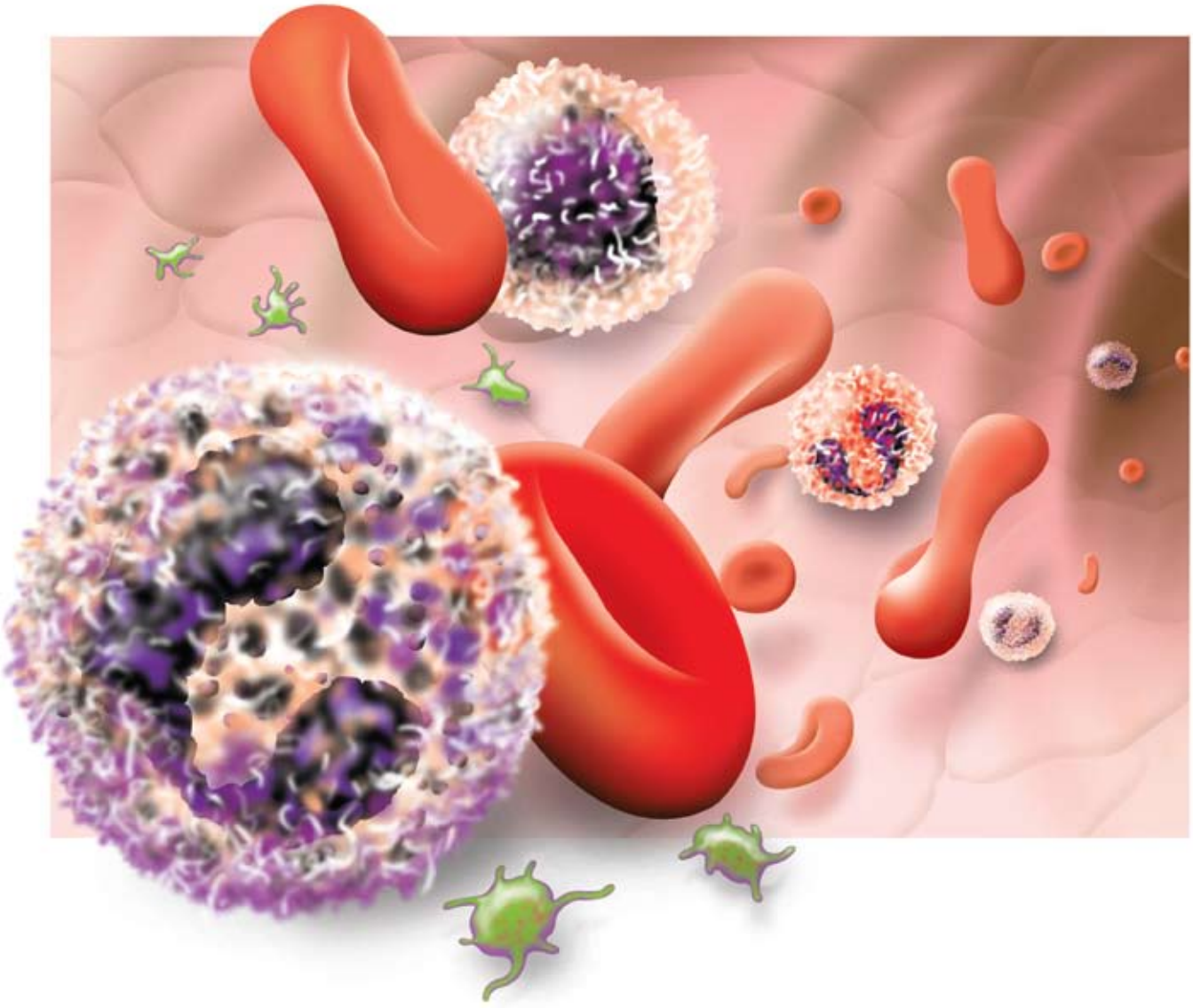
Learning Guide

Hematology



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Abbott Diagnostics Hematology Educational Services

Intended Audience

This Learning Guide is intended to serve the basic educational needs of health care professionals who are involved in the fields of laboratory medicine. Anyone associated with the testing of the formed elements of the blood will find this monograph of special interest.

The monograph features basic information necessary to understand and appreciate the importance of hematology testing in the laboratory and is intended for those who use the hematology laboratory services, including, but not limited to, laboratory technicians, laboratory technologists, supervisors and managers, nurses, suppliers, and other physician office and laboratory support personnel.

How to Use This Learning Guide

Each section begins with a section of learning objectives that will help you focus in the key concepts presented in the section. There is a short section review quiz at the end of each section designed to help you recall the concepts introduced. If you answer the questions incorrectly, review the appropriate portions of the section before moving to the next section.

A glossary of terms is included at the end of this Learning Guide for a quick reference of commonly used terms in the science of hematology.

Acknowledgements

On behalf of Abbott Diagnostics, I would like to acknowledge the work of Patrick Barnes, BGS, MT (ASCP) MA, Laboratory Manager, Hematology, Barnes-Jewish Hospital, St. Louis, MO, for his dedication to making this Learning Guide a useful resource for health care professionals.

I would also like to thank the Marketing and Communications Department for their significant contribution in the design and layout of the Learning Guide.

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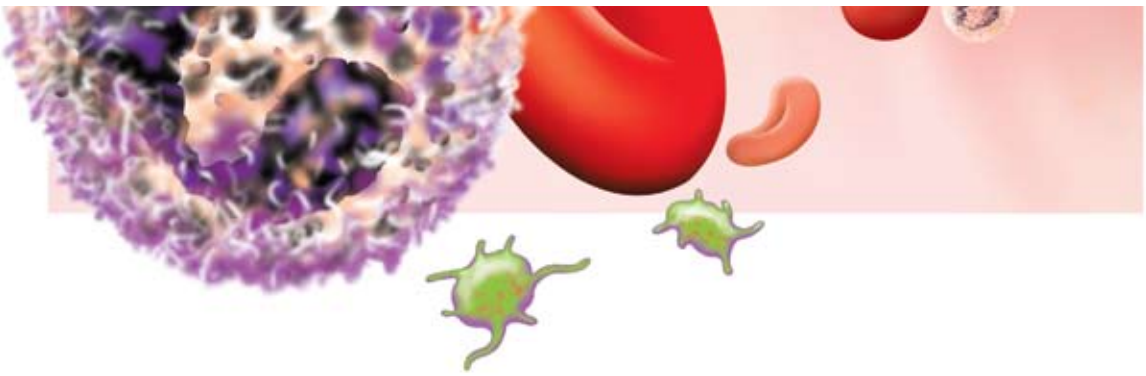
Contents



Introduction

Hematology Learning Guide

Hematology is defined as the study of blood (its blood forming tissues) and its components. Because of its critical role in maintaining life, blood has been referred to by some as ***"the river of life"***. Additionally, because routine examination of the blood by means of the complete blood count (CBC) is the most widely performed test in the clinical laboratory and has also been referred to as ***"a window on the body"***.

Blood is one of the most complex organ systems in the human body. The key parts that make up the hematological system are the blood, bone marrow, spleen, and lymph system. In the adult, blood consists of approximately 55% plasma (liquid component) and 45% formed elements including: erythrocytes (red blood cells – RBC), Leukocytes (white blood cells – WBC), and thrombocytes (platelets). Blood makes up about 7% of your body's weight. Blood is formed from hematopoietic stem cells in the bone marrow. Blood, as a whole, is responsible for the most important functions of life, such as the transport of metabolic components, nutrients, hormones, gas exchange, the immune defense, and coagulation.

The science of hematology literally is part of the evaluation of all disease states. Blood, the fluid that nourishes and cleanses the body, has been at the center of interest and investigation from early humans to the field of modern science it has become today.

We hope this Hematology Learning Guide from Abbott Diagnostics provides a basic understanding in this field of medicine.

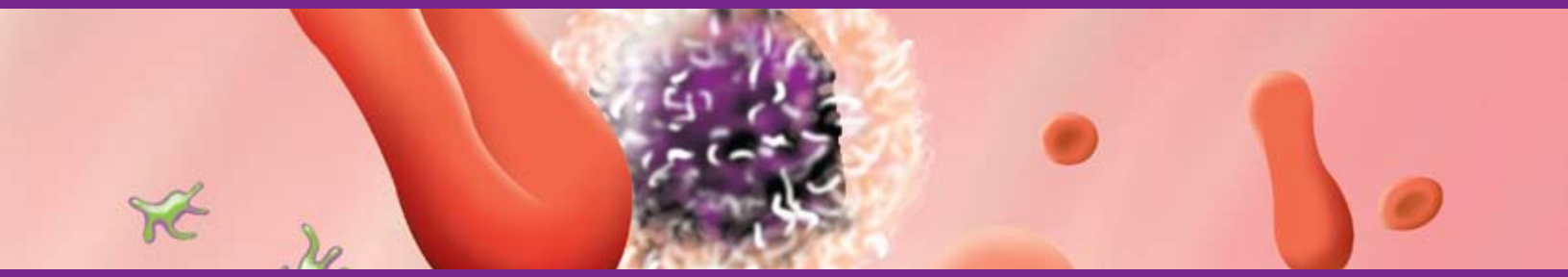
Section 1

PHYSIOLOGIC FEATURES OF THE BODY

Learning Objectives

When you complete this section, you will be able to:

1. Recognize physiologic features of the body in which blood plays an important role
2. Indicate the major categories of body chemicals



Physiologic Features of the Body

To understand the clinical uses of hematology, it is necessary to review some physiologic features of the body.

Water (about 60% of an adult's body mass) is the medium in which the body's chemicals are dissolved, metabolic reactions take place, and substances are transported. The chemical reactions that keep us functioning are mediated by body fluids.

Homeostasis. The generation, movement, metabolism, and storage of body fluids are balanced through complex interlocking chemical processes and feedback controls. This balance, called homeostasis, is a dynamic but steady state maintained by the constant expenditure of energy from cellular metabolism. This ensures that the body's fluids carry out functions such as circulation, digestion, excretion, reproduction, etc.

Metabolism. Interstitial fluid surrounds and bathes cells and is the medium for exchanges of nutrients and wastes between the blood and the cells. To perform their work, cells must receive oxygen and nutrients (carbohydrates, proteins, fats, water, minerals, and vitamins). Metabolism is the process by which cells take up, transform, and use nutrients. Waste material resulting from metabolism must be removed before it becomes toxic to cells.

The digestive system supplies the nutrients, and the respiratory system supplies the oxygen. The cardiovascular system (heart, blood vessels, and blood) is the delivery system.

Body Chemicals. The body's actions and reactions rely on chemicals that have special characteristics. These chemicals can be categorized into broad groups:

- Carbohydrates: energizers and sources of energy
- Lipids (fats): energy stores
- Proteins: structural forms, carriers, and sources of energy
- Enzymes: facilitators
- Hormones: chemical messengers
- Electrolytes: gatekeepers that allow movement of substances through cell walls

Testing for Variances

Normal cellular and metabolic functions are reflected by normal values or concentrations for the cells or chemicals involved in these functions. In disease states, tests show abnormally high or low levels of these chemicals or cells. Note, however, that due to variations in individuals, normal values are almost always given in ranges. Normal ranges may vary somewhat from one source to another and may be reported in a variety of units.

Due to its function as the body's delivery service, blood is a prime indicator of the body's status. Hematology usually refers to the study of gross features of blood such as cell counts, bleeding time, etc.

Now complete the section Quiz.

Review Questions • Section 1

1. Match the following:

__ A The medium in which the body's chemicals are dissolved and metabolic reactions take place	**1**. Metabolism
__ B Dynamic steady state maintained by constant expenditure of body energy	**2**. Water
__ C The process by which cells take up and use nutrients	**3**. Homeostasis
__ D The body's delivery system	**4**. Blood

2. Match the following actions and reactions to the body chemicals:

__ A Energizers and sources of energy	**1**. Lipids
__ B Structural forms, carriers, and sources of energy	**2**. Hormones
__ C Facilitators	**3**. Carbohydrates
__ D Chemical messengers	**4**. Proteins
__ E Energy stores	**5**. Electrolytes
__ F Gatekeepers that allow movement of substances through cell walls	**6**. Enzymes

3. Define hematology:

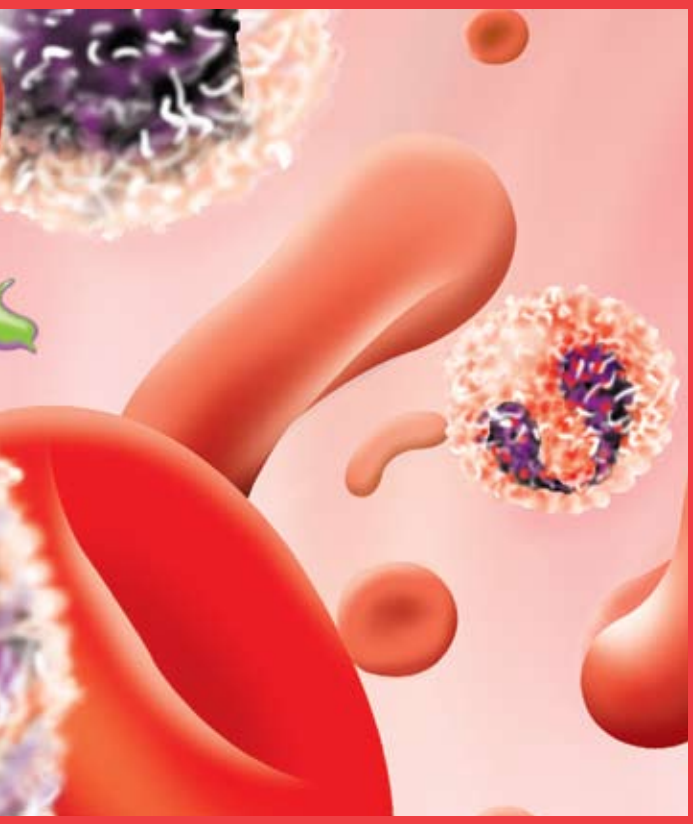
Section 2

OVERVIEW OF BLOOD

Learning Objectives

When you complete this section, you will be able to:

1. Indicate the four major functions of blood
2. Recognize the components of blood
3. Identify the chief characteristics of red blood cells, white blood cells, and platelets
4. Recognize normal values for the cellular elements in blood
5. Recognize a general description of blood cell formation
6. Indicate the role of the spleen



Functions of Blood

Blood is essential to all cell life. It distributes oxygen, nutrients, electrolytes, hormones, and enzymes throughout the body. ***Table 1*** shows four major functions of blood.

