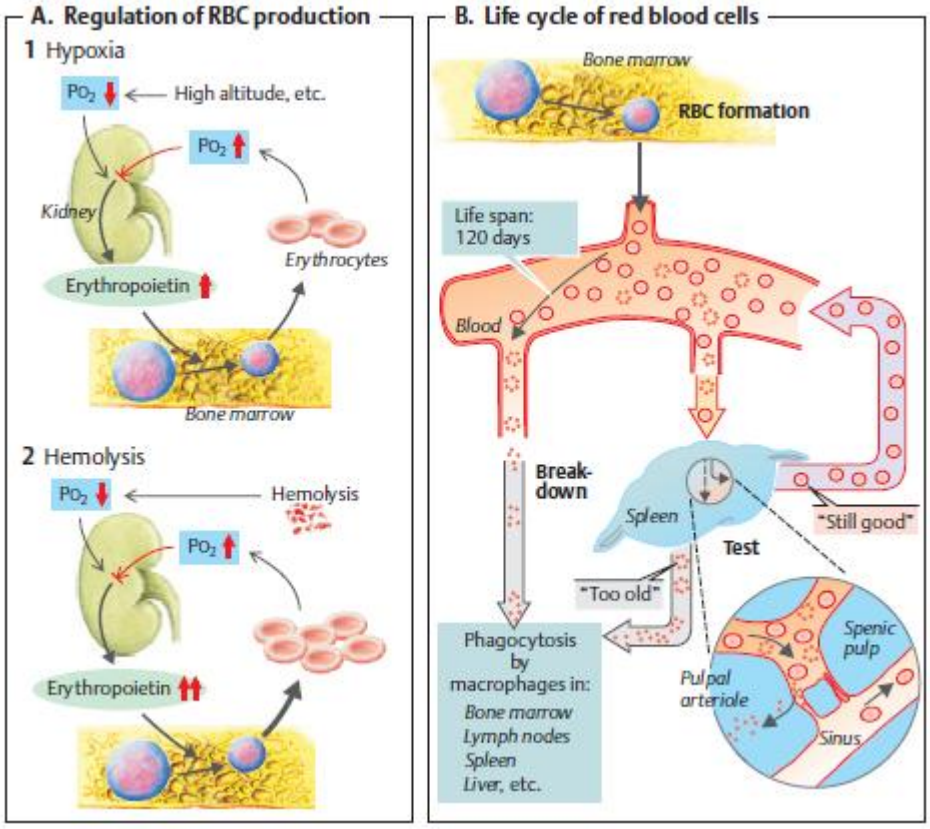
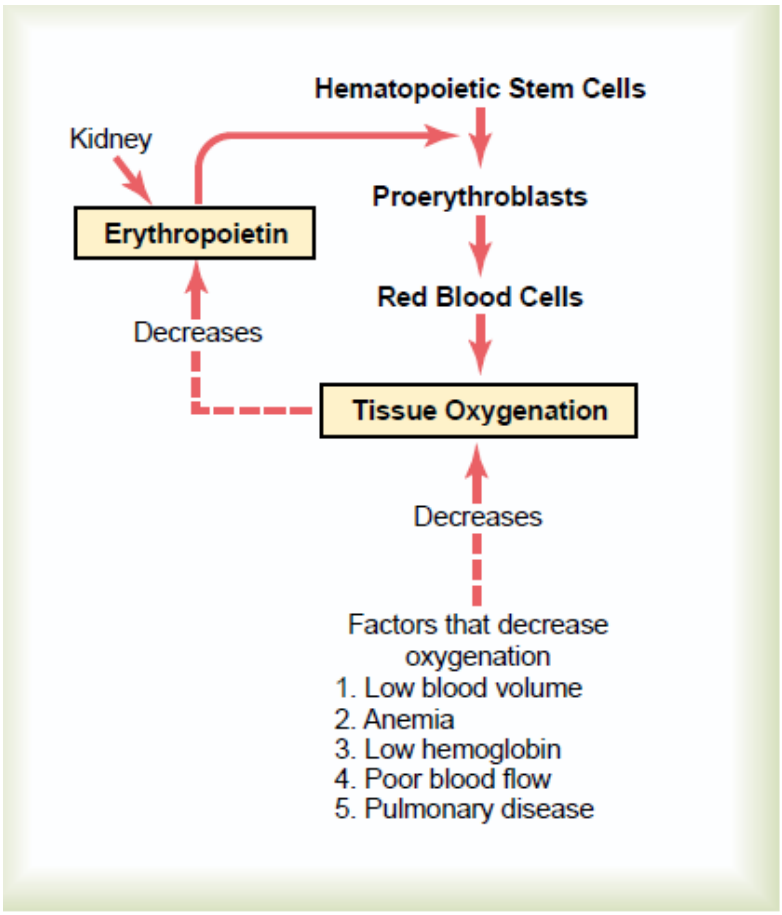
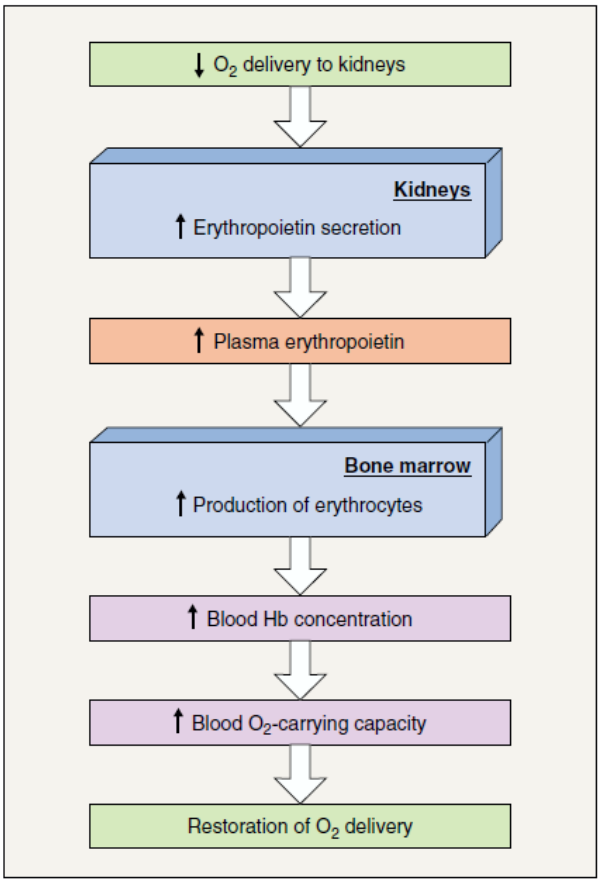


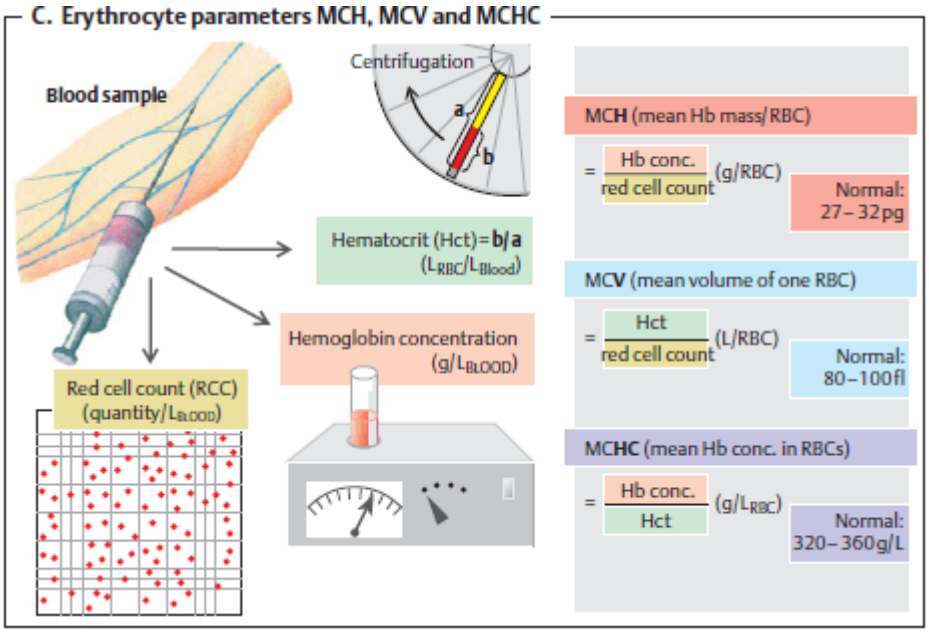
Figure 32–3

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


A. Regulation of RBC production
1 Hypoxia
PO_2
High altitude, etc.
PO_2
Kidney
Erythrocytes
Erythropoietin
Bone marrow
2 Hemolysis
PO_2
Hemolysis
PO_2
Erythropoietin
B. Life cycle of red blood cells
Bone marrow
RBC formation
Life span: 120 days
Blood
Break-down
Spleen
"Still good"
Test
"Too old"
Phagocytosis by macrophages in:
Bone marrow
Lymph nodes
Spleen
Liver, etc.
Spenic pulp
Pulpal arteriole
Sinus


Hematopoietic Stem Cells
Kidney
Erythropoietin
Proerythroblasts
Red Blood Cells
Decreases
Tissue Oxygenation
Decreases
Factors that decrease oxygenation
1. Low blood volume
2. Anemia
3. Low hemoglobin
4. Poor blood flow
5. Pulmonary disease


↓ O_2 delivery to kidneys
Kidneys
↑ Erythropoietin secretion
↑ Plasma erythropoietin
Bone marrow
↑ Production of erythrocytes
↑ Blood Hb concentration
↑ Blood O_2-carrying capacity
Restoration of O_2 delivery



Iron Metabolism and Erythropoiesis

The body's **iron pool** (ca. 2 g in women and 5 g in men) is bound to *hemoglobin* (Hb). About 1/4 exists as *stored iron* (ferritin, hemosiderin),the rest as *functional iron* (myoglobin,iron-containing enzymes). **Iron losses** from the body amount to about 1 mg/day in men and up to 2 mg/day in women due to menstruation, birth, and pregnancy. **Iron absorption** occurs mainly in the duodenum and *varies according to need*. The absorption of iron supplied by the diet usually amounts to about 3 to 15% in healthy individuals, but can increase to over 25% in individuals with iron deficiency. A minimum daily **iron intake** of at least 10–20 mg/day is therefore recommended (women ! children ! men).

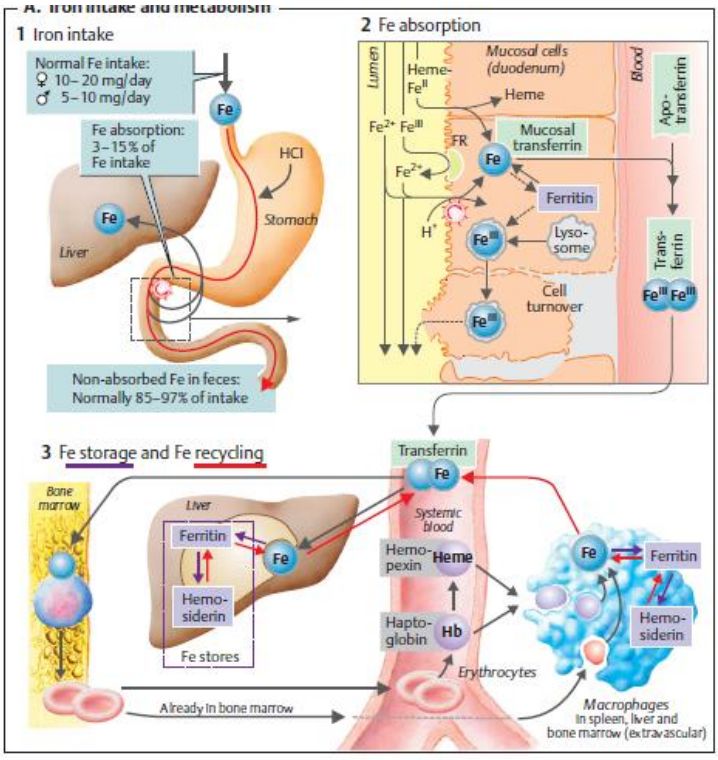
Iron absorption Fe(II) supplied by the diet (hemoglobin,myoglobin found chiefly in meat and fish) is absorbed relatively efficiently as a **heme-Fe(II)** upon protein cleavage.

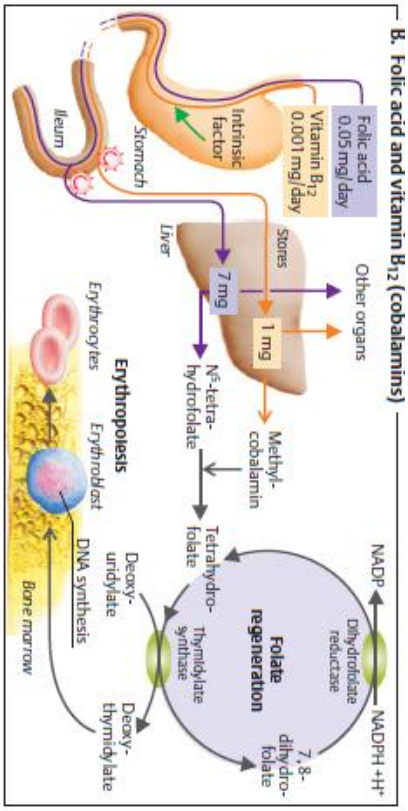
With the aid of *heme oxygenase*, Fe in mucosal cells cleaves from heme and oxidizes to Fe(III). The triferric form either remains in the mucosa as a ferritin-Fe(III) complex and returns to the lumen during cell turnover or enters the bloodstream. **Non-heme-Fe** can only be absorbed as Fe2+. Therefore, non-heme Fe(III) must first be reduced to Fe2+ by *ferrireductase*(and ascorbate on the surface of the luminal mucosa. Fe2+ is probably absorbed through secondary active transport via an Fe2+-H+ symport carrier (DCT1) (competition with Mn2+, Co2+, Cd2+, etc.). A *low chymous pH* is important since it (a) increases the H+gradient that drives Fe2+ via DCT1 into the cell and (b) frees dietary iron from complexes. The absorption of iron into the bloodstream is *regulated by the intestinal mucosa*. When an iron deficiency exists, *aconitase* (an iron-regulating protein) in the cytosol binds with ferritin-mRNA, thereby inhibiting mucosal ferritin translation. As a result, larger quantities of absorbed Fe(II) can enter the bloodstream.

Fe(II) in the blood is oxidized to Fe(III) by ceruloplasmin (and copper). It then binds to *apotransferrin*, a protein responsible for **iron transport in plasma**. *Transferrin* (apotransferrin loaded with 2 Fe(III)), is taken up by endocytosis into erythroblasts and cells of the liver, placenta, etc. with the aid of *transferrin receptors*. Once iron has been released to the target cells, apotransferrin again becomes available for uptake of iron from the intestine and macrophages (see below). **Iron storage and recycling** (**A3**). *Ferritin*, one of the chief forms in which iron is stored in the body, occurs mainly in the intestinal mucosa, liver, bone marrow, red blood cells, and plasma. It contains binding pockets for up to 4500 Fe3+ ions and provides rapidly available stores of iron (ca. 600 mg), whereas iron mobilization from *hemosiderin* is much slower (250mg Fe in macrophages of the liver and bone marrow). Hb-Fe and heme-Fe released from malformed erythroblasts (so-called inefficient erythropoiesis) and hemolyzed red blood cells bind to haptoglobin and hemopexin, respectively. They are then engulfed by macrophages in the bone marrow or in the liver and spleen, respectively, resulting in 97% iron recycling.

An **iron deficiency** inhibits Hb synthesis, leading to hypochromic microcytic anemia:MCH "26 pg, MCV "70 fL, Hb "110 g/L. The primary causes are: blood loss (most common cause); 0.5mg Fe are lost with each mL of blood; insufficient iron intake or absorption; increased iron requirement due to growth,pregnancy, breast-feeding, etc.;decreased iron recycling (due to chronic infection); ! apotransferrin defect (rare cause).

Iron overload most commonly damages the liver, pancreas and myocardium (hemochromatosis). If the iron supply bypasses the intestinal tract (iron injection.





Physiology

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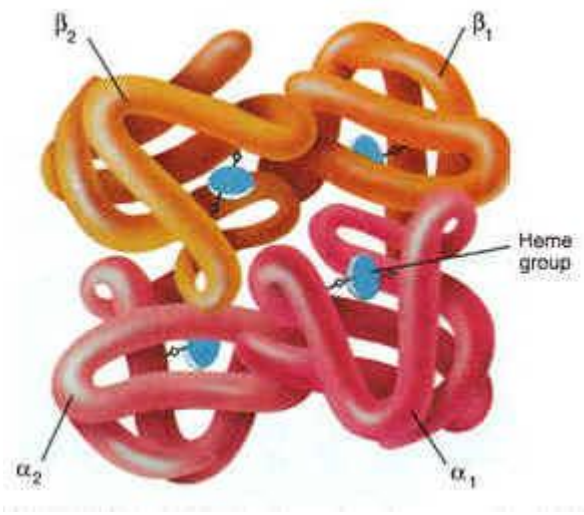
Haemoglobin



Haemoglobin(Hb):-

is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body. In mammals, the protein makes up about 97% of the red blood cells' dry content (by weight), and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 mL O_2 per gram of hemoglobinwhich increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules. Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen. Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism. Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobins may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen away from anaerobic systems, such as the nitrogen-fixing nodules of leguminous plants, before the oxygen can poison the system.

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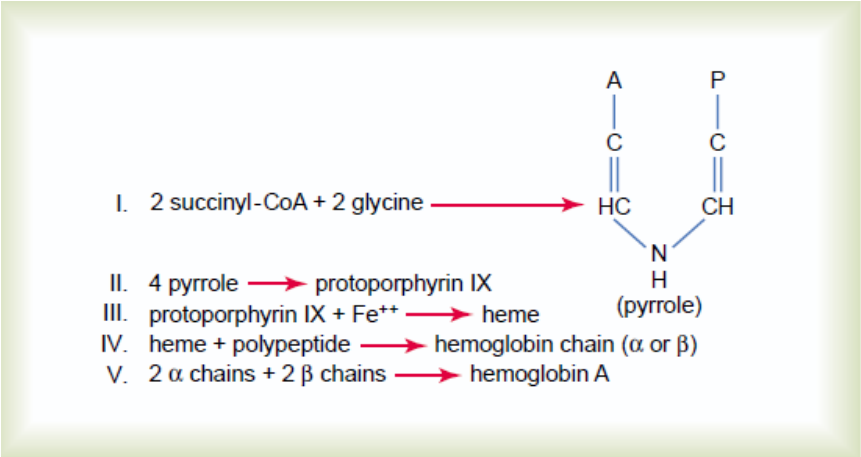
Synthesis:-

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).



Structure:-

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin. This folding pattern contains a pocket that strongly binds the heme group.

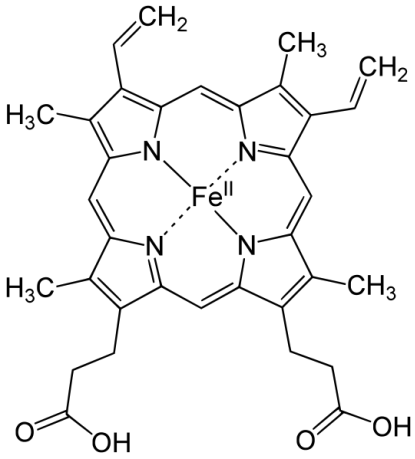
A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methine bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding,

coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporarily turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable of binding oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called *hemoglobin A*, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size.