Four major functions of blood

Transports	From:	To:
Oxygen (O_2)	Lungs	All cells
Carbon dioxide (CO_2)	Cells	Lungs
Nutrients	Digestive organs	All cells
Waste products	All cells	Organs of excretion
Hormones	Endocrine glands	Target organs
Regulates fluid and electrolyte balance between cells and interstitial fluid, and of body and cell temperature		
Protects against toxins produced by bacteria		
Prevents fluid loss through the clotting mechanism		

Table 1. *Primary functions of blood.*

Components of Blood

Blood consists of plasma and formed elements. Plasma, which makes up about 55% of blood, is the straw-colored clear liquid in which cellular elements and dissolved substances (solutes) are suspended. (Serum is the fluid portion of the blood that remains after fibrin and the formed elements have been removed with centrifugation.) Plasma is approximately 92% water and 8% a mixture of both organic and inorganic substances. ***Table 2*** shows the components of plasma and their functions.

Components of Plasma and Their Functions
WATER (92%): Carries formed elements and dissolved substances; absorbs heat
Major proteins. Albumin controls water movement across membranes; affects blood viscosity (thickness), pressure, and volume; transports substances such as drugs. Globulin forms antibodies to fight bacteria and viruses. Fibrinogen forms fibrin and, with platelets, coagulates blood
Nonprotein nitrogen. Products of metabolism: urea, uric add, creatine, creatinine, ammonium salts; toxic if not removed; carried in blood to organs of excretion
Products of digestion. Amino acids, glucose, fatty adds – all needed by cells for energy, repair, and reproduction
Regulatory substances. Enzymes for cellular chemical reactions; hormones to regulate growth and development
Electrolytes. Sodium (Na^+), potassium (K^+), calcium (Ca^{++}), magnesium (Mg^+), chloride (Cl^-), phosphate (PO_4^-), sulfate (SO_4^-), bicarbonate (HCO_3^-), and inorganic salts

Table 2. *Components of plasma.*

Blood Cells and Platelets

The blood has three types of formed elements: erythrocytes (red blood cells), leukocytes (white blood cells), and platelets or thrombocytes (***Figure 1***).

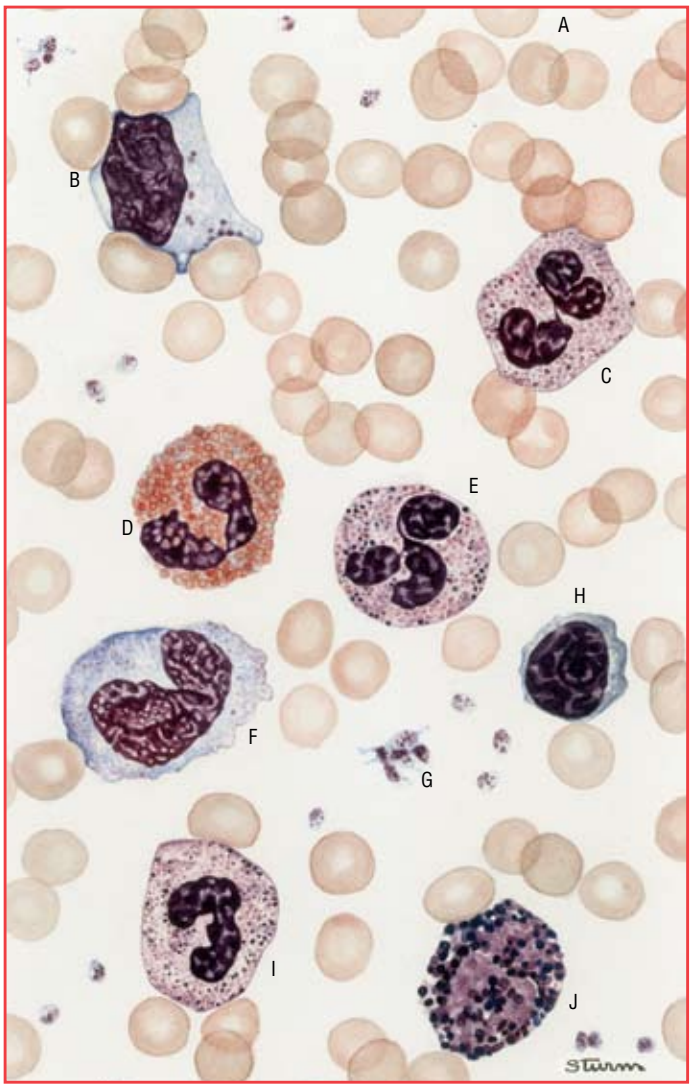


Figure 1. *The blood has three types of formed elements: erythrocytes (red blood cells), leukocytes (white blood cells), and platelets or thrombocytes. Blood cells and platelets. A = erythrocytes, B-F = leukocytes, G = platelets (thrombocytes), H-J = leukocytes.*

Most blood samples are measured in microliters (µL); to give you a frame of reference, a drop of blood is roughly 30 µL.

Erythrocytes. Also called red blood cells (RBCs), erythrocytes are the body's most numerous blood cells. Average normal values indicating numbers of RBCs in the blood may be recorded as 4.60 x 10^6/µL (4.60 million/ µL) for women and 5.20 x 10^6/µL (5.20 million/µL) for men.

To transport oxygen and carbon dioxide through the circulation, each RBC contains approximately 280 million hemoglobin molecules. Each hemoglobin molecule contains four iron atoms. As RBCs pass through the lungs, iron atoms combine with oxygen molecules. RBCs travel the circulatory system until, at the tissues, iron atoms release oxygen into interstitial fluid and hemoglobin molecules take up carbon dioxide. Back at the lungs, RBCs release carbon dioxide and take up oxygen again.

Leukocytes. The body normally contains 4,500-11,000 white blood cells (WBCs) per µL of blood. This value may be reported as 4.5-11.0 x 10^3/µL or k/µL (k = thousand). Unlike RBCs, WBCs occur in many different types. Most WBCs are filled with tiny grains and are called granulocytes (gran = grain). The normal range for granulocytes is 1.8-8.5 x 10^3/µL of blood. Granulocytes include:

- **Neutrophils:** 50%-70% of total WBCs or about 1.8-7.7 x 10^3/µL
- **Eosinophils:** up to 5% of total WBCs or about 0.0-0.450 x 10^3/µL
- **Basophils:** up to 2% of total WBCs or about 0.0-0.2 x 10^3/µL

Lymphocytes and monocytes are non-granular WBCs. The normal range for lymphocytes and monocytes are:

- **Lymphocytes:** 20%-47% of total WBCs or about 1.0-4.8 x 10^3/µL
- **Monocytes:** 3%-10% of total WBCs or about 0.0-0.8 x 10^3/µL

WBCs fight infection; some surround and destroy debris and foreign invaders while others produce antigen/antibody reactions [an antigen is any substance (foreign or part of the body) which causes production of an antibody; an antibody is a protein which neutralizes antigens].

Nice to know…

- The average adult circulation contains 5 liters of blood (roughly 5.28 quarts).
- Blood completes the entire systemic circuit – from left heart through the body to right heart – in 90 seconds.
- Every cubic millimeter of blood contains 5 million RBCs.
- RBCs survive about 4 months; neutrophils survive about 6 hours.

Platelets. Also called thrombocytes, platelets are cell fragments that travel in the bloodstream. The normal range for platelets is 140-440 x 10^3/µL of blood.

Platelets help prevent blood and fluid loss by clumping together to begin the coagulation process. A blood clot is formed when sticky platelets become covered with fibrin – a plasma protein that holds the blood clot together.

Each of the formed elements of blood will be covered in more detail in the following sections.

Hematopoiesis: The Formation of Blood Cells

All blood cells begin as undifferentiated stem cells capable of reproducing themselves. Generations of cells eventually differentiate into cell lines that will mature to produce erythrocytes, leukocytes, and platelets. Stem cells in bone marrow continuously proliferate – usually at a steady state to maintain a constant population of mature blood cells. A disruption in this process can lead to serious illnesses.

As a group, immature cells are large. As they age and mature, they become smaller and change in their reaction to the dyes used to stain them for identification.

Role of the Spleen. Located beneath the diaphragm and behind the stomach, the spleen is an intricate filter that receives 5% of the total blood volume each minute. In the embryo, the spleen is a blood-forming center; it loses this function as the fetus matures.

In the spleen, RBCs and WBCs are "inspected" by specialized WBCs. Old or damaged cells are removed; salvageable cells may be "pitted", that is, unwanted particles are removed without destroying the cells.

Because it is not essential to life, the spleen may be removed (splenectomy) without serious effects.

Now complete the section Quiz.

Review Questions • Section 2

1. What are the four major functions of blood?

2. Which of the following statements are true?

   A. Blood consists of plasma and formed elements.

   B. Plasma is a straw-colored clear liquid containing cellular elements and solutes.

   C. Plasma is approximately 92% water.

   D. All of the above statements are also true of serum.

3. Fill in the following blanks to characterize red blood cells.

   A. The average normal values for RBCs in the blood is_____M/µL, Women; _____M/µL, Men.

   B. To transport oxygen and carbon dioxide through the circulation, each RBC contains _____atoms and_____molecules.

4. Which of the following statements are true?

   A. For WBCs, the normal range is 4.5-11.0 x 10^3/µL of blood.

   B. Most WBCs are granular.

   C. Neutrophils are the most common granulocytes.

   D. Lymphocytes account for 80% of non-granular WBCs.

   E. WBCs primarily fight infection.

5. Which of the following statements are true? Platelets are:

   A. Disk-shaped cell particles

   B. Also called thrombocytes

   C. Numerous: 140-440 x 10^3/µL of blood

   D. Able to clump together to begin the coagulation process

   E. Able to release enzymes to destroy bacteria

6. Which of the following statements are true? Blood cell formation is a process involving:

- A The differentiation and maturation of various cell lines
- B Production of new cells to maintain a constant population of mature cells
- C Proliferation of mature cells

7. Which of the following statements are true? In adults, the spleen serves as a:

- A Blood-forming center
- B Filter for blood cells
- C Means of removing unwanted blood cells
- D Critical inspector of blood cells

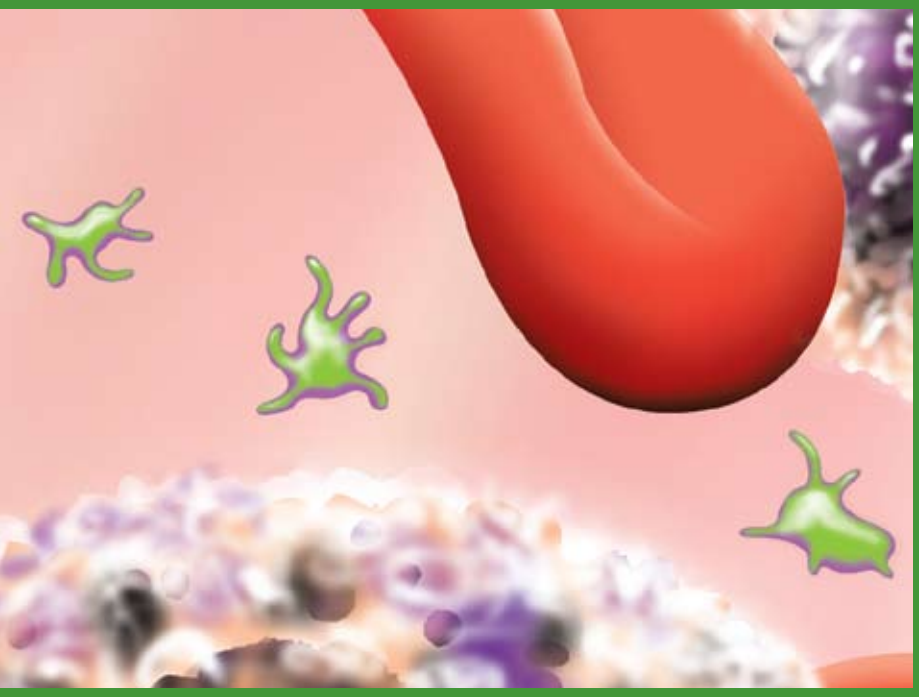
Section 3

RED BLOOD CELLS

Learning Objectives

When you complete this section, you will be able to:

1. Recognize how RBCs are formed
2. Indicate why some RBCs may be immature
3. Recognize conditions that stimulate production of RBCs
4. Name two substances needed for RBC maturation
5. Recognize the roles of hemoglobin, transferrin, and ferritin



Description

An erythrocyte (erythro = red) is a fully mature red cell found in the peripheral blood. Although highly specialized, it is little more than a small bag – a membrane surrounding a solution of protein and electrolytes.

In appearance, an erythrocyte is a bi-concave, disc-shaped cell, somewhat like a doughnut that has no hole. Small enough to pass easily through capillaries in single file, RBCs can change shape into almost any configuration – a characteristic that gives them access to all tissue cells.

Formation of RBCs

RBCs are formed continuously, but their number is precisely regulated. Too few cells will not oxygenate tissue; too many cells will impede blood flow. Mature RBCs cannot reproduce themselves, so several million new cells enter the blood daily from blood-forming centers in bone marrow. The term for red blood cell formation is erythropoiesis.

Life Span. When an RBC is about 120 days old, it is trapped and removed from the blood by the spleen or the liver. Its iron atoms, however, are recycled; approximately 25 milligrams of iron become available daily from the breakdown of old RBCs.

Bone Marrow. Virtually all bones in children up to the age of 5 are blood-forming centers. Bone marrow becomes less productive as age increases. In adults (over age 20), RBCs are formed in the marrow of the vertebra, sternum (breastbone), ribs, and the ends of the long bones.