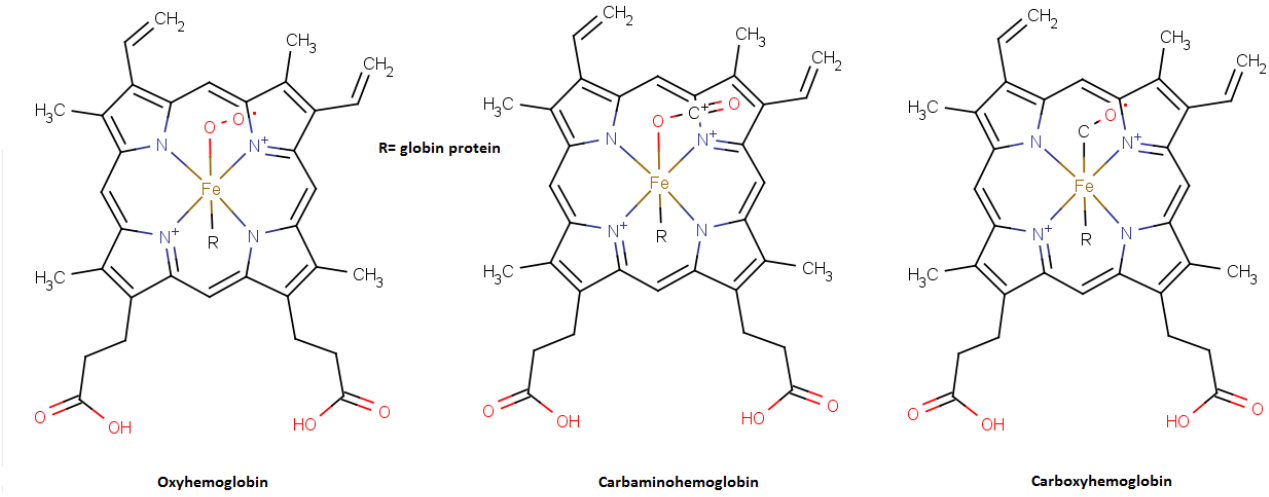


Oxygen saturation:-

In general, hemoglobin can be saturated with oxygen molecules oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin).

Oxyhemoglobin:-

Oxyhemoglobin is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized as a terminal electron acceptor in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH. Hemoglobin exists in two forms, a *taut (tense) form* (T) and a *relaxed form* (R). Various factors such as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. Conversely, a high pH, low CO_2, or low 2,3 BPG favors the relaxed form which can better bind oxygen. The partial pressure of the system also affects O_2 affinity where, at high partial pressures of oxygen (such as those present in the alveoli), the relaxed (high affinity, R) state is favoured. Inversely, at low partial pressures (such as those present in respiring tissues), the (low affinity, T) tense state is favoured. Additionally, the binding of oxygen to the Iron-II heme pulls the iron into the plane of the porphryn ring, causing a slight conformational shift. The shift encourages oxygen to bind to the three remaining hemes within hemoglobin (thus, oxygen binding is cooperative).



Deoxygenated hemoglobin:-

Deoxygenated hemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter. This difference also

accounts for the presentation of cyanosis, the blue to purplish color that tissues develop during hypoxia**.**

Types of Hemoglobin:-

Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants**.**

- In the embryo
- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland I ($\zeta_2\gamma_2$)
- Hemoglobin Portland II ($\zeta_2\beta_2$).

In the fetus:

- Hemoglobin F ($\alpha_2\gamma_2$) (PDB 1FDH).

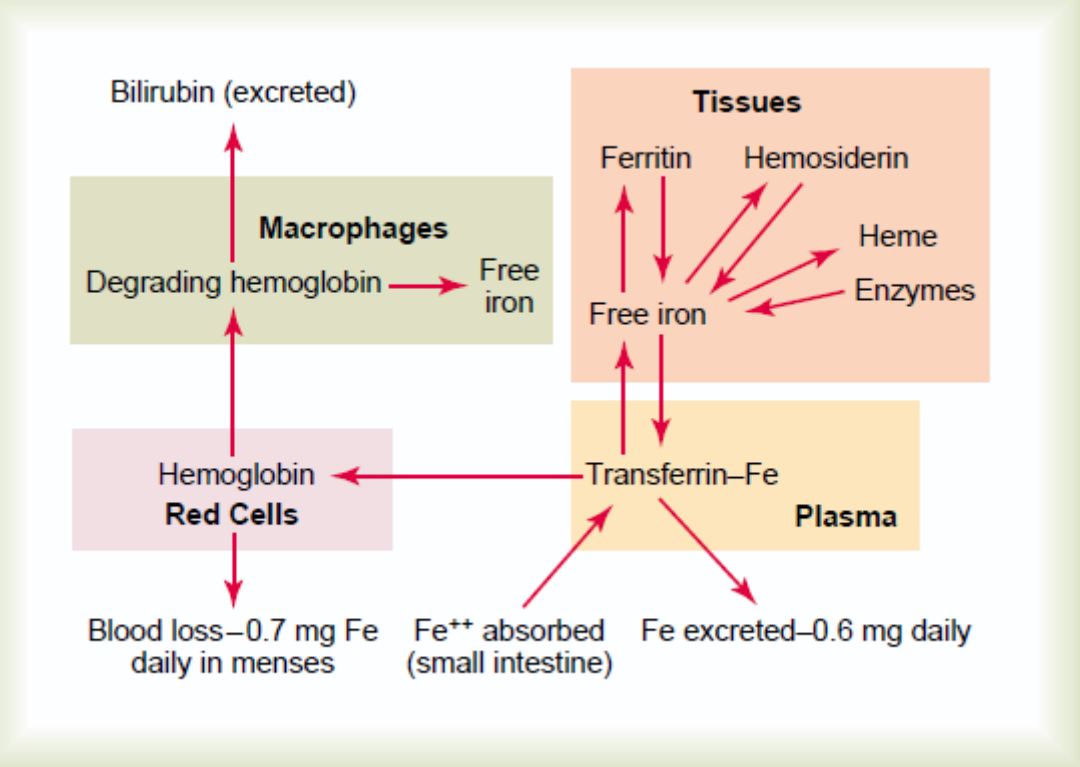
In adults:

- Hemoglobin A ($\alpha_2\beta_2$) (PDB 1BZ0) – The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) – δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5–3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) – In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia

Degradation of Hemoglobin in Animals:-

When red cells reach the end of their life due to aging or defects, they are broken down in spleen, the hemoglobin molecule is broken up and the iron gets recycled. This process also produces one molecule of carbon monoxide for every molecule of heme degraded. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The other major final product of heme degradation is bilirubin. Increased levels of this chemical are detected in the blood if red cells are being destroyed more rapidly than usual. Improperly degraded hemoglobin protein or hemoglobin that has been released from the blood cells too rapidly can clog small blood vessels, especially the delicate blood filtering vessels of the kidneys, causing kidney damage. Iron is removed from heme and salvaged for later use, it is stored as hemosiderin or ferritin in tissues and transported in plasma by beta globulins as transferins. When the porphyrin ring is broken up, the fragments are normally

secreted as a yellow pigment called bilirubin, which is secreted into the intestines as bile. Intestines metabolise bilirubin into urobilinogen. Urobilinogen leaves the body in faeces, in a pigment called stercobilin. Globulin is metabolised into amino acids which are then released into circulation.



Anemias

Anemia means deficiency of hemoglobin in the blood, which can be caused by either too few red blood cells or too little hemoglobin in the cells. Some types of anemia and their physiologic causes are the following.

Blood Loss Anemia

After rapid hemorrhage, the body replaces the fluid portion of the plasma in 1 to 3 days, but this leaves a low concentration of red blood cells.

If a second hemorrhage does not occur, the red blood cell concentration usually returns to normal within 3to 6 weeks.

In chronic blood loss, a person frequently cannot absorb enough iron from the intestines to form hemoglobin as rapidly as it is lost. Red cells are then produced that are much smaller than normal and have too little hemoglobin inside them, giving rise to *microcytic,hypochromic anemia*.

Aplastic Anemia

Bone marrow aplasia means lack of functioning bone marrow. For instance, a person exposed to gamma ray radiation from a nuclear bomb blast can sustain complete destruction of bone marrow, followed in a few weeks by lethal anemia. Likewise, excessive x-ray treatment, certain industrial chemicals, and even drugs to which the person might be sensitive can cause the same effect.

Megaloblastic Anemia

Based on the earlier discussions of vitamin B12, folic acid, and intrinsic factor from the stomach mucosa, one can readily understand that loss of any one of these can lead to slow reproduction of erythroblasts in the bone marrow. As a result, the red cells grow too large, with odd shapes, and are called *megaloblasts.* Thus, atrophy of the stomach mucosa, as occurs in *pernicious anemia,* or loss of the entire stomach after surgical total gastrectomy can lead to megaloblastic anemia. Also, patients who have intestinal sprue, in which folic acid, vitamin B12, and other vitamin B compounds are poorly absorbed, often develop megaloblastic anemia. Because in these states the erythroblasts cannot proliferate rapidly enough to form normal numbers of red blood cells, those red cells that are formed are mostly oversized, have bizarre shapes, and have fragile membranes. These cells rupture easily, leaving the person in dire need of an adequate number of red cells.

Hemolytic Anemia

Different abnormalities of the red blood cells, many of which are hereditarily acquired, make the cells fragile, so that they rupture easily as they go through the capillaries, especially through the spleen. Even though the number of red blood cells formed may be normal, or even much greater than normal in some hemolytic diseases, the life span of the fragile red cell is so short that the cells are destroyed faster than they can be formed, and serious anemia results. Some of these types of anemia are the following.

In *hereditary spherocytosis,* the red cells are very small and *spherical* rather than being biconcave discs.

These cells cannot withstand compression forces because they do not have the normal loose, baglike cell membrane structure of the biconcave discs. On passing through the splenic pulp and some other tight vascular beds, they are easily ruptured by even slight compression.

In *sickle cell anemia,* which is present in 0.3 to 1.0 per cent of West African and American blacks, the cells have an abnormal type of hemoglobin called *hemoglobin S,* containing faulty beta chains in the hemoglobin molecule, as explained earlier in the chapter. When this hemoglobin is exposed to low concentrations of oxygen, it precipitates into long crystals inside the red blood cell. These crystals elongate the cell and give it the appearance of a sickle rather than a biconcave disc. The precipitated hemoglobin also damages

the cell membrane, so that the cells become highly fragile, leading to serious anemia. Such patients frequently experience a vicious circle of events called a sickle cell disease "crisis," in which low oxygen tension in the tissues causes sickling, which leads to ruptured red cells, which causes a further decrease in oxygen tension and still more sickling and red cell destruction.