Cell Generations. RBCs develop in a series of cell generations in the bone marrow (***Figure 2***). After several generations, new cells called basophilic erythroblasts emerge. (A note about naming: WBCs are dyed or stained for viewing microscopically. A cell with basophilic staining properties is a cell that stains specifically with basic dyes; a cell with eosinophilic staining properties is a cell that stains with eosin, a red acidic dye. Erythroblast literally means an immature red cell.)

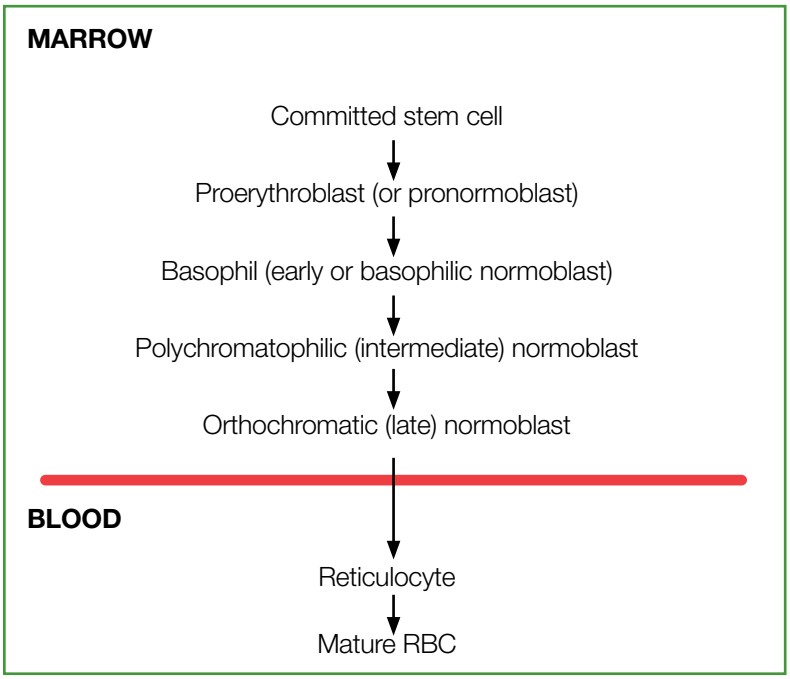


Figure 2. *Formation of RBCs.*

In the next cell generations, hemoglobin begins to give the cells their typical red color and the nucleus is extruded from the cell. The cell is now called a reticulocyte (reticula = network) because staining causes strands of residual RNA cell content to clump into a network.

Reticulocytes pass into the capillaries by diapedesis (squeezing through the pores of the membrane) and become mature RBCs within one or two days. These cells normally make up about 1% of circulating RBCs.

Regulation of RBC Production

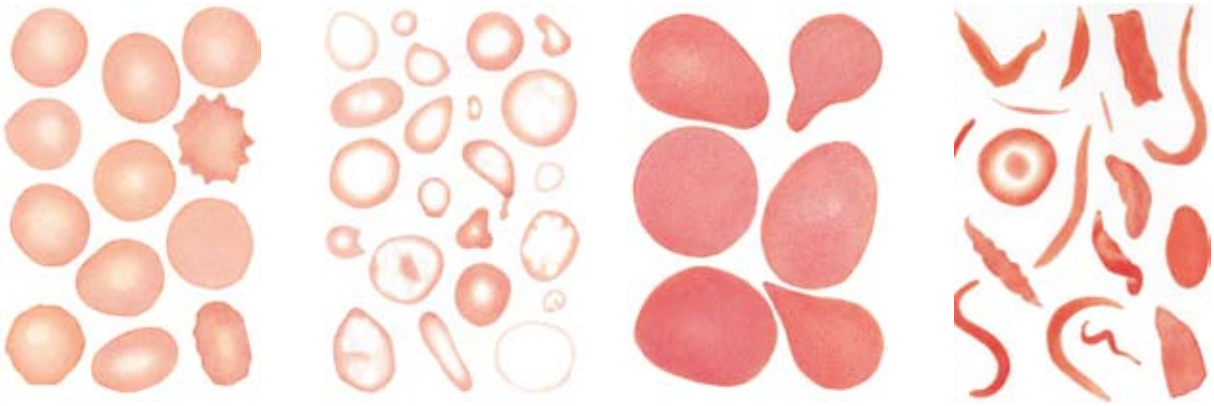
Tissue oxygenation regulates RBC production. If the amount of oxygen transported to the tissues decreases, the rate of RBC production increases (***Table 3***).

Conditions That Stimulate RBC Production
Low blood volume; blood loss due to hemorrhaging
Anemia; low RBC content due to destruction or lack of production of RBCs; low hemoglobin in blood
Destruction of bone marrow as in radiation therapy
Lack of available oxygen due to high altitudes
Poor blood flow due to circulatory diseases such as heart failure
Lung diseases that decrease absorption of oxygen

Table 3. *Stimulation of RBC production.*

The Feedback Mechanism. Tissue hypoxia (abnormally low oxygen level) triggers the kidneys to increase production of erythropoietin. This hormone stimulates bone marrow to produce stem cells and speeds the passage of newly formed RBCs through the various generations. Bone marrow continues producing RBCs as long as tissue hypoxia is present or until tissue is adequately oxygenated. Feedback signals to bone marrow diminish production of erythropoietin to the level needed to maintain the normal production of RBCs.

RBC production can be judged by counting reticulocytes in the blood and by ferrokinetics, that is, the amount and traffic of iron in the blood.



Normal | **Iron-deficient anemia** | **Megaloblastic anemia** | **Sickle cell anemia (Hb SS)**

Figure 3. *Normal compared to several types of abnormal RBCs.*

If bone marrow produces many RBCs rapidly, immature cells are released into the blood. The number of reticulocytes can increase to as high as 30%-50% of total circulating RBCs. Erythroblasts (NRBCs – nucleated red blood cells) may also appear in the circulating blood.

Maturation of RBCs depends on the presence of two substances – vitamin B12 (cyanocobalamin) and folic acid (another member of the vitamin B complex). Both are needed for cell synthesis of DNA (deoxyribonucleic acid, the genetic material that controls heredity). Both are obtainable from a normal diet and must pass through the gastrointestinal (GI) tract.

Lack of one or both of these substances inhibits RBC production and causes blood cells forming in the bone marrow to enlarge. Called megaloblasts (literally, large immature cells) in the forming stage and macrocytes (large cells) as adult RBCs, these abnormal cells are irregular in shape, have flimsy membranes, and often contain excess hemoglobin. Macrocytes carry oxygen but have a very short life.

Poor absorption of vitamin B12 is due to lack of secretion of intrinsic factor by the gastric mucosa. Intrinsic factor must be present for absorption and utilization of vitamin B12. Folic acid deficiency may be due to lack of vitamin C. These absorption and deficiency problems can arise in alcoholics, geriatric patients, and in pregnant or lactating women.

Hemoglobin

Hemoglobin, the oxygen-carrying component of RBCs, is composed of two pairs of protein chains called globin and four smaller units called heme, which contain iron. Iron binds and releases oxygen (O_2). Decreases in hemoglobin reduce the amount of O_2 carried by the blood to cells.

The oxygen-carrying capacity of hemoglobin can be affected by the formation of gases that can prevent O_2 from reaching cells and by abnormalities of hemoglobin production and destruction.

Iron

Although iron is important in cellular metabolism and oxidation, the body needs only trace amounts of two types of iron:

- Functional iron, a component of hemoglobin and myoglobin (a pigment in muscles); approximately 70% of body iron
- Iron stored in the forms of ferritin and hemosiderin

Iron that is not part of hemoglobin is bound to the blood protein transferrin, which transports it to storage tissues (liver, bone marrow, and spleen). In storage tissues, iron binds to another protein to form ferritin. Normally about 35% of the circulating transferrin is saturated with iron. The degree to which transferrin is saturated with iron indicates the iron supply for developing RBCs.

To keep hemoglobin and other functional iron levels constant, the body draws iron from storage. Because it is readily mobilized when iron is lost (through hemorrhage) or inadequate (poor diet), ferritin is depleted early in iron deficiency. Accurate ferritin measurement often reveals iron-deficient anemia before other laboratory values change.

Now complete the section Quiz.

Review Questions • Section 3

1. Which of the following statements are true?

   A. New RBCs are continually being formed.

   B. RBCs are recycled constantly through the liver.

   C. RBCs increase by reproducing themselves.

   D. In children, virtually all bones are blood-forming centers.

2. Which of the following are events in the formation of RBCs?

   A. Stem cells in the bone marrow form proerythroblasts.

   B. Hemoglobin forms in red cells after it enters the blood.

   C. Immature cells extrude the cell nucleus.

   D. Reticulocytes are the first generation RBCs.

3. Fill in the blank. Tissue_____ is the basic regulator of RBC production.

4. Conditions that stimulate RBC production include:

   A. Low blood volume

   B. Anemia

   C. Chemotherapy

   D. High altitudes

   E. Circulatory diseases

5. True or False? If the statement is false, write the correction in the space below.

   T

   F

Bone marrow continues producing RBCs as long as tissue hypoxia is present or until adequate tissue oxygenation is accomplished. Production of erythropoietin then diminishes to the level needed to maintain the normal production of RBCs.

6. Why are immature RBCs sometimes present in the blood?

   A. Too rapid production of RBCs

   B. Lack of folic acid

   C. Lack of vitamin B12 (cyanocobalamin)

   D. Inadequate dietary iron

7. The oxygen-carrying component of RBCs can be affected by:

   A. Gases in the blood

   B. Some drugs

   C. Genetic abnormalities

   D. Abnormal red cell destruction

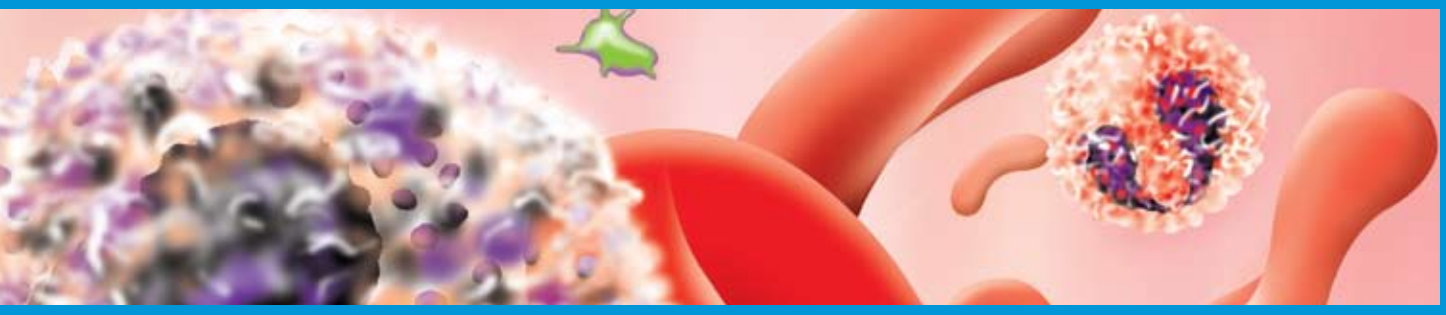
Section 4

DISORDERS OF RED BLOOD CELLS

Learning Objectives

When you complete this section, you will be able to:

1. Define anemia
2. Recognize terms used to describe RBC changes in anemia
3. Indicate types of anemia
4. Describe polycythemia



Anemia

Anemia indicates a deficiency of RBCs. It is a clinical sign, not a diagnosis. The terms that physicians may use to describe types of defective RBCs and anemias are listed and defined below.

anisochromatic: Unequally distributed hemoglobin in RBCs; the periphery is pigmented but the centers are almost colorless (*aniso-* means unequal, -chromic means color)

anisocytosis: A condition in which RBCs vary considerably in size

hypochromic: Pale; less than normal hemoglobin

macrocytic: Larger than normal non-nucleated RBC; macrocytic hyperchromic cells contain large amounts of hemoglobin due to their size

microcytic: Smaller than normal non-nucleated RBC

normochromic: Normal color; normal hemoglobin

normocytic: Normal healthy cell

poikilocytosis: A condition in which RBCs have irregular shapes (*poikilo-* means irregular)

The following are some of the most common anemias. They are arbitrarily grouped according to RBC color and size; other sources may group them in different ways.

1. Anemia Due to Bleeding: Hypochromic-Microcytic Anemia

A temporary anemia can occur after a rapid hemorrhage due to trauma such as a car accident, surgery, or childbirth. The body quickly replaces plasma, but it usually needs several weeks to replace RBCs. With chronic blood loss, as in undiagnosed internal hemorrhaging, anemia develops due to lack of sufficient functional iron and RBCs contain too little hemoglobin.

2. Anemia Due To Deficient RBC Formation: Hypochromic-Microcytic Anemia

Iron-deficient anemia is very common. It is often due to defective production of heme or globin. The iron deficiency may, in turn, be due to:

- Lack of dietary iron (strict vegetarianism or poor diet)
- Defects in iron utilization. Called sideroblastic anemia, the condition is characterized by ineffective RBC formation and the presence of ferritin in developing RBCs
- Defects in iron reutilization. This is the second most common form of anemia and is related to a variety of chronic diseases, infections, and cancers
- Defects in iron transport; a very rare condition called atransferrinemia

3. Anemias Due to Deficient RBC Formation: Normochromic-Normocytic Anemias

Anemia Due to Renal Failure. Chronic kidney failure shortens the life span of RBCs, probably due to an interaction with uremia (excess nitrogen waste in the blood). Replacing RBCs is hindered because failing kidneys release erythropoietin inefficiently.

Aplastic or Hypoplastic Anemia. Both aplastic and hypoplastic indicate incomplete or defective development. They describe an anemia that arises from partial failure of the bone marrow. Some physicians prefer the term pancytopenia, which means reduced numbers of all types of blood cells. Causes include x-rays, toxic chemicals (benzene, DDT), therapeutic drugs (chloramphenicol, phenylbutazone), or infections such as viral hepatitis.

4. Anemias Due to Excessive RBC Destruction

Hemolytic Anemias. Hemolysis (*lysis* = break down) is a process in which red cells fall apart and spill out their hemoglobin. Some hemoglobin normally leaks out of cells and dissolves in plasma. Anemia arises when hemolysis occurs in response to a number of factors: spleen dysfunction, immunologic abnormalities, trauma, blood transfusion reactions, malaria, use of some therapeutic drugs, exposure to toxic chemicals, or infections.