Once the process starts, it progresses rapidly, eventuating in a serious decrease in red blood cells within a few hours and, often, death.

In *erythroblastosis fetalis,* Rh-positive red blood cells in the fetus are attacked by antibodies from an Rh-negative mother. These antibodies make the Rh-positive cells fragile, leading to rapid rupture and causing the child to be born with serious anemia. The extremely rapid formation of new red cells to make up for the destroyed cells in erythroblastosis fetalis causes a large number of early *blast*forms of red cells to be released from the bone marrow into the blood.

Effects of Anemia on Function of the Circulatory System

The viscosity of the blood depends almost entirely on the blood concentration of red blood cells. In severe anemia, the blood viscosity may fall to as low as 1.5 times that of water rather than the normal value of about 3. This decreases the resistance to blood flow in the peripheral blood vessels, so that far greater than normal quantities of blood flow through the tissues and return to the heart, thereby greatly increasing cardiac output. Moreover, hypoxia resulting from diminished transport of oxygen by the blood causes the peripheral tissue blood vessels to dilate, allowing a further increase in the return of blood to the heart and increasing the cardiac output to a still higher level-sometimes three to four times normal. Thus, one of the major effects of anemia is greatly *increased cardiacoutput,* as well as *increased pumping workload on the heart.*

The increased cardiac output in anemia partially offsets the reduced oxygen-carrying effect of the anemia, because even though each unit quantity of blood carries only small quantities of oxygen, the rate of blood flow may be increased enough so that almost normal quantities of oxygen are actually delivered to the tissues. However, when a person with anemia begins to exercise, the heart is not capable of pumping much greater quantities of blood than it is already pumping. Consequently, during exercise, which greatly increases tissue demand for oxygen, extreme tissue hypoxia results, and *acute cardiac failure* ensues.

Polycythemia

Secondary Polycythemia. Whenever the tissues become hypoxic because of too little oxygen in the breathed air, such as at high altitudes, or because of failure of oxygen delivery to the tissues, such as in cardiac failure, the blood-forming organs automatically produce large quantities of extra red blood cells. This condition is called *secondary polycythemia,* and the red cell count commonly rises to 6 to 7 million/mm3, about 30 per cent above normal.

A common type of secondary polycythemia, called *physiologic polycythemia,* occurs in natives who live at altitudes of 14,000 to 17,000 feet, where the

atmospheric oxygen is very low.The blood count is generally 6 to 7 million/mm3; this allows these people to perform

Leukocytes

Our bodies are exposed continually to bacteria, viruses, fungi, and parasites, all of which occur normally and to varying degrees in the skin, the mouth, the respiratory passageways, the intestinal tract, the lining membranes of the eyes, and even the urinary tract. Many of these infectious agents are capable of causing serious abnormal physiologic function or even death if they invade the deeper tissues. In addition, we are exposed intermittently to other highly infectious bacteria and viruses besides those that are normally present, and these can cause acute lethal diseases such as pneumonia, streptococcal infection, and typhoid fever.

Our bodies have a special system for combating the different infectious and toxic agents.This is comprised of blood leukocytes (white blood cells) and tissue cells derived from leukocytes.These cells work together in two ways to prevent disease: (1) by actually destroying invading bacteria or viruses by *phagocytosis*, and (2) by forming *antibodies* and *sensitized lymphocytes,* one or both of which may destroy or inactivate the invader.

Leukocytes (White Blood Cells)

The leukocytes, also called *white blood cells,* are the *mobile units* of the body's protective system. They are formed partially in the bone marrow (*granulocytes* and *monocytes* and a few *lymphocytes*) and partially in the lymph tissue (*lymphocytes*and *plasma cells*). After formation, they are transported in the blood to different parts of the body where they are needed.

The real value of the white blood cells is that most of them are specifically transported to areas of serious infection and inflammation, thereby providing a rapid and potent defense against infectious agents. As we see later, the granulocytes and monocytes have a special ability to "seek out and destroy" a foreign invader.

General Characteristics of Leukocytes

Types of White Blood Cells. Six types of white blood cells are normally present in the blood. They are *polymorphonuclear neutrophils, polymorphonucleareosinophils, polymorphonuclear basophils, monocytes, lymphocytes,* and, occasionally, *plasma cells.* In addition, there are large numbers of *platelets,* which are fragments of another type of cell similar to the white blood cells found in the bone marrow, the *megakaryocyte.* The first three types of cells, the polymorphonuclear cells, all have a granular appearance, as shown in cell numbers 7,Specific types of cells are associated with different illnesses and reflect the special function of that cell type in body defense. A fall in white cell count, which is called leukopenia, occurs in states such as debilitation, anaphylaxis, and overwhelming infection. In general, newborns have a high white blood cell count that gradually falls to the adult level during childhood. An exception is the lymphocyte count, which is low at birth, reaches

its highest levels in the first four years of life, and thereafter falls gradually to a stable adult level. *See also* blood cell formation.

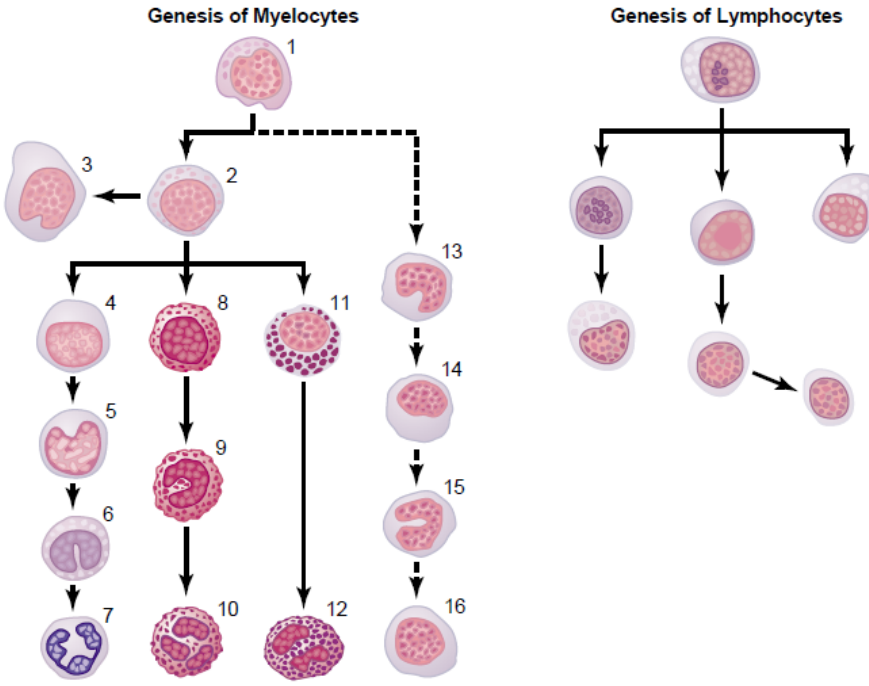


Figure 33–1

Genesis of white blood cells. The different cells of the myelocyte series are 1, myeloblast; 2, promyelocyte; 3, megakaryocyte; 4, neutrophil myelocyte; 5, young neutrophil metamyelocyte; 6, "band" neutrophil metamyelocyte; 7, polymorphonuclear neutrophil; 8, eosinophil myelocyte; 9, eosinophil metamyelocyte; 10, polymorphonuclear eosinophil; 11, basophil myelocyte; 12, polymorphonuclear basophil; 13–16, stages of monocyte formation.

10, and 12 in Figure 33–1, for which reason they are called *granulocytes,* or, in clinical terminology, "polys," because of the multiple nuclei.

The granulocytes and monocytes protect the body against invading organisms mainly by ingesting them—that is, by *phagocytosis.* The lymphocytes and plasma cells function mainly in connection with the immune system; this is discussed in Chapter 34. Finally, the function of platelets is specifically to activate the blood clotting mechanism, which is discussed in Chapter 36.

Concentrations of the Different White Blood Cells in the Blood. The adult human being has about 7000 white blood cells per *microliter* of blood (in comparison with 5 million red blood cells). Of the total white blood cells, the normal percentages of the different types are approximately the following:

Polymorphonuclear neutrophils	62.0%
Polymorphonuclear eosinophils	2.3%
Polymorphonuclear basophils	0.4%
Monocytes	5.3%
Lymphocytes	30.0%

The number of platelets, which are only cell fragments, in each microliter of blood is normally about 300,000.

Genesis of the White Blood Cells

Early differentiation of the pluripotential hematopoietic stem cell into the different types of committed stem cells is shown in Figure 32–2 in the previous

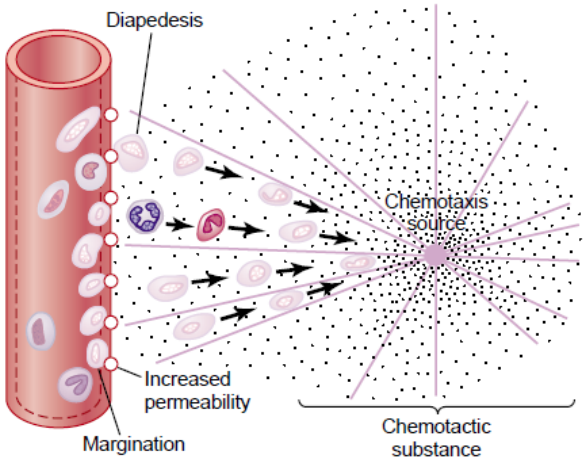


Figure 33–2

Movement of neutrophils by *diapedesis* through capillary pores and by *chemotaxis* toward an area of tissue damage.

chapter. Aside from those cells committed to form red blood cells, two major lineages of *white blood cells* are formed, the myelocytic and the lymphocytic lineages. The left side of Figure 33–1 shows the *myelocytic lineage,* beginning with the *myeloblast;* the right shows the *lymphocytic lineage,* beginning with the *lymphoblast.*

The granulocytes and monocytes are formed only in the bone marrow. Lymphocytes and plasma cells are produced mainly in the various lymphogenous tissues—especially the lymph glands, spleen, thymus, tonsils, and various pockets of lymphoid tissue

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The granulocytes and monocytes are formed only in the bone marrow. Lymphocytes and plasma cells are produced mainly in the various lymphogenous tissues—especially the lymph glands, spleen, thymus, tonsils, and various pockets of lymphoid tissueelsewhere in the body, such as in the bone marrow and in so-called Peyer's patches underneath the epithelium in the gut wall.