Genetic hemolytic conditions include a deficiency in the enzyme G6PD (glucose 6-phosphate dehydrogenase). In some inherited conditions, RBC abnormalities cause the cells to rupture easily, especially in the spleen. In the resulting anemias, sufficient or even excessive RBCs are formed, but their life span is very short. Types of hemolytic anemias due to RBC defects include:

- Hereditary spherocytosis. Microcytic spherical RBCs rupture easily.
- Sickle cell anemia. Hemoglobin S, an abnormal hemoglobin, precipitates into crystals when exposed to oxygen, damaging the cell membrane and causing it to assume a sickle shape. Anisocytosis and poikilocytosis are present. This serious condition is present in about 1% of West African and American Blacks.
- Thalassemia. Faulty or incomplete formation of hemoglobin causes production of tremendous numbers of hypochromic, microcytic, fragile RBCs that often rupture before leaving the bone marrow. Anisocytosis and poikilocytosis are present.
- Erythroblastosis fetalis. RBCs in the fetus are attacked by antibodies from the mother, causing the child to be born with serious anemia.

5. Megaloblastic Anemia (Pernicious Anemia)

These patients remain in constant need of RBCs due to production of oversized, strangely shaped, normochromic RBCs that rupture easily. Causes are inadequate vitamin B12 or folic acid, or both (***Table 4***). Pregnancy increases folic acid requirements and can lead to "megaloblastic anemia of pregnancy" in the last trimester and in pregnancies involving multiple fetuses.

Underlying Causes of Vitamin B12 and/or Folic Acid Deficiencies
Insufficient diet (due to poverty, strict vegetarianism)
Damage to the stomach lining
Gastrectomy; total removal of the stomach
Inflammatory conditions of intestines; such as sprue, a tropical disease
Intestinal parasites
Malabsorption syndromes
Alcoholism
Pregnancy and lactation

Table 4. *Causes of B12 and/or folic acid deficiencies.*

Polycythemia

This is the opposite of anemia; the term literally means "many cells in the blood". The blood becomes highly viscous (thick) and flows sluggishly. The condition results when tissues become hypoxic. Examples include:

- Secondary polycythemia. People who live in high altitudes automatically produce large quantities of RBCs due to the sparse oxygen in the air. A patient with cardiac failure is also likely to develop this condition; the inefficient heart is not able to deliver enough oxygenated blood to tissues.
- Polycythemia vera (also erythremia). This tumorous condition of the blood-forming organs causes production of massive amounts of RBCs, WBCs, and platelets. The most common cell line elevated in polycythemia vera is the RBC often referred to as red cell dyscrasia.

Cold Agglutination

This is a disorder in which RBCs – in their own serum or in other serum – clump together (agglutinate) in response to slight cooling (below 86° F). The clumping is caused by a group of antibodies called agglutinins. Cold agglutinins may be present in elderly people or may be due to infections such as those causing atypical pneumonia or infectious mononucleosis. The agglutination may be mild and transient or may progress into a disease called "cold antibody disease" or "cold agglutinin disease". Hemolysis, hemoglobinuria, and hemosiderinuria may be present.

The presence of cold agglutinins in the blood is likely to cause discrepancies in the results of blood tests.

Now complete the section Quiz.

Review Questions • Section 4

1. Write a brief definition of anemia.

2. Match the following terms with their definitions.

__ A Normochromic	1. Smaller than normal
__ B Normocytic	2. Larger than normal
__ C Hypochromic	3. Pale color
__ D Microcytic	4. Normal cell
__ E Macrocytic	5. Normal color

3. Anemias may arise directly or indirectly due to:

A Bleeding
B Iron deficiency
C Kidney failure
D Removal of the spleen
E Alcoholism

4. Sickle cell anemia is:

A One of the hemolytic anemias
B Characterized by RBCs with different shapes and sizes
C A megaloblastic anemia
D Caused by radiation exposure

5. A probable cause for hypochromic-microcytic anemia is:

A Defective production of heme or globin
B Pancytopenia
C Hemorrhage
D Thalassemia

6. A pregnant patient who practices strict vegetarianism may be a candidate for:

A Polycythemia
B Megaloblastic anemia
C Sideroblastic anemia

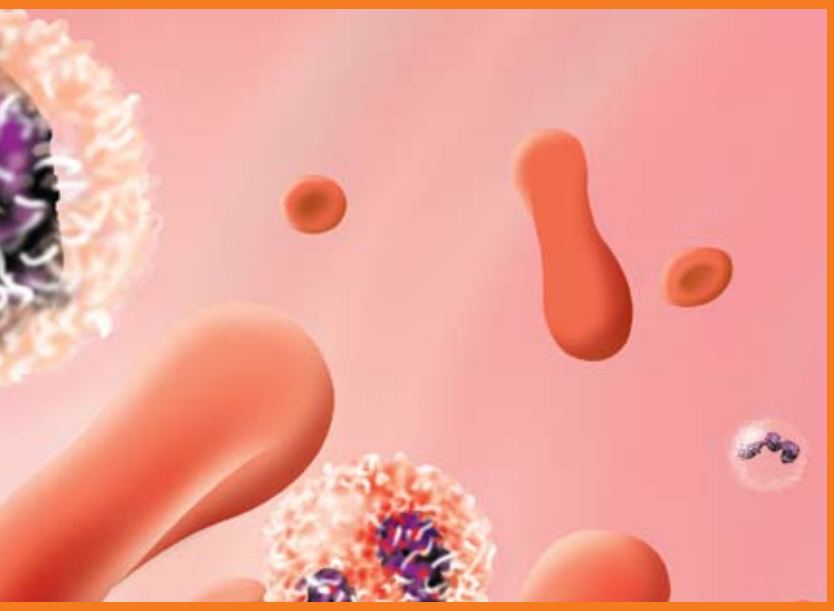
Section 5

WHITE BLOOD CELLS

Learning Objectives

When you complete this section, you will be able to:

1. Recognize descriptions of the various WBCs
2. Indicate their primary functions



Description and Classification

White blood cells are described and classified in several ways:

1. By function:
   Defense cells; phagocytes (granulocytes, macrophages)
   Cells that produce antibodies and cellular immunity (lymphocytes, plasma cells)
2. By the shape of the nucleus: Polymorphonuclear or mononuclear
3. By the site of origin: Myeloid (*MYE-* = bone marrow), lymphoid (lymph refers to the lymphatic system and the fluid collected from tissues that flows through the lymph vessels and is added to venous blood)
4. By staining characteristics: Granulocytes, non-granulocytes; neutrophils, eosinophils (eosin is an acid dye), basophils

Formation

Granulocytes begin with the stem cells. Lymphocytes have their origin with common progenitor cells, the origin of the stem cells (***Figure 4***).

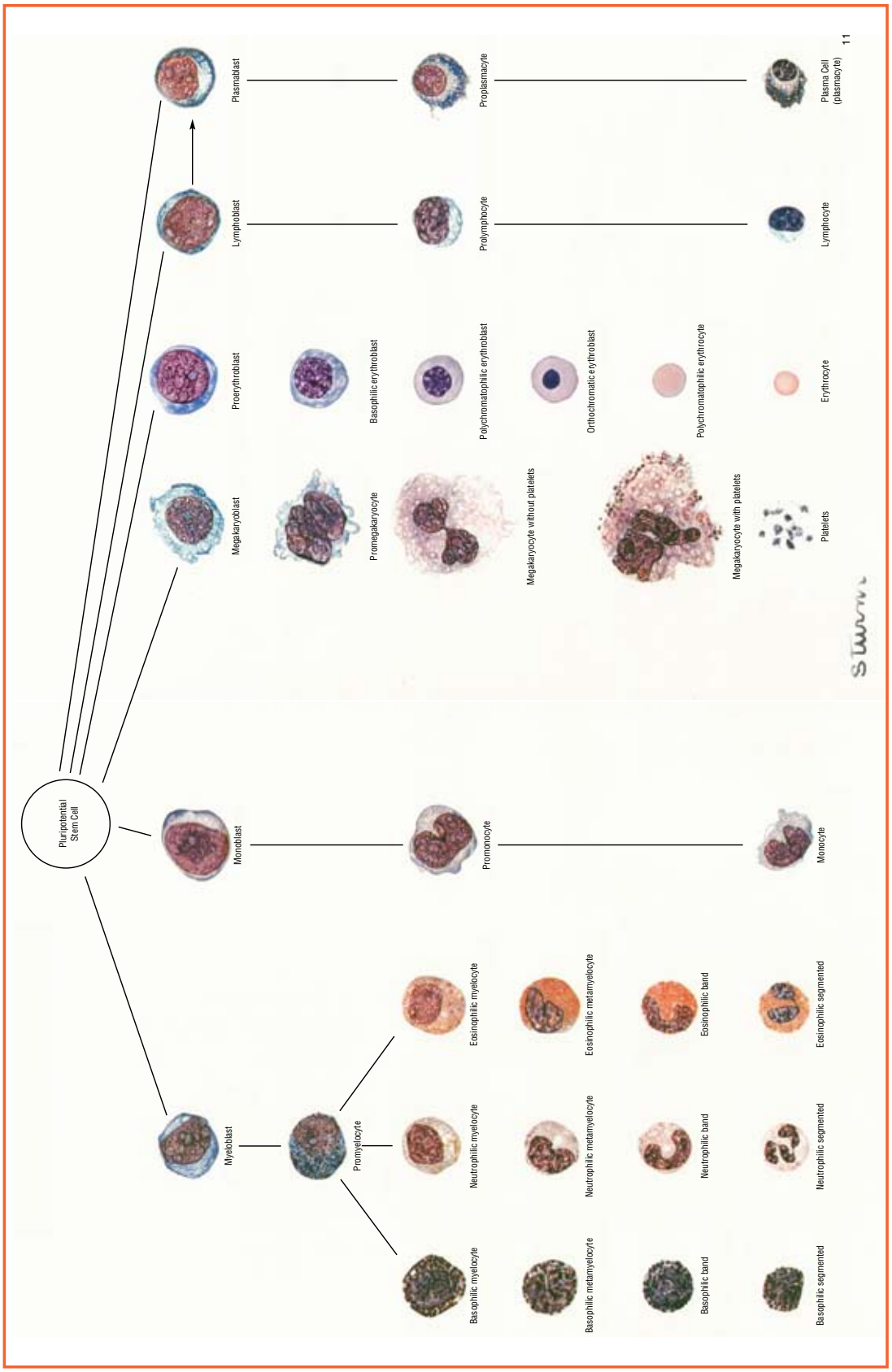


Figure 4. *The developmental progression of WBCs.*

Granulocytes

Granulopoiesis is the formation of granulocytes, the most numerous white cell. As a granulocyte matures, the cell nucleus undergoes many changes; it shrinks, indents, assumes a band form, and segments. Granules containing enzymes and antibacterial agents appear; they are clearly evident in ***Figure 5***. Myelocytes are distinguished according to the staining characteristics of their granules: neutrophilic, eosinophilic, and basophilic. Mature granulocytes are polymorphonuclear cells (PMNs) (sometimes called polys).

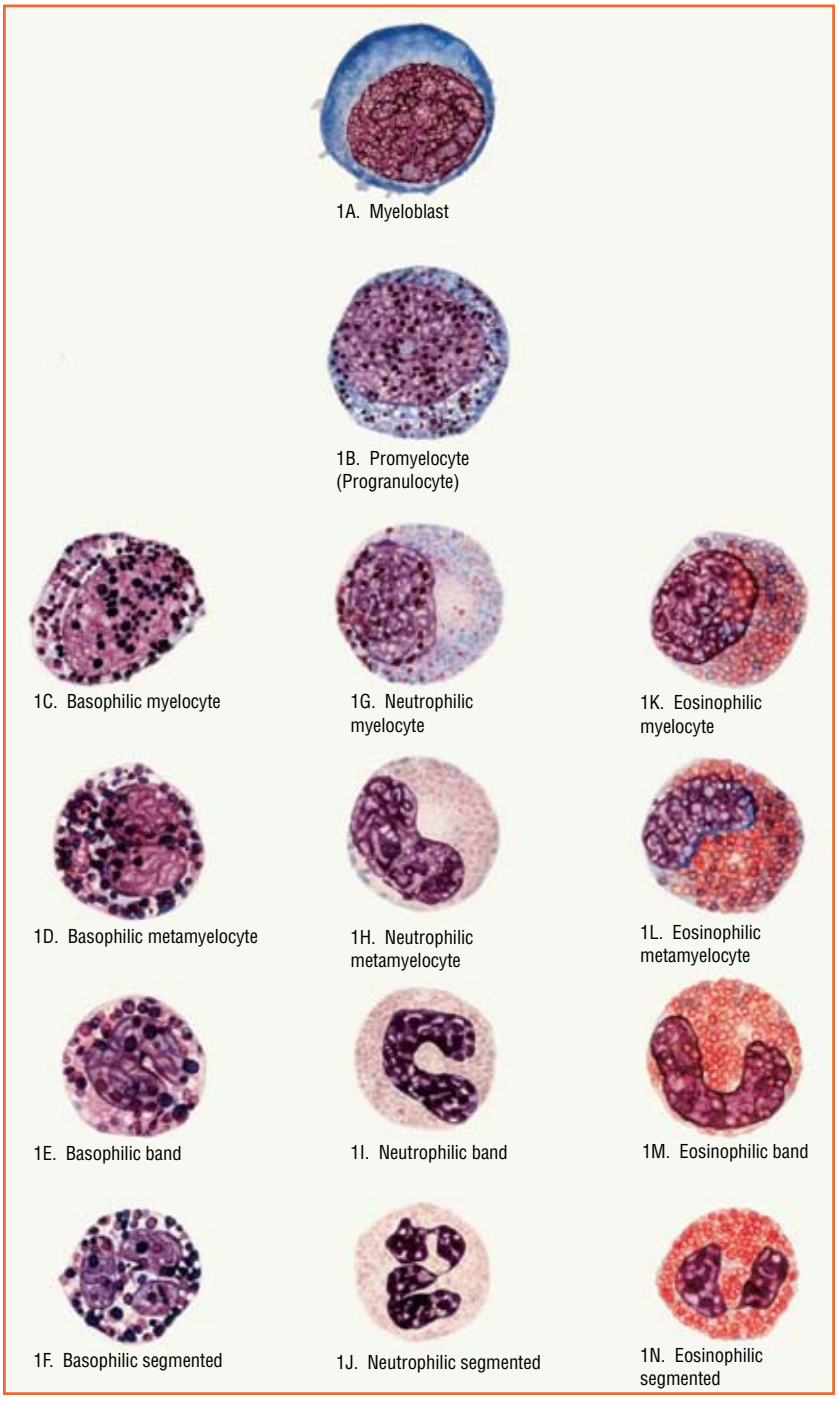


Figure 5. *Granulocytes. Granules are evident in each cell. C-F are basophils, G-J are neutrophils, and K-N are eosinophils. E, I, and M are band forms. F, J, and N are segmented forms.*

Normally, granulocytes are regulated at a constant level. During infection, the number of granulocytes rises dramatically.

Functions of Granulocytes. Neutrophils seek out and kill bacteria – a process called phagocytosis (see below). Eosinophils attack some parasites and inactivate mediators released during allergic reactions. Basophils contain histamine and are important in immunity and hypersensitivity reactions; they also contain heparin (an anti-clotting substance), but their role in blood clotting is uncertain.

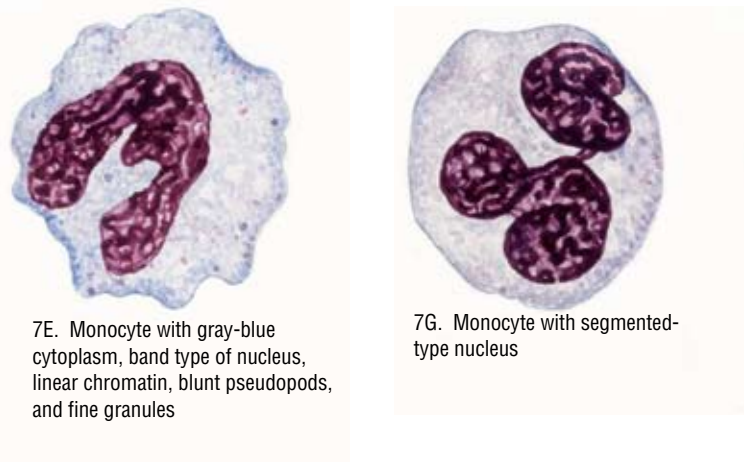
Quick Review: Phagocytosis

Meaning	Literally, Cell Eating
Cells	Phagocytes – neutrophils (PMNs, polys), macrophages
Process	Foreign substances (antigens) invade
Chemotaxis	Plasma factors (including lymphocytes and basophils) attract phagocytes to the invaded area
Opsonization	Plasma factors (immunoglobulins) coat antigens to make them "tasty" for phagocytes

Phagocytes bind to and ingest antigens. (Neutrophils can ingest 5-25 bacteria before dying; macrophages live longer because they can extrude toxic substances.)

Monocytes/Macrophages

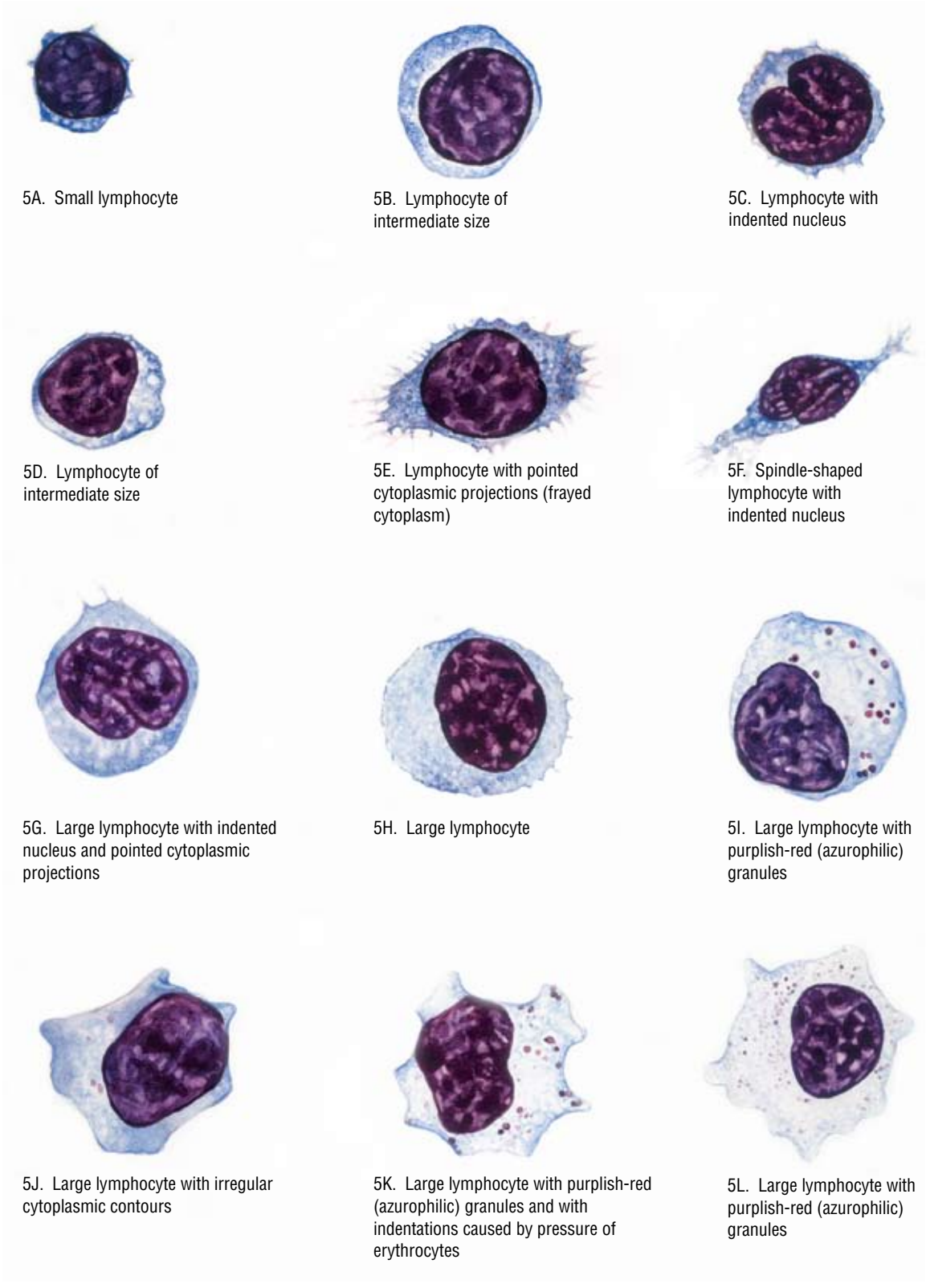
After a short time in the blood, monocytes (***Figure 6***) enter tissue, grow larger, and become tissue macrophages. Once it was incorrectly thought that endothelial cells (cells lining blood vessels) performed the same function as macrophages. This is the origin of the name reticuloendothelial system (RES); macrophage system is more accurate.



7E. Monocyte with gray-blue cytoplasm, band type of nucleus, linear chromatin, blunt pseudopods, and fine granules

7G. Monocyte with segmented-type nucleus

Figure 6. *Monocytes. E = band form. G = segmented form.*



5A. Small lymphocyte

5B. Lymphocyte of intermediate size

5C. Lymphocyte with indented nucleus

5D. Lymphocyte of intermediate size

5E. Lymphocyte with pointed cytoplasmic projections (frayed cytoplasm)

5F. Spindle-shaped lymphocyte with indented nucleus

5G. Large lymphocyte with indented nucleus and pointed cytoplasmic projections

5H. Large lymphocyte

5I. Large lymphocyte with purplish-red (azurophilic) granules

5J. Large lymphocyte with irregular cytoplasmic contours

5K. Large lymphocyte with purplish-red (azurophilic) granules and with indentations caused by pressure of erythrocytes

5L. Large lymphocyte with purplish-red (azurophilic) granules

Figure 7. *Large and small lymphocytes.*

Activated by the immune system, macrophages are the body's first line of defense. Powerful and long-lived, macrophages can ingest large particles – whole RBCs, parasites, bacteria – and rid the body of dead or damaged cells and other debris. Macrophages fixed in tissue often look different and have different names, e.g., Kupffer cells in the liver are macrophages.

Macrophages and Neutrophils. Areas of inflammation attract both macrophages and neutrophils. The few tissue macrophages present begin to attack invaders. Within a few hours, the second line of defense appears: the number of neutrophils in the blood increases substantially as substances in the blood stimulate bone marrow to release stored neutrophils. The third line and the long-term chronic defense is the proliferation of macrophages by cell reproduction in the tissue, by attracting monocytes from the blood, and by increased production of monocytes.

Quick Review: Cells of the RES or Tissue Macrophage System	
Descriptions	Locations
Fixed macrophages: (reticulum cells) large cells, small nucleus	Spleen, lymph nodes, bone marrow, liver, skin (histiocytes), lungs (macrophages), etc.
Free macrophages: large wandering cells	Spleen, lymph nodes, lungs, many other tissues
Circulating monocytes: large, motile cells with indented nuclei	Blood

Table 5. *Cells of the RES or tissue macrophage system.*

Lymphocytes Play a Key Role in Immunity *(Figure 7)*

T lymphocytes (T cells). Involved in cellular immunity, T cells carry receptors for molecules on other cells or in body fluids. T cell receptors allow them to interact with macrophages and other cells and substances in the body. T cells defend against foreign substances such as viruses that invade body cells, fungi, parasites, transplanted tissue, and cancer cells. Through a variety of T cells (helper T cells, suppressor T cells), the body initiates, carries through, and terminates antigen-antibody reactions to provide immunity.

B lymphocytes (B cells) are involved in humoral immunity, which consists of antibodies circulating in the blood and lymphatic system. B lymphocytes produce antibodies typically against bacteria and viruses.

Example: AIDS. In AIDS (acquired immune deficiency syndrome), the virus (HIV) infects the helper T lymphocyte cells and destroys them. It can also pass unrecognized from cell to cell by changing the surface of helper T cells. HIV may lie dormant until another infection triggers T cells to increase and the virus to multiply. With HIV, not only is part of the body's defense system lost, but the very cells that should defend the body are working against it.

Now complete the section Quiz.

Review Questions • Section 5

1. Match the following.

__ A Phagocytes	1. Produce antibodies
__ B Myeloid cells	2. PMNs
__ C Granulocytes	3. Destroy bacteria
__ D Staining characteristics	4. Bone marrow cells
__ E Lymphocytes	5. Eosinophilic, basophilic

2. After several cell generations, neutrophils, eosinophils, and basophils become:

- A Monocytes
- B Polymorphonuclear cells
- C Granulocytes
- D Lymphocytes

3. Which of the following statements is true of monocytes?

- A They are lymphocytes.
- B They become tissue macrophages.
- C They are part of the reticuloendothelial system (RES).
- D They are large WBCs.

4. True or False? The primary function of lymphocytes is immunity.

- T
- F

Section 6

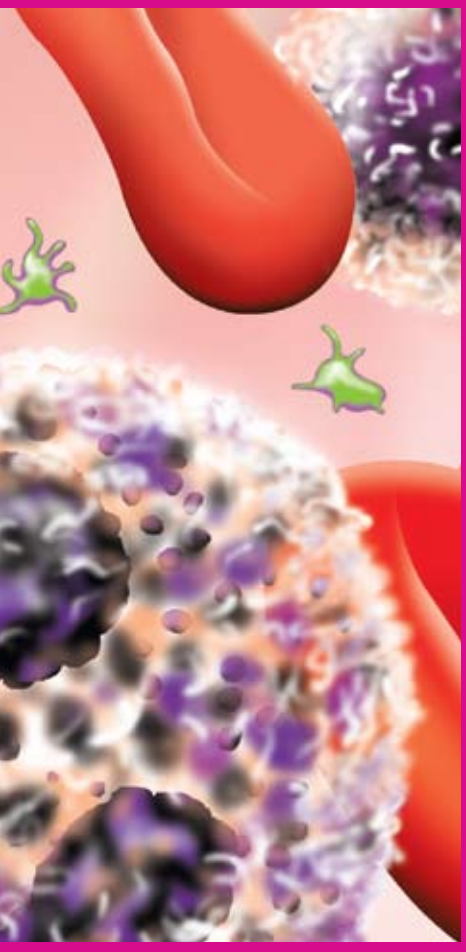
DISORDERS OF WHITE BLOOD CELLS

Learning Objectives

When you complete this section, you will be able to:

- Recognize descriptions of the following WBC disorders: leukocytosis, neutropenia, lymphocytosis, agranulocytosis, lymphoma, leukemia

Common disorders affecting the development or function of WBCs are covered briefly in the following pages.



Disorders Affecting Phagocytosis

These disorders make the body vulnerable to recurrent infections such as bacteremia (bacterial infection of blood), meningitis, and pulmonary infections. Genetic deficiencies that adversely affect phagocytosis can lead to systemic lupus erythematosus (SLE, a chronic disorder of connective tissue), advanced liver disease, and immune diseases.

Neutrophil Disorders

Leukocytosis, Granulocytosis, Neutrophilia

All three terms indicate increased circulating neutrophils (but not an increase in eosinophils or basophils). This is a very commonly encountered disorder in clinical medicine.

Causes: Bacterial infection; inflammation or tissue death (as in a myocardial infarction); uremia, acidosis, and other pathologic changes in the content of blood; cancer; acute hemorrhage; removal of the spleen.

Leukopenia, Neutropenia, Granulocytopenia

-penia is a suffix denoting a depression in amount. Neutropenia results from either decreased neutrophil production or abnormal destruction of neutrophils. The term usually indicates a neutrophil count below 1.50 x 10^3/μL. Agranulocytosis is severe neutropenia. Neutropenia increases susceptibility to serious bacterial or fungal infection.