The white blood cells formed in the bone marrow are stored within the marrow until they are needed in the circulatory system. Then, when the need arises, various factors cause them to be released (these factors are discussed later). Normally, about three times as many white blood cells are stored in the marrow as circulate in the entire blood.This represents about a 6-day supply of these cells.

The lymphocytes are mostly stored in the various lymphoid tissues, except for a small number that are temporarily being transported in the blood.

As shown in Figure 33–1, megakaryocytes (cell 3) are also formed in the bone marrow.These megakaryocytes fragment in the bone marrow; the small fragments, known as *platelets* (or *thrombocytes*), then pass into the blood. They are very important in the initiation of blood clotting.

Life Span of the White Blood Cells

The life of the granulocytes after being released from the bone marrow is normally 4 to 8 hours circulating in the blood and another 4 to 5 days in tissues where they are needed. In times of serious tissue infection, this total life span is often shortened to only a few hours because the granulocytes proceed even more rapidly to the infected area, perform their functions, and, in the process, are themselves destroyed.

The monocytes also have a short transit time, 10 to 20 hours in the blood, before wandering through the capillary membranes into the tissues. Once in the tissues, they swell to much larger sizes to become *tissue macrophages,* and, in this form, can live for months unless destroyed while performing phagocytic functions. These tissue macrophages are the basis of the *tissue macrophage system,* discussed in greater detail later, which provides continuing defense against infection.

Lymphocytes enter the circulatory system continually, along with drainage of lymph from the lymph nodes and other lymphoid tissue. After a few hours,

they pass out of the blood back into the tissues by diapedesis. Then, still later, they re-enter the lymph and return to the blood again and again; thus, there is continual circulation of lymphocytes through the body.

The lymphocytes have life spans of weeks or months; this life span depends on the body's need for these cells. The platelets in the blood are replaced about once every 10 days; in other words, about 30,000 platelets are formed each day for each microliter of blood.

Neutrophils and Macrophages Defend Against Infections

It is mainly the neutrophils and tissue macrophages that attack and destroy invading bacteria, viruses, and other injurious agents. The neutrophils are mature cells that can attack and destroy bacteria even in the circulating blood. Conversely, the tissue macrophages begin life as blood monocytes, which are immature cells while still in the blood and have little ability to fight infectious agents at that time. However, once they enter the tissues, they begin to swell—sometimes increasing their diameters as much as fivefold—to as great as 60 to 80 micrometers, a size that can barely be seen with the naked eye. These cells are now called *macrophages,* and they are extremely capable of combating intratissue disease agents.

White Blood Cells Enter the Tissue Spaces by Diapedesis.

Neutrophils and monocytes can squeeze through the pores of the blood capillaries by *diapedesis.* That is, even though a pore is much smaller than a cell, a small portion of the cell slides through the pore at a time; the portion sliding through is momentarily constricted to the size of the pore,

White Blood Cells Move Through Tissue Spaces by Ameboid

Motion. Both neutrophils and macrophages can move through the tissues by ameboid motion. Some cells move at velocities as great as 40 mm/min, a distance as great as their own length each minute.

White Blood Cells Are Attracted to Inflamed Tissue Areas by Chemotaxis.

Many different chemical substances in the tissues cause both neutrophils and macrophages to move toward the source of the chemical. This phenomenon, shown in Figure 33–2, is known as *chemotaxis.*When a tissue becomes inflamed, at least a dozen different products are formed that can cause chemotaxis toward the inflamed area. They include (1) some of the bacterial or viral toxins, (2) degenerative products of the inflamed tissues themselves, (3) several reaction products of the "complement complex" activated in inflamed tissues, and (4) several reaction products caused by plasma clotting in the inflamed area, as well as other substances. Chemotaxis depends on the concentration gradient of the chemotactic substance.

The concentration is greatest near the source, which directs the unidirectional movement of the white cells. Chemotaxis is effective up to 100 micrometers

away from an inflamed tissue.Therefore, because almost no tissue area is more than 50 micrometers away from a capillary, the chemotactic signal can easily move hordes of white cells from the capillaries into the inflamed area.

Phagocytosis

The most important function of the neutrophils and macrophages is *phagocytosis*, which means cellular ingestion of the offending agent. Phagocytes must be selective of the material that is phagocytized; otherwise, normal cells and structures of the body might be ingested. Whether phagocytosis will occur depends especially on three selective procedures.

First, most natural structures in the tissues have smooth surfaces, which resist phagocytosis. But if the surface is rough, the likelihood of phagocytosis is increased. Second, most natural substances of the body have protective protein coats that repel the phagocytes.

Conversely, most dead tissues and foreign particles have no protective coats, which makes them subject to phagocytosis.

Third, the immune system of the body develops *antibodies* against infectious agents such as bacteria. The antibodies then adhere to the bacterial membranes and thereby make the bacteria especially susceptible to phagocytosis. To do this, the antibody molecule also combines with the C3 product of the *complement cascade*, which is an additional part of the immune system. The C3 molecules, in turn, attach to receptors on the phagocyte membrane, thus initiating phagocytosis. This selection and phagocytosis process is called *opsonization.*

Phagocytosis by Neutrophils.

The neutrophils entering the tissues are already mature cells that can immediately begin phagocytosis. On approaching a particle to be phagocytized, the neutrophil first attaches itself to the particle and then projects pseudopodia in all directions around the particle. The pseudopodia meet one another on the opposite side and fuse. This creates an enclosed chamber that contains the phagocytized particle. Then the chamber invaginates to the inside of the cytoplasmic cavity and breaks away from the outer cell membrane to form a free-floating *phagocytic vesicle* (also called a *phagosome*) inside the cytoplasm. A single neutrophil can usually phagocytize 3 to 20 bacteria before the neutrophil itself becomes inactivated and dies.

Phagocytosis by Macrophages. Macrophages are the endstage product of monocytes that enter the tissues from the blood. When activated by the immune system they are much more powerful phagocytes than neutrophils, often capable of phagocytizing as many as 100 bacteria.They also have the ability to engulf much larger particles, even whole red blood cells or, occasionally, malarial parasites, whereas neutrophils are not capable of phagocytizing particles much larger than bacteria. Also, after digesting particles, macrophages can extrude the residual products and often survive and function for many more months.

Once Phagocytized, Most Particles Are Digested by Intracellular

Enzymes. Once a foreign particle has been phagocytized, lysosomes and other cytoplasmic granules in the neutrophil or macrophage immediately come in contact with the phagocytic vesicle, and their membranes fuse, thereby dumping many digestive enzymes and bactericidal agents into the vesicle. Thus, the phagocytic vesicle now becomes a *digestive vesicle,*and digestion of the phagocytized particle begins immediately.

Both neutrophils and macrophages contain an abundance of lysosomes filled with *proteolytic enzymes* especially geared for digesting bacteria and other foreign protein matter. The lysosomes of macrophages (but not of neutrophils) also contain large amounts of *lipases*, which digest the thick lipid membranes possessed by some bacteria such as the tuberculosis bacillus.

Both Neutrophils and Macrophages Can Kill Bacteria.

In addition to the digestion of ingested bacteria in phagosomes, neutrophils and macrophages contain *bactericidal agents* that kill most bacteria even when the lysosomal enzymes fail to digest them.This is especially important, because some bacteria have protective coats or other factors that prevent their destruction by digestive enzymes. Much of the killing effect results from several powerful *oxidizing agents* formed by enzymes in the membrane of the phagosome or by a special organelle called the *peroxisome.*These oxidizing agents include large quantities of *superoxide* (O_2^-), *hydrogen peroxide* (H_2O_2), and *hydroxyl ions* ($-OH^-$), all of which are lethal to most bacteria, even in small quantities. Also, one of the lysosomal enzymes, myeloperoxidase, catalyzes the reaction between H_2O_2 and chloride ions to form hypochlorite, which is exceedingly bactericidal.

Some bacteria, however, notably the tuberculosis bacillus, have coats that are resistant to lysosomal digestion and also secrete substances that partially resist the killing effects of the neutrophils and macrophages. These bacteria are responsible for many of the chronic diseases, an example of which is tuberculosis.

Monocyte-Macrophage Cell System (Reticuloendothelial System)

In the preceding paragraphs, we described the macrophages mainly as mobile cells that are capable of wandering through the tissues. However, after entering the tissues and becoming macrophages, another large portion of monocytes becomes attached to the tissues and remains attached for months or even years until they are called on to perform specific local protective functions. They have the same capabilities as the mobile macrophages to phagocytize large quantities of bacteria, viruses, necrotic tissue, or other foreign particles in the tissue.And, when appropriately stimulated, they can break away from their attachments and once again become mobile macrophages that respond to chemotaxis and all the other stimuli related to the inflammatory process.Thus, the body has a widespread “monocyte-macrophage system” in virtually all tissue areas.

The total combination of monocytes, mobile macrophages, fixed tissue macrophages, and a few specialized endothelial cells in the bone marrow, spleen, and lymph nodes is called the *reticuloendothelialsystem.* However, all or almost all these cells originate from monocytic stem cells; therefore, the reticuloendothelial system is almost synonymous with the monocyte-macrophage system. Because the term *reticuloendothelial system* is much better known in medical literature than the term *monocytemacrophage system*, it should be remembered as a generalized phagocytic system located in all tissues, especially in those tissue areas where large quantities of particles, toxins, and other unwanted substances must be destroyed.

Tissue Macrophages in the Skin and Subcutaneous Tissues (Histiocytes).

Although the skin is mainly impregnable to infectious agents, this is no longer true when the skin is broken. When infection begins in a subcutaneous tissue and local inflammation ensues, local tissue macrophages can divide in situ and form still more macrophages. Then they perform the usual functions of attacking and destroying the infectious agents, as described earlier.

Macrophages in the Lymph Nodes.

Essentially no particulate matter that enters the tissues, such as bacteria, can be absorbed directly through the capillary membranes into the blood. Instead, if the particles are not destroyed locally in the tissues, they enter the lymph and flow to the lymph nodes located intermittently along the course of the lymph flow. The foreign particles are then trapped in these nodes in a meshwork of sinuses lined by *tissue macrophages.*

The general organization of the lymph node, showing lymph entering through the lymph node capsule by way of *afferent lymphatics,* then flowing through the *nodal medullary sinuses*, and finally passing out the *hilus* into *efferent lymphatics*that eventually empty into the venous blood.

Large numbers of macrophages line the lymph sinuses, and if any particles enter the sinuses by way of the lymph, the macrophages phagocytize them and prevent general dissemination throughout the body.

Alveolar Macrophages in the Lungs.

Another route by which invading organisms frequently enter the body is through the lungs. Large numbers of tissue macrophages are present as integral components of the alveolar walls. They can phagocytize particles that become entrapped in the alveoli. If the particles are digestible, the macrophages can also digest them and release the digestive products into the lymph. If the particle is not digestible, the macrophages often form a "giant cell" capsule around the particle until such time—if ever—that it can be slowly dissolved. Such capsules are frequently formed around tuberculosis bacilli, silica dust particles, and even carbon particles.

Macrophages (Kupffer Cells) in the Liver Sinusoids.

Still another favorite route by which bacteria invade the body is through the gastrointestinal tract. Large numbers of bacteria from ingested food constantly

pass through the gastrointestinal mucosa into the portal blood. Before this blood enters the general circulation, it passes through the sinusoids of the liver; these sinusoids are lined with tissue macrophages called *Kupffer cells*. These cells form such an effective particulate filtration system that almost none of the bacteria from the gastrointestinal tract succeeds in passing from the portal blood into the general systemic circulation. Indeed, motion pictures of phagocytosis by Kupffer cells have demonstrated phagocytosis of a single bacterium in less than 1/100 of a

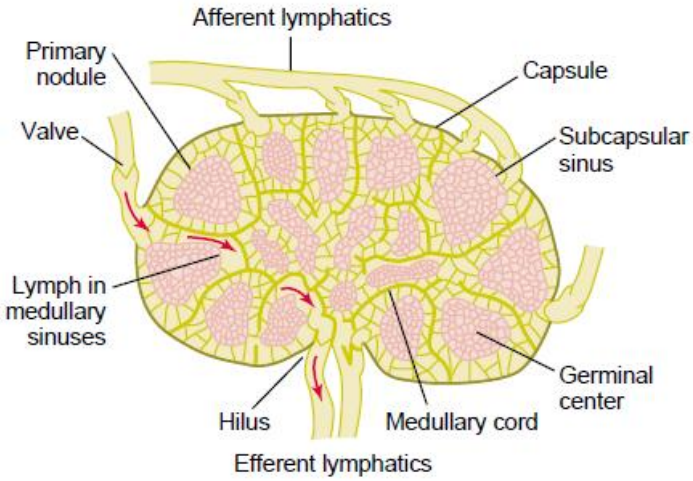

Figure 33–3

Functional diagram of a lymph node. (Redrawn from Ham AW: Histology, 6th ed. Philadelphia: JB Lippincott, 1969.) (Modified from Gartner LP, Hiatt JL: Color Textbook of Histology, 2nd ed. Philadelphia, WB Saunders, 2001.)

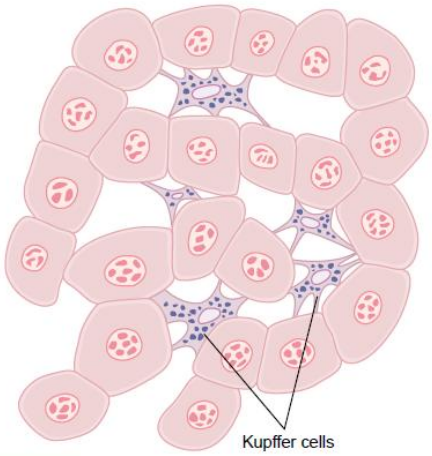

Figure 33–4

Kupffer cells lining the liver sinusoids, showing phagocytosis of India ink particles into the cytoplasm of the Kupffer cells. (Redrawn from Copenhaver WM, et al: Bailey's Textbook of Histology, 10th ed. Baltimore: Williams & Wilkins, 1971.)

Macrophages of the Spleen and Bone Marrow

If an invading organism succeeds in entering the general circulation,
there are other lines of defense by the tissue macrophage system, especially by macrophages of the spleen and bone marrow. In both these tissues, macrophages have become entrapped by the reticular meshwork of the two organs, and when foreign particles come in contact with these macrophages, they are phagocytized.

The spleen is similar to the lymph nodes, except that blood, instead of lymph, flows through the tissue spaces of the spleen. Figure 33–5 shows a small peripheral segment of spleen tissue. Note that a small artery penetrates from the splenic capsule into the *splenic pulp* and terminates in small capillaries. The capillaries are highly porous, allowing whole blood to pass out of the capillaries into *cords of red pulp.*The blood then gradually *squeezes* through the trabecular

meshwork of these cords and eventually returns to the circulation through the endothelial walls of the *venous sinuses.*

The trabeculae of the red pulp are lined with vast numbers of macrophages, and the venous sinuses are also lined with macrophages. This peculiar passage of blood through the cords of the red pulp provides an exceptional means of phagocytizing unwanted debris in the blood, including especially old and abnormal red blood cells.

Inflammation: Role of Neutrophils and Macrophages Inflammation

When tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat, or any other phenomenon, multiple substances are released by the injured tissues and cause dramatic secondary changes in the surrounding uninjured tissues. This entire complex of tissue changes is called *inflammation.*

Inflammation is characterized by (1) vasodilation of the local blood vessels, with consequent excess local blood flow; (2) increased permeability of the capillaries, allowing leakage of large quantities of fluid into the interstitial spaces; (3) often clotting of the fluid in the interstitial spaces because of excessive amounts of fibrinogen and other proteins leaking from the capillaries; (4) migration of large numbers of granulocytes and monocytes into the tissue; and (5) swelling of the tissue cells. Some of the many tissue products that cause these reactions are *histamine*, *bradykinin*, *serotonin*,*prostaglandins*, several different *reaction productsof the complement system*, *reaction products of the blood clotting system,* and multiple substances called *lymphokines* that are released by sensitized T cells (part of the immune system. Several of these substances strongly activate the macrophage system, and within a few hours, the macrophages begin to devour the destroyed tissues. But at times, the macrophages also further injure the still-living tissue cells.

"Walling-Off" Effect of Inflammation

One of the first results of inflammation is to "wall off " the area of injury from the remaining tissues. The tissue spaces and the lymphatics in the inflamed area are blocked by fibrinogen clots so that after a while, fluid barely flows through the spaces. This walling-off process delays the spread of bacteria or toxic products. The intensity of the inflammatory process is usually proportional to the degree of tissue injury. For instance, when *staphylococci* invade tissues, they release extremely lethal cellular toxins. As a result, inflammation develops rapidly—indeed, much more rapidly than the staphylococci themselves can multiply and spread. Therefore, local staphylococcal infection is characteristically walled off rapidly and prevented from spreading through the body. Streptococci,

in contrast, do not cause such intense local tissue destruction. Therefore, the walling-off process develops slowly over many hours, while many streptococci reproduce and migrate. As a result, streptococci often
have a far greater tendency to spread through the body and cause death than do staphylococci, even though staphylococci are far more destructive to the tissue.

Macrophage and Neutrophil Responses During Inflammation

Tissue Macrophage Is a First Line of Defense Against Infection

Within minutes after inflammation begins, the macrophages already present in the tissues, whether histiocytes in the subcutaneous tissues, alveolar macrophages in the lungs, microglia in the brain, or others, immediately begin their phagocytic actions.

When activated by the products of infection and inflammation, the first effect is rapid enlargement of each of these cells. Next, many of the previously sessile macrophages break loose from their attachments and become mobile, forming the first line of defense against infection during the first hour or so. The numbers of these early mobilized macrophages often are not great, but they are lifesaving.

Neutrophil Invasion of the Inflamed Area Is a Second Line of Defense

Within the first hour or so after inflammation begins, large numbers of neutrophils begin to invade the inflamed area from the blood. This is caused by products from the inflamed tissues that initiate the following reactions: (1) They alter the inside surface of the capillary endothelium, causing neutrophils to stick to the capillary walls in the inflamed area. This effect is called *margination*. They cause the intercellular attachments between the endothelial cells of the capillaries and small venules to loosen, allowing openings large enough for neutrophils to pass by *diapedesis* directly from the blood into the tissue spaces. (3) Other products of inflammation then cause *chemotaxis* of the neutrophils toward the injured tissues, as explained earlier.

Thus, within several hours after tissue damage begins, the area becomes well supplied with neutrophils. Because the blood neutrophils are already
mature cells, they are ready to immediately begin their scavenger functions for killing bacteria and removing foreign matter.

Acute Increase in Number of Neutrophils in the Blood-“Neutrophilia.”

Also within a few hours after the onset of acute, severe inflammation, the number of neutrophils in the blood sometimes increases fourfold to fivefold-from a normal of 4000 to 5000 to 15,000 to 25,000 neutrophils per microliter. This is called *neutrophilia,*which means an increase in the number of neutrophils in the blood. Neutrophilia is caused by products of inflammation that enter the blood stream, are transported to the bone marrow, and there act on the stored neutrophils of the marrow to mobilize these into the circulating blood.This makes even more neutrophils available to the inflamed tissue area.

Second Macrophage Invasion into the Inflamed Tissue Is a Third Line of Defense

Along with the invasion of neutrophils, monocytes from the blood enter the inflamed tissue and enlarge to become macrophages. However, the number of monocytes in the circulating blood is low: also, the storage pool of monocytes in the bone marrow is much less than that of neutrophils. Therefore, the buildup of macrophages in the inflamed tissue area is much slower than that of neutrophils, requiring several days to become effective. Furthermore, even after invading the inflamed tissue, monocytes are still immature cells, requiring 8 hours or more to swell to much larger sizes and develop tremendous quantities of lysosomes; only then do they acquire the full capacity of *tissue macrophages* for phagocytosis. Yet, after several days to several weeks, the macrophages finally come to dominate the phagocytic cells of the inflamed area because of greatly increased bone marrow production of new monocytes, as explained later.