Causes: Decreased production of neutrophils may be due to a genetic disorder, aplastic anemia, or cancer. It is a potential adverse effect of several therapeutic agents (cancer therapy, phenothiazine, anticonvulsants, some antibiotics). Abnormal neutrophil destruction may be due to infection, therapeutic drugs, hemodialysis, or disorders of the spleen.

Lymphocyte Disorders

Lymphocytosis

Increased numbers of lymphocytes in the blood may occur in infectious mononucleosis (mono), a self-limited viral infection common in young people, and acute infectious lymphocytosis, a benign self-limited viral disorder.

The Leukemias

Literally, a cancer of the white blood cells in which uncontrolled, increasing numbers of abnormal WBCs occur in blood forming and other tissues. Types of leukemia are identified by the dominant cell involved:

- Immature lymphocytes predominate in acute lymphocytic (or lymphoblastic) leukemias (ALL) and chronic lymphocytic leukemias (CLL)
- Myeloid cells predominate in acute myelocytic leukemia (AML)
- Granulocytes predominate in chronic myelocytic leukemia (CML)

Causes: Heredity, immune deficiencies, chronic marrow dysfunction, environmental factors (radiation, toxic chemicals), viruses.

Lymphomas

This group of neoplasms that arise in the reticuloendothelial system (RES) and lymphatic system include:

- Hodgkin's disease, a chronic disease involving the lymphatic system and a wide variety of changes in WBCs; eosinophilia may be present, and anemia may develop in advanced cases
- Malignant lymphomas and non-Hodgkin's lymphomas

Now complete the section Quiz.

Review Questions • Section 6

1. Match the following.

__ A Very common disorder	1. Neutrophilia
__ B Leukemia	2. A form of lymphoma
__ C Agranulocytosis	3. Severe neutropenia
__ D Infectious mononucleosis	4. A type of lymphocytosis
__ E Granulocytosis	5. A cancer causing increased WBCs in the blood
__ F Hodgkin's disease	

2. True or False? Repeated white cell counts could be helpful in managing patients who are taking therapeutic agents that have the potential adverse effect of neutropenia.

T

F

3. What do leukocytosis, granulocytosis, and neutrophilia have in common?

4. Disorders of WBCs are frequently associated with:

A Drug therapy

B Radiation exposure

C Cancer

D Inherited disorders

E Dietary deficiencies

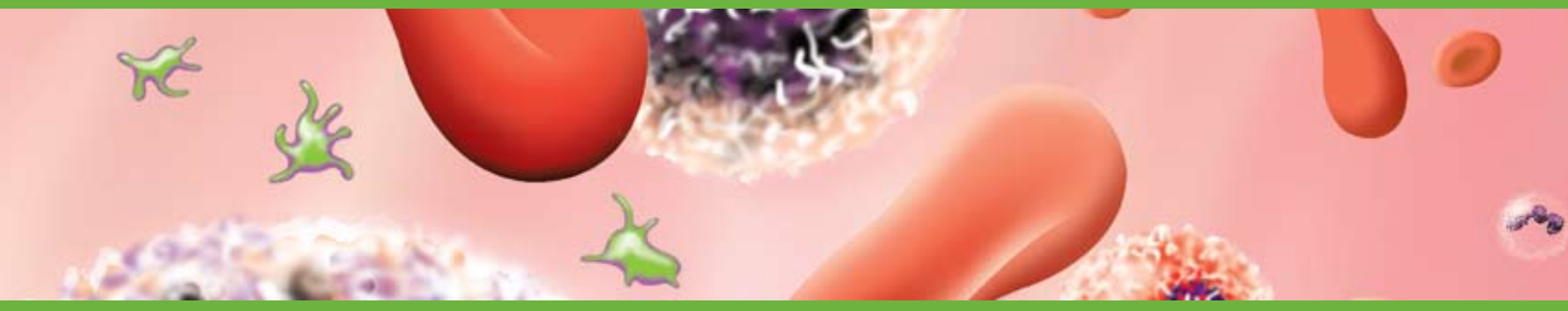
Section 7

PLATELETS AND HEMORRHAGIC DISORDERS

Learning Objectives

When you complete this section, you will be able to:

1. Describe platelets
2. Indicate the functions of platelets
3. Recognize hemorrhagic disorders involving platelets



Description and Formation

Platelets (thrombocytes) are small, granulated bodies with various shapes (round, oval, spindle, discoid). Megakaryocytes, giant cells in the bone marrow, form platelets by pinching off and extruding pieces of cytoplasm.

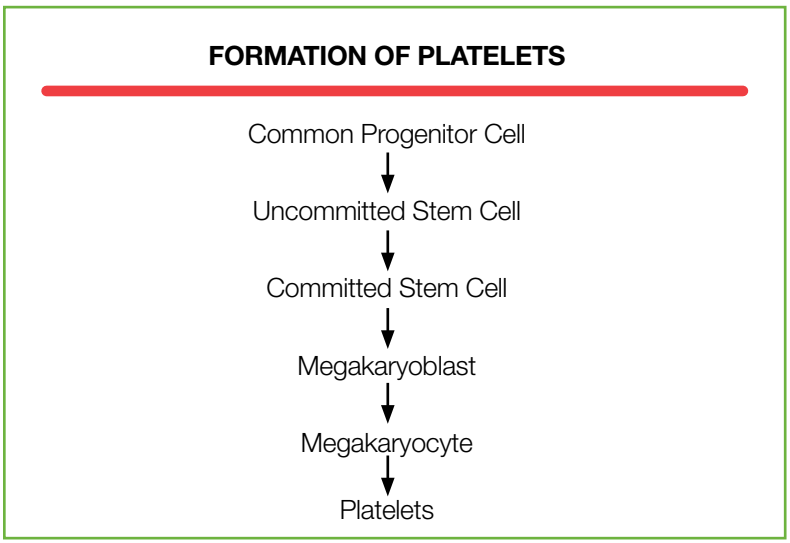


Figure 8. *Formation of platelets.*

Functions. Hemostasis, the process of stopping bleeding, is the primary function of platelets. To accomplish this, platelets contain lysosomes (chemicals capable of breaking down other substances), clotting factors, and a growth factor that stimulates healing. Traveling in the circulation, platelets join with other blood components to limit blood loss.

Platelets may also help maintain the integrity of the vascular lining and stimulate proliferation of vascular smooth muscle.

Coagulation. Blood coagulation is a complex process.

Activated by factors at the site of an injured blood vessel, platelets aggregate (collect) to form a plug, change shape, discharge their granules, and initiate the generation of thrombin, an enzyme that converts fibrinogen to fibrin. Thrombin causes them to become sticky and adhere irreversibly to each other, as well as to the break in the vessel wall. The granules attract more platelets, and thrombin begins formation of a true clot with a net of fibrin.

At the same time, anti-clotting factors act on the interior of the blood vessel to ensure that the clot will not block blood flow. When healed, the vessel releases factors that lyse the fibrin network. Failure of anti-clotting factors can lead to thrombosis (clot formation in the circulation) or to disseminated intravascular coagulation (DIC), a serious condition in which fibrin is generated in the circulating blood.

Hemorrhagic Disorders

These are disorders of hemostasis, that is, patients with these disorders have a tendency to bleed. Some hemorrhagic disorders involve blood vessels, but most involve the body's ability to stop or contain bleeding.

Thrombocytopenia is a reduced platelet count. ***Table 6*** lists the types and causes of this condition.

Thrombocytopenia	
Types	Causes Include
Decreased production of platelets	Toxic agents, infection, radiation, anemias, genetic disorders
Abnormal distribution of platelets	Enlarged spleen (which traps platelets), various cancers
Dilution loss of platelets	Massive blood transfusion
Abnormal destruction of platelets	Disseminated intravascular coagulation (DIC), vasculitis (blood vessel inflammation), thrombotic thrombocytopenia purpura (TTP), heparin, quinine, some antibiotics, leukemia, lymphoma

Table 6. *Causes of thrombocytopenia.*

Thrombocytosis, elevated platelet count, may cause hemorrhage or thrombosis. It occurs in cancers, inflammation, splenectomy, iron deficiency, and qualitative disorders of platelets.

The pattern of bleeding often indicates the type of problem to investigate. Types of bleeding include:

- Ecchymosis (bruising), diffuse bleeding into the skin
- Petechiae, pinpoint bleeding into the skin without trauma
- Mucosal bleeding: epistaxis (nosebleed), menorrhagia (excessive menstrual bleeding), bleeding gums, or gastrointestinal bleeding

Note: Hemophilia is a bleeding disorder due to hereditary deficiencies in the blood clotting factors; it is not a disorder of platelets.

Now complete the section Quiz.

Review Questions • Section 7

1. The primary function of platelets is:

2. Platelets are:

- A Small, granulated cells
- B Formed by megakaryocytes, giant cells in the bone marrow
- C Pieces of cytoplasm
- D Found only in small numbers in the blood

3. Which of the following could describe thrombocytopenia?

- A A disorder of platelet production due to a genetic disorder
- B Abnormal distribution of platelets due to an enlarged spleen
- C Too few platelets in the blood due to transfusion
- D The probable cause of leukemia and lymphoma

4. True or False? If false, indicate how the statement should be corrected.

- T
- F

Thrombocytosis, elevated platelet count, occurs in some cancers and in qualitative disorders of platelets.

5. True or False? If false, indicate how the statement should be corrected.

- T
- F

"Hemorrhagic disorder" simply means a tendency to bleed.

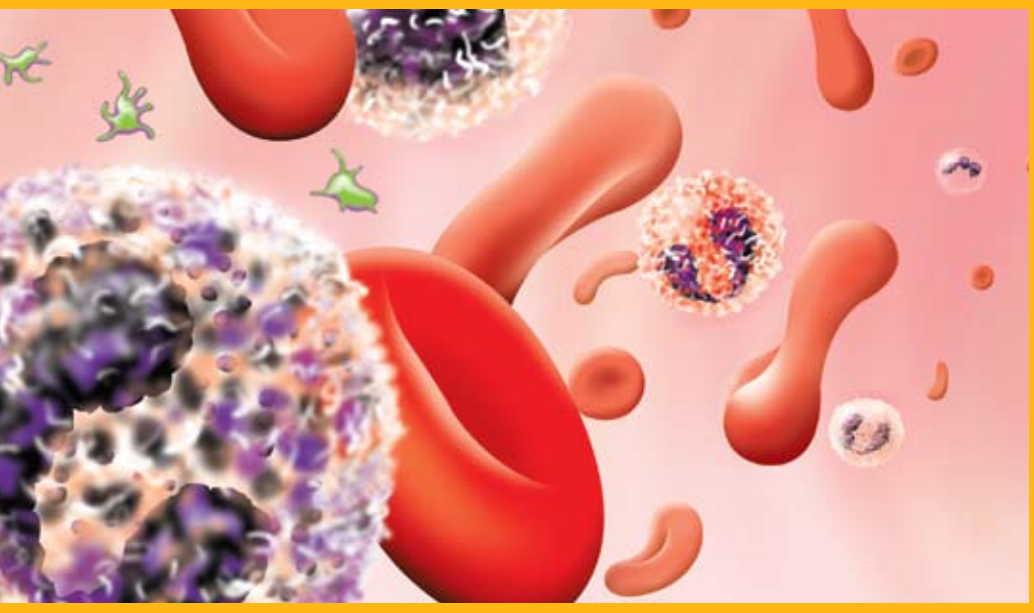
Section 8

HEMATOLOGY TESTS

Learning Objectives

When you complete this section, you will be able to:

1. Recognize blood components that are often tested
2. Indicate types of technologies used in testing
3. List the tests that comprise a complete blood count
4. Recognize normal values for the cellular elements in blood



A Word About Tests

Because few laboratory tests are disease-specific, a single abnormal test value is not considered diagnostic of a disease state. Many variables affect almost every test; these include:

- The patient's state of stress
- Lack of patient compliance with procedure (e.g., fasting)
- Improper sample handling
- Improper lab procedures
- Lack of accuracy in the procedure or instrument used

Reason for Repeating Tests. Lab tests are repeated to confirm an abnormal finding. When used to determine the prognosis of a disease or to monitor the progress of therapy, tests that are repeated over time can indicate a trend, e.g., the disease is growing worse or getting better.

How Blood Tests Are Done

Hematology tests include a wide variety of laboratory studies, ranging from coagulation factors to various cell evaluations. The tests discussed here are those that are most likely to be done with a hematology analyzer.

The Sample. A sample of whole blood is taken, usually from a vein. Amounts differ according to the number and types of tests to be run and the testing instruments to be used. Typically, RBCs are counted and lysed (broken down); then WBCs are measured.

Dilutions. Because blood clots quickly, the measured blood sample is diluted with either a lysing agent or an anti-clotting agent, depending on the test(s) to be completed. A lysing agent destroys the RBCs and allows counting of WBCs. The dilution is an important step in preparing samples for testing for several reasons.

First, concentrations of the anticoagulant must be adequate for the volume of blood. Insufficient dilution may allow formation of small clots that lower cell counts; excessive dilution can cause cells to shrink or swell. Anticoagulants that are widely used are EDTA (ethylenediaminetetraacetic acid) and heparin. EDTA (purple-top vacutainer) is used often for routine cell counts.

Second, even relatively large blood samples do not provide a sufficient quantity to flow through an analyzer for measurement. Blood must be mixed with a diluent that will allow the cells to be evenly suspended in sufficient liquid to flow at a constant rate for measurement.

Originally, blood counts were performed manually by visualizing the cells through a microscope and counting after diluting and pipeting a small volume onto a pre-defined glass slide containing etched counting areas. For identifying and counting the WBC subpopulations, a blood sample was smeared on a slide and stained, then placed under a microscope. The hematologist or technician identified and counted the cells in a systematic prescribed method, classifying the first 100 cells seen and assigning a percentage figure to each type found. Although generally accurate, the process was very technologist-dependent and time-consuming.

Now cells are almost always counted electronically by an analyzer utilizing specific technologies and methods. When abnormalities are suspected or determined, however, a hematologist or technician will still do an actual visual count and identification of the cells.

Counts and other tests should be run within 3-4 hours of obtaining blood samples (within 1-2 hours for platelet counts).