As already pointed out, macrophages can phagocytize far more bacteria (about five times as many) and far larger particles, including even neutrophils themselves and large quantities of necrotic tissue, than can neutrophils.Also, the macrophages play an important role in initiating the development of antibodies.

Increased Production of Granulocytes and Monocytes by the Bone Marrow Is a Fourth Line of Defense

The fourth line of defense is greatly increased production of both granulocytes and monocytes by the bone marrow. This results from stimulation of the granulocytic and monocytic progenitor cells of the marrow. However, it takes 3 to 4 days before newly formed granulocytes and monocytes reach the stage of leaving the bone marrow. If the stimulus from the inflamed tissue continues, the bone marrow can continue to produce these cells in tremendous quantities for months and even years, sometimes at a rate 20 to 50 times normal.

Feedback Control of the Macrophage and Neutrophil Responses

Although more than two dozen factors have been implicated in control of the macrophage response to inflammation, five of these are believed to play dominant roles. They are shown in Figure 33–6 and consist of (1) *tumor necrosis factor* (TNF), (2) *interleukin-1* (IL-1), (3) *granulocyte-monocyte colony-stimulating factor* (GM-CSF), (4) *granulocyte colony-stimulating factor* (G-CSF), and (5) *monocyte colony-stimulating factor* (M-CSF).These factors

are formed by activated macrophage cells in the inflamed tissues and in smaller quantities by other inflamed tissue cells.

The cause of the increased production of granulocytes and monocytes by the bone marrow is mainly the three colony-stimulating factors, one of which, GM-CSF, stimulates both granulocyte and monocyte production; the other two, G-CSF and M-CSF, stimulate granulocyte and monocyte production, respectively.

This combination of TNF, IL-1, and colony-stimulating factors provides a powerful feedback mechanism that begins with tissue inflammation and proceeds to formation of large numbers of defensive white blood cells that help remove the cause of the inflammation.

Formation of Pus

When neutrophils and macrophages engulf large numbers of bacteria and necrotic tissue, essentially all the neutrophils and many, if not most, of the macrophages eventually die. After several days, a cavity is often excavated in the inflamed tissues that contains varying portions of necrotic tissue, dead neutrophils, dead macrophages, and tissue fluid. This mixture is commonly known as *pus.* After the infection has been suppressed, the dead cells and necrotic tissue in the pus gradually autolyze over a period of days, and the end products are eventually absorbed

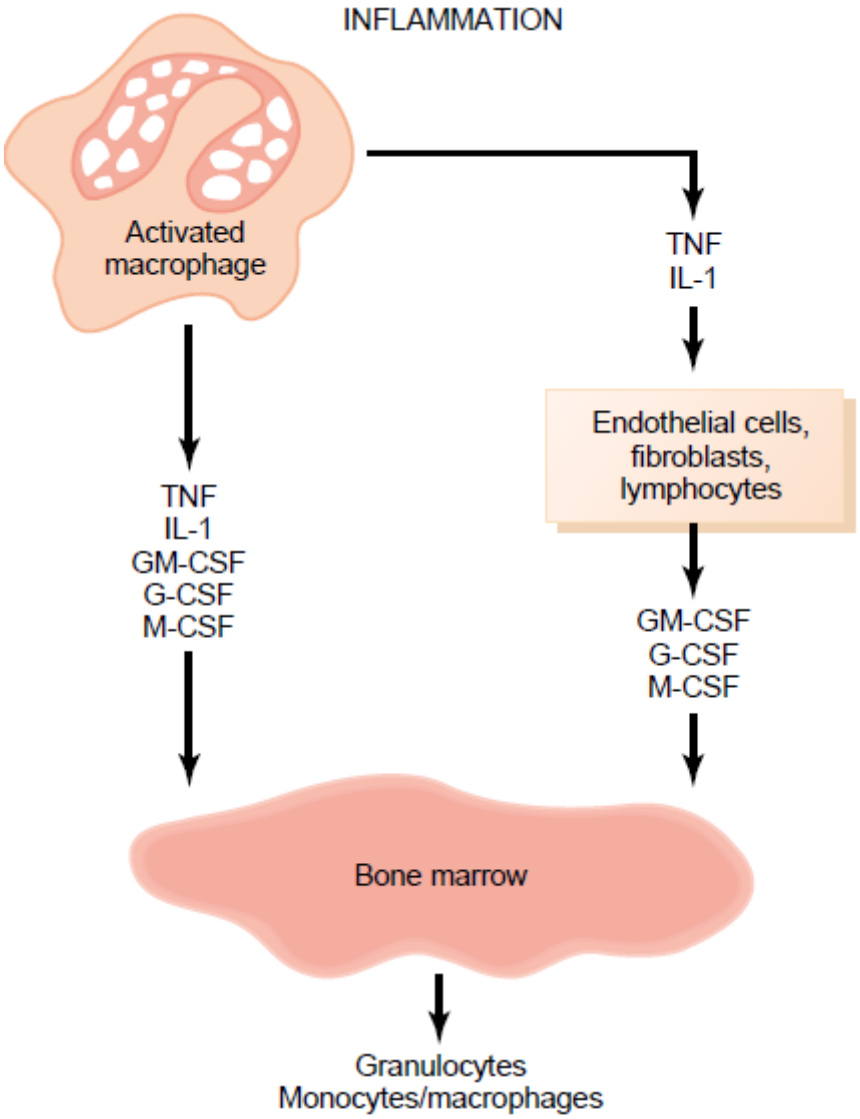

Figure 33–6

Control of bone marrow production of granulocytes and monocyte-macrophages in response to multiple growth factors released from activated macrophages in an inflamed tissue. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-monocyte colony-stimulating factor; IL-1, interleukin-1; M-CSF, monocyte colony-stimulating factor; TNF, tumor necrosis factor.

into the surrounding tissues and lymph until most of the evidence of tissue damage is gone.

Eosinophils

The eosinophils normally constitute about 2 per cent of all the blood leukocytes. Eosinophils are weak phagocytes, and they exhibit chemotaxis, but in comparison with the neutrophils, it is doubtful that the eosinophils are significant in protecting against the usual types of infection.

Eosinophils, however, are often produced in large numbers in people with parasitic infections, and they migrate in large numbers into tissues diseased by parasites.

Although most parasites are too large to be phagocytized by eosinophils or any other phagocytic cells, eosinophils attach themselves to the parasites by way of special surface molecules and release substances that kill many of the parasites. For instance, one of the most widespread infections is *schistosomiasis*, a parasitic infection found in as many as one third of the population of some Third World countries; the parasite can invade any part of the body. Eosinophils attach themselves to the juvenile forms of the parasite and kill many of them. They do so in several ways: (1) by releasing hydrolytic enzymes from their granules, which are modified lysosomes; (2) probably by also releasing highly reactive forms of oxygen that are especially lethal to parasites; and (3) by releasing from the granules a highly larvacidal polypeptide called *major basic protein.*

In a few areas of the world, another parasitic disease that causes eosinophilia is *trichinosis.* This results from invasion of the body's muscles by the *Trichinella* parasite ("pork worm") after a person eats undercooked infested pork.

Eosinophils also have a special propensity to collect in tissues in which allergic reactions occur, such as in the peribronchial tissues of the lungs in people with asthma and in the skin after allergic skin reactions.

This is caused at least partly by the fact that many mast cells and basophils participate in allergic reactions, as we discuss in the next paragraph. The mast cells and basophils release an *eosinophil chemotactic factor* that causes eosinophils to migrate toward the inflamed allergic tissue. The eosinophils are believed to detoxify some of the inflammation-inducing substances released by the mast cells and basophils and probably also to phagocytize and destroy allergen-antibody complexes, thus preventing excess spread of the local inflammatory process.

Basophils

The basophils in the circulating blood are similar to the large tissue *mast cells* located immediately outside many of the capillaries in the body. Both mast cells and basophils liberate *heparin* into the blood, a substance that can prevent blood coagulation.

The mast cells and basophils also release *histamine,*as well as smaller quantities of *bradykinin* and *serotonin.* Indeed, it is mainly the mast cells in inflamed tissues that release these substances during inflammation.

The mast cells and basophils play an exceedingly important role in some types of allergic reactions because the type of antibody that causes allergic reactions, the immunoglobulin E (IgE) type has a special propensity to become attached to mast cells and basophils. Then, when the specific antigen for the specific IgE antibody subsequently reacts with the antibody, the resulting attachment of

antigen to antibody causes the mast cell or basophil to rupture and release exceedingly large quantities of *histamine, bradykinin, serotonin, heparin, slow-reactingsubstance of anaphylaxis,* and a number of *lysosomalenzymes.* These cause local vascular and tissue reactions that cause many, if not most, of the allergic manifestations.

Leukopenia

white blood cells, leaving the body unprotected against many bacteria and other agents that might invade the tissues.

Normally, the human body lives in symbiosis with many bacteria, because all the mucous membranes of the body are constantly exposed to large numbers of bacteria. The mouth almost always contains various spirochetal, pneumococcal, and streptococcal bacteria, and these same bacteria are present to a lesser extent in the entire respiratory tract. The distal gastrointestinal tract is especially loaded with colon bacilli. Furthermore, one can always find bacteria on the surfaces of the eyes, urethra, and vagina. Any decrease in the number of white blood cells immediately allows invasion of adjacent tissues by bacteria that are already present.

Within 2 days after the bone marrow stops producing white blood cells, ulcers may appear in the mouth and colon, or the person might develop some form of severe respiratory infection. Bacteria from the ulcers rapidly invade surrounding tissues and the blood.

Without treatment, death often ensues in less than a week after acute total leukopenia begins.

Irradiation of the body by x-rays or gamma rays, or exposure to drugs and chemicals that contain benzene or anthracene nuclei, is likely to cause aplasia of the bone marrow. Indeed, some common drugs, such as chloramphenicol (an antibiotic), thiouracil (used to treat thyrotoxicosis), and even various barbiturate hypnotics, on very rare occasions cause leukopenia, thus setting off the entire infectious sequence of this malady.

After moderate irradiation injury to the bone marrow, some stem cells, myeloblasts, and hemocytoblasts may remain undestroyed in the marrow and are capable of regenerating the bone marrow, provided sufficient time is available.A patient properly treated with transfusions, plus antibiotics and other drugs to ward off infection, usually develops enough new bone marrow within weeks to months for blood cell concentrations to return to normal.

The Leukemias

Uncontrolled production of white blood cells can be caused by cancerous mutation of a myelogenous or lymphogenous cell. This causes *leukemia,* which is usually characterized by greatly increased numbers of abnormal white blood cells in the circulating blood.

Types of Leukemia

Leukemias are divided into two general types: *lymphocytic leukemias* and *myelogenousleukemias.*The lymphocytic leukemias are caused by cancerous production of lymphoid cells, usually beginning in a lymph node or other lymphocytic tissue and spreading to other areas of the body. The second type of leukemia, myelogenous leukemia, begins by cancerous production of young myelogenous cells in the bone marrow and then spreads throughout the body so that white blood cells are produced in many extramedullary tissues—especially in the lymph nodes, spleen, and liver.

In myelogenous leukemia, the cancerous process occasionally produces partially differentiated cells, resulting in what might be called *neutrophilic leukemia*, *eosinophilic leukemia*, *basophilic leukemia*, or *monocytic leukemia.* More frequently, however, the leukemia cells are bizarre and undifferentiated and not identical to any of the normal white blood cells.

Usually, the more undifferentiated the cell, the more *acute* is the leukemia, often leading to death within a few months if untreated.With some of the more differentiated cells, the process can be *chronic*, sometimes developing slowly over 10 to 20 years. Leukemic cells, especially the very undifferentiated cells, are usually nonfunctional for providing the normal protection against infection.

Effects of Leukemia on the Body

The first effect of leukemia is metastatic growth of leukemic cells in abnormal areas of the body.

Leukemic cells from the bone marrow may reproduce so greatly that they invade the surrounding bone, causing pain and, eventually, a tendency for bones to fracture easily.

Almost all leukemias eventually spread to the spleen, lymph nodes, liver, and other vascular regions, regardless of whether the origin of the leukemia is in the bone marrow or the lymph nodes. Common effects in leukemia are the development of infection, severe anemia, and a bleeding tendency caused by thrombocytopenia (lack of platelets). These effects result mainly from displacement of the normal bone marrow and lymphoid cells by the nonfunctional leukemic cells.

Finally, perhaps the most important effect of leukemia on the body is excessive use of metabolic substrates by the growing cancerous cells. The leukemic tissues reproduce new cells so rapidly that tremendous demands are made on the body reserves for foodstuffs, specific amino acids, and vitamins. Consequently, the energy of the patient is greatly depleted, and excessive utilization of amino acids by the leukemic cells causes especially rapid deterioration of the normal protein tissues of the body.Thus, while the leukemic tissues grow, other tissues become debilitated.

After metabolic starvation has continued long enough, this alone is sufficient to cause death.

The Factor Effect to Stimulate WBC:-

Leukocyte inducing factor (Plasma factor).

White blood cell growth factors, also called hematopoietic (blood-forming) colony-stimulating factors (CSFs), are proteins that help the body make white blood cells. White blood cells help fight infection. Many cancer treatments, such as chemotherapy, damage white blood cells. This can cause neutropenia, a very low level of white blood cells that increases the risk of getting an infection. When neutropenia occurs with a fever, it is called febrile neutropenia and may be a sign of an infection. Infections can be very serious for people with cancer because they often do not have enough white blood cells to fight the infection on their own and will usually need to be treated in the hospital with antibiotics. CSFs increase levels of white blood cells to help a person avoid infection. However, most patients receiving chemotherapy will not need CSFs. This is because most chemotherapy is only associated with less severe neutropenia, and the risk of severe neutropenia can usually be predicted ahead of time.

Platelets

It's a fregment of cell from megakaryocytes which add to the blood after RBCs (1-3µ) no nucli, occure after distruction of megakaryocyte250000-400000.

1-make plug to wall of vein (platetate piug).

2-release of thromboplastin activator which act to produce coagulation.

3-serotonine release (vasoconstruction) decrease the blood slow in the destroyed wall.(vascular spasm).

4-ADP release (actin retruction of clott)(blood coagulation this above hemastasis.

Haemostasis:-

Aims to decrease the amount of blood and also keep the injury wound clean.

Blood clott

Its convers of protein (fibrinogen) to gel.

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 may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.

Platelets release a multitude of growth factors including platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades.

- The physiological range for platelets is $(150 – 400) \times 10^3$ per mm^3.
- Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes.
- Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys.
- Each megakaryocyte produces between 5,000 and 10,000 platelets.
- Around 10^{11} platelets are produced each day by an average healthy adult.
- Reserve platelets are stored in the spleen, and are released when needed by sympathetically induced splenic contraction.
- The lifespan of circulating platelets is 5 to 9 days.
- Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.

Thrombus formation:-

The function of platelets is the maintenance of hemostasis. This is achieved primarily 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. These processes are regulated through thromboregulation.

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 by producing nitric oxide, endothelial-ADPase, and PGI_2. Endothelial-ADPase clears away the platelet activator, ADP.

Endothelial cells produce a protein called von Willebrand factor (vWF), a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen is not exposed to the bloodstream. vWF is secreted constitutively into the plasma by the endothelial cells, and is stored in granules within the endothelial cell and in platelets.

When the endothelial layer is injured, collagen, vWF and tissue factor from the subendothelium is exposed to the bloodstream. When the platelets contact collagen or vWF, they are activated (e.g. to clump together). They are also activated by thrombin (formed with the help of tissue factor). They can also be activated by a negatively charged surface, such as glass. Non-physiological flow conditions (especially high values of shear stress) caused by arterial stenosis or artificial devices (Mechanical Heart Valves, blood pumps etc.) can also lead to platelet activation.
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. Calcium ions are essential for binding of these coagulation factors.

Mechanism of clot

After injury there will be vasoconstriction due to decrease to blood flow by influence on sympathetic stimulation (adrenaline). This secretion will act as stronger vasoconstriction platelets will accumulate in injury site and make temporary plug to prevent more bleeding. Then coagulation occur.

Coagulation (homeostasis):-

Three stages (phases) of coagulation occur:-

1- **Enzymatic phase :** The enzyme play important role after injury thromboplastine release which occur from two sources:-

a- from damage tissue which are more active rapidly after release.(skin factor).

b- from destroyed of platelets itself (inactive enzyme). So there is must factor to inflence on this enzyme to make it active.

*Antihemophilic factor.

* Plasma thromboplastine anticidant +Vit K also help this (above to produce it). Then thromboplastine activated.

c- Convert of prothrobine to thrombin:-

Prothrombine manfuctured in liver with cooperative Ca++ with thromboplastine activator act to convert prothrombine to thrombin with help from two factors found in plasma.

1-Exciterater globulin.

2-proconvertive.

d-Convert the fibrinogen to fibrin by thrombin.

2-**Clotting phase:**

The fibrin like not have RBC& WBC & platelets adhesion with fibrin fiber so given clot(plug) in site of injury.

3-**rettration Phase:**

ADP play important role which lead retraction of clot & squeeze the serum.

*Antithromboplastine found in blood circulation which prevent for thromboplastine formation or make it inactive.
*Antithrombin: even thromboplastine activator the antithrombin will prevent prothrombin convert to thrombin.
* heparin:-anticoagulant produce in liver & lung but not in basophile.
Heparin anti convert prothrmbine to thrombin.
α_2-Macroglobin simillar to heparin & antithrombin. Its bind with coagulant factors & prevent there protyolytive.
If thrombus occur:-
And will analysis of thrombus in bloodvcirculation by plasmin secretion. Tis plasmin occur from plasminogen which found in plasma which activated by after enzyme called enterokinase. So plasmine will analysis of thrombus.
Trypsine will sometime digest the thrombus.
How to prevent coagulation in blood:- (in vitro)

1- Isolate fibrin by remove of blood glass rod the fibrin fiber separated from blood to glass rod and the blood called defibirinated.
Some type used metalic net float in blood & accumulation of fibers on it.
2- Oxalate $\longrightarrow$ precipitation of Ca++ $\longrightarrow$ prevent the aggulatation.(oxalated blood).
3- Dicumarine:-
Its important when animal feed on spoiled clover which lead to death due to bleed (no blood aggulation).(so its use in human fraction).
4- Heparin:- may be used commonly in blood transfusion because of its harmless & strong.
5- EDTA:- ethyline ditetraacetic acid.

Disorder in aggulation Process

1- May forma thrombus in blood circulatory.
2- Aggulation process may not happen naturally because avoid of thromboplastin activator which release from platelets. In this stage hemophilia will occur due to an activator of thromboplastine (antihemophilic factor depressed factor VIII).

Hemophilia:-Heredity disease in genes occur in male and female will carrier.

3-Reduce of platelates
Thrombocytopenia purpura:-This occur when blood leave its capillary to be & give blue color.
Cyanosis :- Occur in case of polycythemia.

$$\text{Blood Volume} = \frac{\text{plasma volume}}{\text{Hematocrit (PCV)}}$$

7% of total body weight are blood.($70cm^3/kg$)

Two ways to calculate blood volume:-

1-Direct way:-

Depend on animal bleeding until death and calculate blood volume so little blood found in blood vessels so wash it by injection of normal saline and then calculate it with bleeding blood.(some remain in heart).

2-Dilution way(Indirect):-

Depend on

$$\text{Dilution Volume} = \frac{\text{adding substance(mg)}}{\text{Substance concentration in dilution(mg/cm}^3\text{)}}$$

So the blood volume can calculated when know plasma volumeor RBC volume (haematocrit PCV) in adult 3L

$$\%\text{of plasma then blood volume} = \frac{\text{plasma volume}}{\text{Plasma percentage in blood}} \times 100$$

% of blood

$$\text{Blood volume} = \frac{\text{RBC volume}}{\text{RBC percentage in blood}} \times 100$$

Plasma volume calculate by two ways:-

1- Dilution :-by use dye (Evan blue) or endocynin green injection of one theses dye in peripheral blood volume and leave it for 10 min to be mixture with all blood and do standard dilution from the same dye to do the comparism collect blood sample along the exam period to determind the concentration then draw aline.