Cell Counting Technologies

Automated hematology has been used in large laboratories for many years. In the late '70s it became easy and cost-effective enough to move into physician office practices as well.

Automated technologies include the following:

Impedance technology. This technology was originally known as the Coulter principle. There are several variations and adaptations to the impedance principle in use today from a variety of commercial manufacturers.

Impedance technology is based on the fact that blood cells are poor conductors of electricity. Cells are diluted with an electrolyte, directed to a moving stream, and pass through a small orifice (opening) within a transducer (detection device). As each cell passes through the small opening of the aperture, an electrical field exists in the transducer; and when the cells pass through the aperture orifice (small opening), the increase in electrical impedance (resistance) is measured (***Figure 9***).

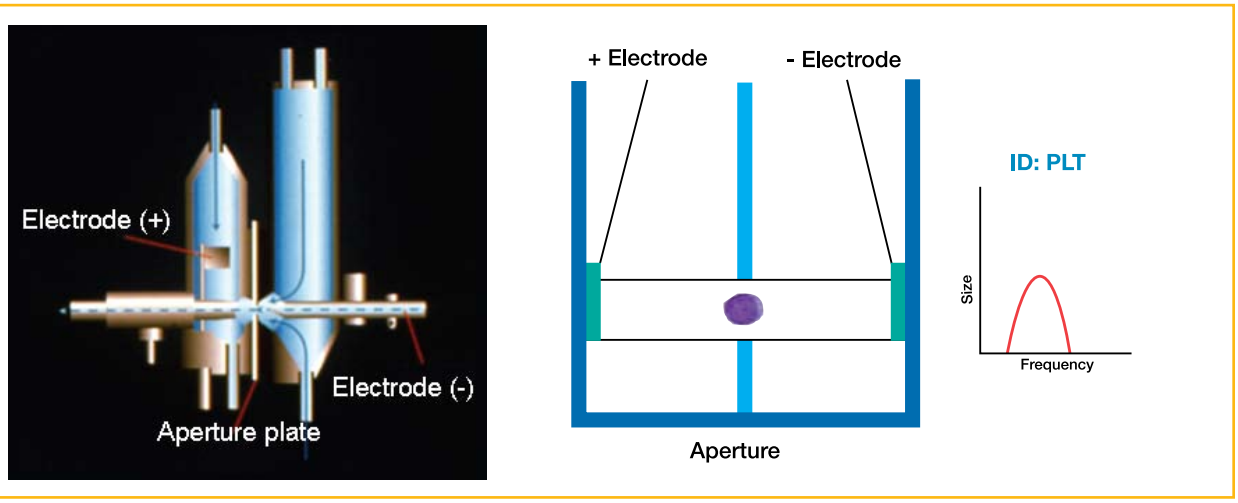


Figure 9. *Simple impedance detection device (transducer).*

Impedance technology provides a one-dimensional method for enumeration of the formed cellular elements as well as for cell sizing due to the resulting impedance signal being proportional to the cell's (event) size.

Different approaches to size discrimination (thresholding) and the cell dilution allow for accurate enumeration of RBCs, WBCs, and PLTs even though the absolute numbers (of cells) and size vary broadly within the blood. Displays of the cellular measurements are usually provided in a graphical form called a histogram (representing number of events versus relative size of the events). (***Figure 10***)

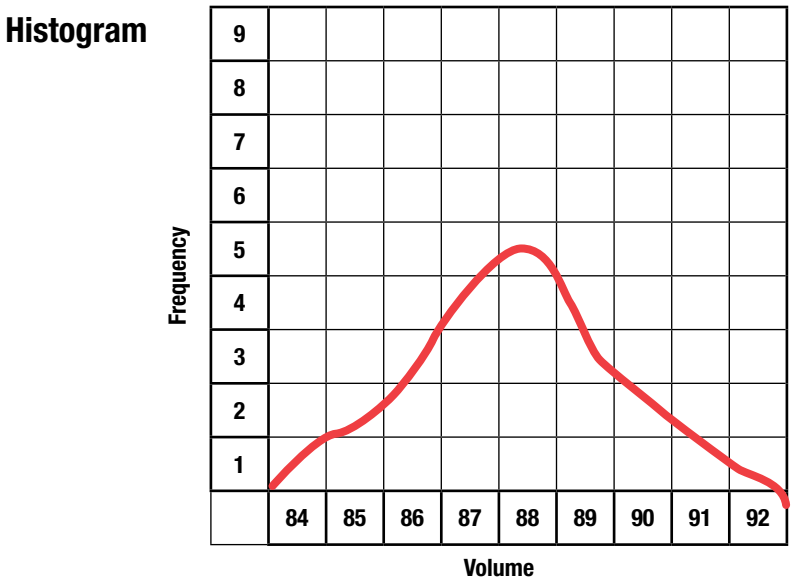


Figure 10. *One-dimensional histogram, frequency of events versus cell volume.*

Optical principles. Automated systems are now based on flow-through (also called flow cytometry) optical technologies that identify cells on the basis of light scatter properties broadly equating to the cell's physical characteristic differences.

Blood cell recognition is optimized by diluting specifically to the number of cells present in the blood so as to count the number of cells appropriate for statistical accuracy.

A focused light beam illuminates a small area of a flow cell. Cells are rapidly injected in single file (hydrodynamic focusing) through the illuminated area (interrogation zone) where the cells intersecting the light beam scatter light in all directions in a manner that is measurable and unique to each cell type (***Figure 11***).

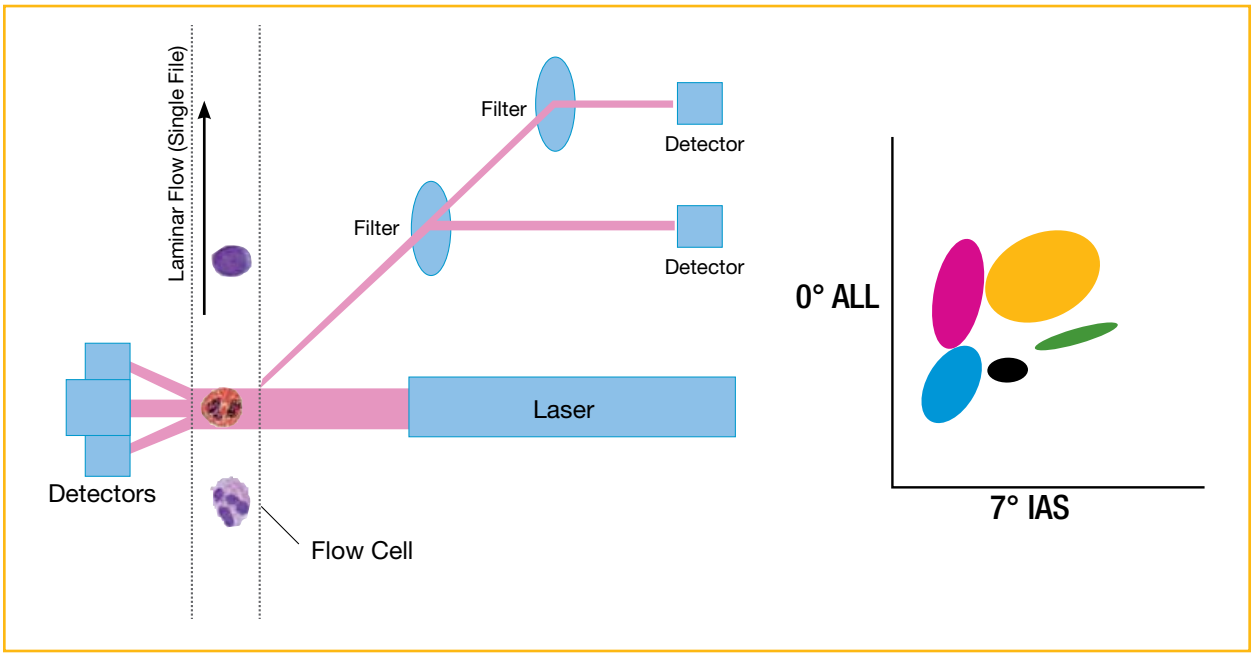


Figure 11. *Optical light scatter technology with two-dimensional graphic scatterplot.*

The resulting light scatter information can be collected at multiple locations incident to the light beam providing a multi-dimensional analysis of the unique light scatter properties from each cellular event. Analysis and displays of the optical light scatter measurements are provided in a two-dimensional graphical form called a scatterplot. (***Figure 11***). Multiple scatterplots may be generated for complete visualization of all the cellular components including sub-populations of each cell line.

Radio Frequency and Multi-Color Fluorescence Emission Detection Methods.

In addition to impedance and optical flow cytometry, other electrical signals and advanced pre-analysis, rapid staining methods have further advanced identification of cellular characteristics and subsequent cellular sub-populations.

Radio Frequency (RF) involves applying a high energy electrical field across an impedance aperture resulting in wave transformations that characterize internal structure, mostly related to WBC structure.

Multi-Color Fluorescence emission has provided the greatest recent advancements in cell identification. Fluorescent flow cytometry is a measurement technology developed by specifically staining internal components of cells (RNA/DNA) or tagging a specific antigenic site of a cell with a fluorochrome conjugated antibody that fluoresces at higher wavelengths when illuminated by a specific wavelength light source (usually a laser diode). Specificity of cell lineage identification has been advanced with these methods, providing greater insight in hematology disease identification and management.

Hematology Tests

Complete Blood Count (CBC)

A complete blood count (CBC) is the most widely requested and single most important lab test on blood. Many CBCs are done as routine screens – tests that provide general information about the patient's status. Most CBCs include the following cellular measurements:

- WBC count
- WBC differential, 3-part: lymphocytes, mid-cells, granulocytes

OR

5-part: lymphocytes, monocytes, neutrophils, eosinophils, basophils

- RBC count
- Hemoglobin (Hb)
- Hematocrit (Hct)
- RBC indices (MCV, MCH, MCHC)
- Platelet count

Normal ranges (also called reference ranges) depend on geographic location, the patient's sex, and age; ranges are used as guidelines and vary based on specific populations.

White Blood Cell (Leukocyte) Count

This is a count of the number of WBCs present in a known volume of blood. Automated systems often count as many as 20,000 WBCs to ensure accuracy and precision. The WBC count is further identified by the major WBC sub-populations (WBC differential). WBC counts and the WBC differential are generally measured by optical technologies or a combination of optical, impedance, radio frequency, or multi-color fluorescence in modern hematology analyzers.

Less sophisticated hematology analyzers provide WBC sub-populations as 3-part WBC differentials reporting a percentage and absolute value for lymphocyte, mid-cells, and granulocyte populations. Advanced hematology analyzers minimally provide a 5-part WBC differential reporting percentages and absolute values for lymphocytes, monocytes, neutrophils, eosinophils, and basophils. The modern hematology analyzer also indicates (flags) suspected abnormalities, from which the technologist may perform a microscopic smear review or an actual manual differential count to ensure accuracy of the reported results (***Table 7***).

WBC Values **Normal Range, Total WBCs: 4.5-11.0 x 10^3/µL (adult population)***		
Normal ranges, differential leukocyte counts:		
3-part differential	Lymphocytes Mid-Cells Granulocytes	1.0-4.8 x 10^3/µL 0-0.9 x 10^3/µL 1.8-8.1 x 10^3/µL
5-part differential	Lymphocytes Monocytes Neutrophils Eosinophils Basophils	1.0-4.8 x 10^3/µL 0-0.8 x 10^3/µL 1.8-7.7 x 10^3/µL 0-0.5 x 10^3/µL 0-0.2 x 10^3/µL
Other normal WBC values:	Band neutrophils (%) Segmented neutrophils (%)	0-6% 40-70%

Table 7. *WBC values.*
**Reference ranges should be established by each laboratory.*

Red Blood Cell (Erythrocyte) Count. This is a count of the number of RBCs present in a known volume of blood. Automated systems often count as many as 20,000-50,000 RBCs to ensure precision. The RBC count is also used to calculate the RBC indices.

RBC Count Normal Range: 4.00-5.9 x 10^6/µL*	
High Polycythemia, severe dehydration, trauma, surgery, burns	***Low*** Anemia, hemorrhage, excessive fluid intake

Table 8. *RBC count.*
**Reference ranges should be established by each laboratory.*

Hematocrit (Hct). This is the volume of RBCs in a whole blood sample expressed as a percentage (%).

Hct Values Normal Values: 36%-53%*	
High Polycythemia, severe dehydration, trauma, surgery, burns	***Low*** Anemia, hemorrhage, excessive fluid intake

Table 9. *Hct Values*
**Reference ranges should be established by each laboratory.*

Hemoglobin (Hb). Hemoglobin values may be obtained in several ways. The most common method adds potassium cyanide (or similar compounds) to convert Hb to cyanmethemoglobin, which is measured with a spectrophotometer.

The results, recorded as grams per deciliter (g/dL), indicate the oxygen-carrying capacity of the RBCs.

Hb Values Normal Values: 12.0-17.5 g/dL*	
High Polycythemia, severe dehydration, trauma, surgery, burns	***Low*** Anemia, hemorrhage, excessive fluid intake

Table 10. *Hb Values.*
**Reference ranges should be established by each laboratory.*

RBC Indices

The RBC indices –mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) – indicate the volume and character of hemoglobin and, therefore, aid in the differential diagnosis of the type of anemia present.

Mean Cell Volume (MCV). The MCV is the average volume of RBCs; it is calculated from the hematocrit (volume of packed red cells) and the erythrocyte count. This is the most important RBC index in the differential diagnosis of anemias.

The value can be derived from the following equation:

MCV = Hct (%) x 10/RBC (x 10^6/µL)

MCV Values Normal Range: 80.0-100 fL*	
High Pernicious anemia, tapeworm infestation, certain medications	***Low*** Anemias, iron deficiency, liver disease, hemorrhage

Table 11. *MCV Values.*
**Reference ranges should be established by each laboratory.*

Today's modern hematology analyzers actually do cell sizing, therefore, the MCV is actually a measured parameter and Hct is calculated from the MCV.

Mean Cell Hemoglobin (MCH). This is a calculation of the ratio of hemoglobin to the erythrocyte count. The formula for the calculation is:

MCH = Hb (g/dL)/RBC (x 10^6/µL) x 10

MCH Values Normal Range: 26-34 pg*
Low Anemias, iron deficiency, liver disease, hemorrhage

Table 12. *MCH Values.*
**Reference ranges should be established by each laboratory.*

Mean Cell Hemoglobin Concentration (MCHC). This calculation of the ratio of hemoglobin to hematocrit indicates the concentration of hemoglobin in the average red cell. The calculation is:

MCHC = [Hb (g/dL)/Hct (%)] x 100

MCHC Values: Normal Range: 31-37 g/dL*
Low Anemia due to inadequate RBC formation, pernicious anemia, tapeworms, certain medications

Table 13. *MCHC Values.*
**Reference ranges should be established by each laboratory.*

Reticulocyte Count. For a count of these young RBCs, a few drops of blood are smeared on a slide, stained with methylene blue, and counterstained with Wright's stain before being counted under a microscope. One thousand RBCs are counted and the number of cells having a blue-stained reticulum are expressed as a percentage. Now, most automated systems provide reticulocyte counts using various stains and dyes.

Reticulocyte Count Normal Range: 0.5%-1.5% of RBCs*	
High Reticulocytosis indicates bone marrow activity in response to: blood loss, therapy for anemia, hemolytic anemia in pregnancy, high altitudes	***Low*** Low or normal counts in anemic patients indicate failure of bone marrow, infection, inflammation, aplastic anemia, severe iron deficiency, megaloblastic anemia

Table 14. *Reticulocyte count.*
**Reference ranges should be established by each laboratory.*

Platelet Count. Because their normal numbers are so high, platelets were often estimated. Now, automated systems provide platelet counts from a variety of methods (i.e., impedance, optical, and immuno-platelet).

Platelet Count Normal Range: 140-440 x 10^3/µL*	
High Rheumatoid arthritis, many cancers, hemorrhage, polycythemia, some anemias	***Low*** Diseases of the spleen, leukemias, aplastic anemia, alcoholism, severe infections, cardiac surgery, blood transfusion

Table 15. *Platelet count Values.*
**Reference ranges should be established by each laboratory.*

Now complete the section Quiz.

Review Questions • Section 8

1. The concentration of hemoglobin in the blood:

   A. Is measured by comparing the patient's blood sample with a standard

   B. Is recorded as g/dL

   C. Indicates the oxygen-carrying capacity of the RBCs

   D. Decreases in all cases of anemia

2. The hematocrit:

   A. Measures the volume of RBCs expressed as a percentage of the volume of whole blood in a sample

   B. Differentiates types of WBCs in a sample

   C. Is part of a CBC

   D. Involves centrifuging the sample to separate the plasma, white blood cells, and red blood cells

3. True or False? The RBC indices indicate the volume and character of hemoglobin and help identify the type of anemia present.

   T

   F

4. Which of the following statements apply to a histogram?

   A. It can be used for WBCs, RBCs, and platelets.

   B. Variations in cell sizes are expressed as the coefficient of variation of the distribution width.

   C. It shows exactly where each cell type is in a sample.

References and Resources

Bell A, et al. *The Morphology of Human Blood Cells*, 7th Edition, Abbott Park, IL: Abbott Laboratories; 2007.

Marshall L, et al, eds. *Williams Hematology*, 7th Edition, New York, NY: McGraw-Hill Companies; 2006.

Steine-Martin E, et al, eds. *Clinical Hematology*, 2nd Edition, Philadelphia, PA: Lippincott-Raven Publishers; 1998.

Correct Responses to Review Questions

Section 1 Review Correct Responses

1. a-2, b-3, c-1, d-4
2. a-3, b-4, c-6, d-2, e-1, f-5
3. Hematology is the study of gross features of blood such as cell counts, bleeding time, etc.

Section 2 Review Correct Responses

1. transportation, regulation, protection, and prevention.
2. a, b, c
3. a. 4.60 – Women, 5.20 – Men, b. iron, hemoglobin
4. a, b, c, e
5. b, c, d
6. a, b
7. b, c, d

Section 3 Review Correct Responses

1. a, d
2. a, b
3. oxygenation
4. a, b, d, e
5. True
6. a, b, c
7. a, b, c, d

Section 4 Review Correct Responses

1. Anemia is a clinical sign that indicates a deficiency of RBCs.
2. a-5, b-4, c-3, d-l, e-2
3. a, b, c, e
4. a, b
5. a, c
6. b

Section 5 Review Correct Responses

1. a-3, b-4, c-2, d-5, e-1
2. b, c
3. b, c, d
4. True

Section 6 Review Correct Responses

1. a-1, b-5, c-3, d-4, e-1, f-2
2. True
3. All three terms indicate increased circulating neutrophils.
3. a, b, c, d

Section 7 Review Correct Responses

1. Hemostasis
2. b, c
3. a, b, c
4. True
5. True

Section 8 Review Correct Responses

1. a, b, c
2. a, c, d
3. True
4. a, b

Glossary of Terms

anemia: Low red blood cell (RBC) count or hemoglobin in blood; caused by blood loss or by impaired production or destruction of RBCs

antibody: A protein molecule in serum or body fluids that reacts with, protects against, and helps destroy foreign or natural substances (antigens)

antigen: Any substance, either foreign or natural, that stimulates lymphocytes (white blood cells) to initiate an immune response; includes bacteria, viruses, fungi, toxins, living and dead tissue, etc.

aplastic: Characterized by incomplete or defective development

basophil: A cell that stains specifically with basic dyes

-blast: A suffix indicating an immature cell

calibrator: A known quantity of an analyte used to establish a normal curve

catalyst: A substance whose presence changes the velocity of a reaction but does not form part of the final product of the reaction; the verb form is catalyze

chemistry: In clinical testing, refers to the solutes dissolved in the plasma such as uric acid, etc.

coefficient of variation (CV): A statistical term that indicates the precision or reproducibility of a measurement; expressed as percentages, CVs indicate the degree of small variations between the same tests run on the same sample (the smaller the number, the more precise the instrument or test)

congestive heart failure (CHF): A collection of symptoms indicating that the heart is unable to pump effectively; inadequate blood circulation results in decreased oxygenation of tissues and breathlessness

control: In chemistry, a known quantity of an analyte that is tested as if it were an unknown to find out how well the instrument is performing

correlation coefficient (r): A value indicating accuracy; values closest to 1.00 are best

-cyte or cyto-: Combining forms meaning cell

dialysis (also called hemodialysis): The process of removing toxins and excess water from the blood; used in kidney failure

diapedesis: The process by which cells squeeze through the pores of a membrane

diffusion: The movement of particles from an area of greater concentration to an area of lesser concentration

ecchymosis: Bruising

electrolyte: Any substance that, in solution, becomes an ion and conducts electricity; examples are sodium and potassium

-emia: Suffix that can be interpreted as "in the blood"

end point: Term for a reaction that is measured at the end; for example, a test converts all of a substance

present in a specimen into a chromogen – which has a specific color – and then measures the color

enzymes: Proteins that catalyze chemical reactions; many enzymes are present in one organ; enzymes appear in blood because they have a natural function there or because disease in the tissue in which they originate caused them to be dispersed via the blood

epistaxis: Nosebleed

erythro-: Prefix meaning red

erythroblast: Literally, an immature red blood cell

erythropoiesis: Red blood cell formation

ferritin: The primary form of storage iron

fibrin: A plasma protein that acts with other blood factors to create blood clots

glucose: The sugar that is the energy source for all body cells

granulopoiesis: The formation of granulocytes

hema- or hemo-: Prefixes indicating blood

hematology: Literally, the study of blood; refers to the gross features of blood such as cell counts, bleeding time, etc.

hematopoiesis: Formation of blood cells

hemoglobin: The oxygen-carrying component of red blood cells; composed of two pairs of protein chains called globin and four smaller units called heme, which contain iron

hemolysis: Destruction of red blood cells, often by separation of hemoglobin; caused by many substances or by freezing or heating

hemolytic-uremic syndrome (HUS): A sudden disorder that involves thrombocytopenia, hemolysis, and acute renal failure; HUS primarily occurs in infants and children following an infection but occasionally occurs in adults; most patients recover, but some require renal dialysis

hemophilia: A bleeding disorder due to hereditary deficiencies in the blood clotting factors

hemostasis: The process of stopping bleeding

hepat-: Prefix indicating the liver

hepatitis: An inflammation of the liver

histiocytes: Macrophages in the connective tissue; part of the reticuloendothelial system

histogram: A graphic means of comparing magnitudes of frequencies or numbers of items; usually shown in bar graphs or columns

homeostasis: The body's tendency to maintain a uniform or stable state

hormones: Endocrine gland secretions that travel to and act on specific target organs

hyper-: Prefix indicating abnormally increased values or activity

hypo-: Prefix indicating abnormally decreased values or activity

hypochromia: A decrease of hemoglobin in the red blood cells so that they are abnormally pale

hypoxia: Abnormally low oxygen level

idiopathic: Occurring without known cause

idiopathic thrombocytopenic purpura (ITP): A chronic blood disorder with no apparent cause (except in children when it may follow a viral infection); the body develops an antibody directed against platelet antigens, causing bleeding (petechiae, purpura, or mucosal bleeding) that may be minimal or extensive; treatment options include steroids, infusion of platelet concentrates, or splenectomy

interstitial fluid: Fluid surrounding cells; transports nutrients, gases, and wastes between blood and cells

ion: An atom or molecule that carries either a positive (cation) or negative (anion) electrical charge

-itis: Suffix indicating inflammation

lymphoid: Associated with lymph; refers to the lymphatic system and to the fluid collected from tissues that flows through the lymph vessels and is added to venous blood

lyse: To break up to cause cells to disintegrate

lysin: An antibody that acts destructively on cells depending on the antigen that stimulated its production

lysis: The destruction of red blood cells, bacteria, and other antigens by a specific lysin

macro-: Prefix meaning large; for example, a macrocyte is a large cell

megalo-: Prefix meaning large; for example, a megalocyte is a large cell

menorrhagia: Excessive menstrual bleeding

meta-: Prefix indicating after or behind; this prefix has the same meaning as the prefix *post-*

metabolism: Physical and chemical processes by which a living organism breaks down complex substances into simpler substances for nutritional use or disposal

microcyte: A red blood cell that is five microns or less in diameter

microcytic, hypochromic: Adjectives describing a form of anemia with red blood cells that are small and pale

mye-: Prefix meaning bone marrow

myeloid: Associated with the bone marrow

neoplasm: Any new or abnormal growth

nephr-: Prefix indicating the kidney

nephritis: Inflammation of the kidney

normo-: Prefix meaning normal or usual

normochromia: Indicating red blood cells having normal coloring

normocyte: A red blood cell that is normal in size, shape, and color

normocytic, normochromic: Adjectives describing normal cells with normal coloring; used to indicate the status of red blood cells

-oma: Suffix indicating a tumor or neoplasm; for example, lymphoma means tumors affecting the lymph system

-osis: Suffix indicating a condition; for example, thrombocytosis is a condition affecting thrombocytes (platelets)

pancytopenia: A condition in which the numbers of all types of blood cells are reduced

panel tests: Usually, a group of three to five tests involving one organ system (heart, liver, kidneys, etc.) or having reference to one condition; used to confirm a diagnosis, to monitor a condition or disease state, or to modify therapy

-penia: Suffix indicating a decreased amount

petechiae: Pinpoint bleeding into the skin from broken blood vessels – usually on arms or thighs – without trauma

pH: means of indicating the acidity of body fluids; pH is measured on a scale of 0 (highly acidic) to 14 (highly alkaline), with 7.0 as neutral; normal range for blood pH is 7.35 to 7.45

phagocyte: A cell that ingests bacteria, foreign particles, and other cells to protect the body

polycythemia: Literally, many cells in the blood; in this condition, which is the opposite of anemia, the blood becomes highly viscous (thick) and flows sluggishly

polymorphonuclear: Having various forms of nuclei

porphyria: A group of inherited conditions in which the production of heme is deficient

pro-: Prefix indicating before or a precursor

profile: In chemistry, a group of tests that can be used to screen for an abnormality that may not be readily detected in another way; often involves 12 to 28 tests performed on a venipuncture sample

purpura: Hemorrhagic disease characterized by the escape of blood into the tissues, under the skin, and through the mucous membranes; results in spontaneous bruises and small red patches on the skin

rate reaction: Term referring to a measure of the rate of activity caused by the presence of an analyte (the constituent or substance that is analyzed); the more analyte in the specimen, the more activity occurs in a specific period of time

reference method: Method of comparing accuracy between two or more testing systems or kits

reference range: Normal values; ranges of values for each assay for people in a defined population

renal: Pertaining to the kidneys

reticul-: Prefix indicating a network

reticulocyte: A young red blood cell containing a network of basophilic substances

rheumatoid arthritis: An incurable inflammatory disease of the joints and connective tissue; an autoimmune disease

serology: The study of antigen-antibody reactions in blood samples (as opposed to within the body)

serum: The fluid portion of the blood after fibrin and the formed elements have been removed

splenectomy: Surgical removal of the spleen

systemic: Pertaining to or affecting the body as a whole

systemic lupus erythematosus (SLE): An incurable inflammatory disease affecting many body systems; an autoimmune disease

thrombin: An enzyme that converts fibrinogen to fibrin

thrombo-: Prefix indicating blood clot

thrombocytopenia: Reduced platelet count

thrombocytosis: Elevated platelet count

thrombotic thrombocytopenic purpura (TTP): An acute and potentially fatal disorder, TTP involves fragmented RBCs, severe thrombocytopenia, hemolysis, elevated reticulocyte count, fever, and possible damage to many organs caused by lack of adequate blood flow. Cause is unknown; without therapy (repeated administration of large volumes of plasma), TTP is usually fatal

timed reaction: Term for test measurements such as blood coagulation tests in which clotting times are calculated from reaction rates

transferrin: A blood protein synthesized by the liver; transferrin binds to iron, transporting and releasing it to storage tissues (liver, bone marrow, and spleen)

uremia: An excess of nitrogen waste in the blood

venipuncture: Blood taken from a vein

Notes:

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