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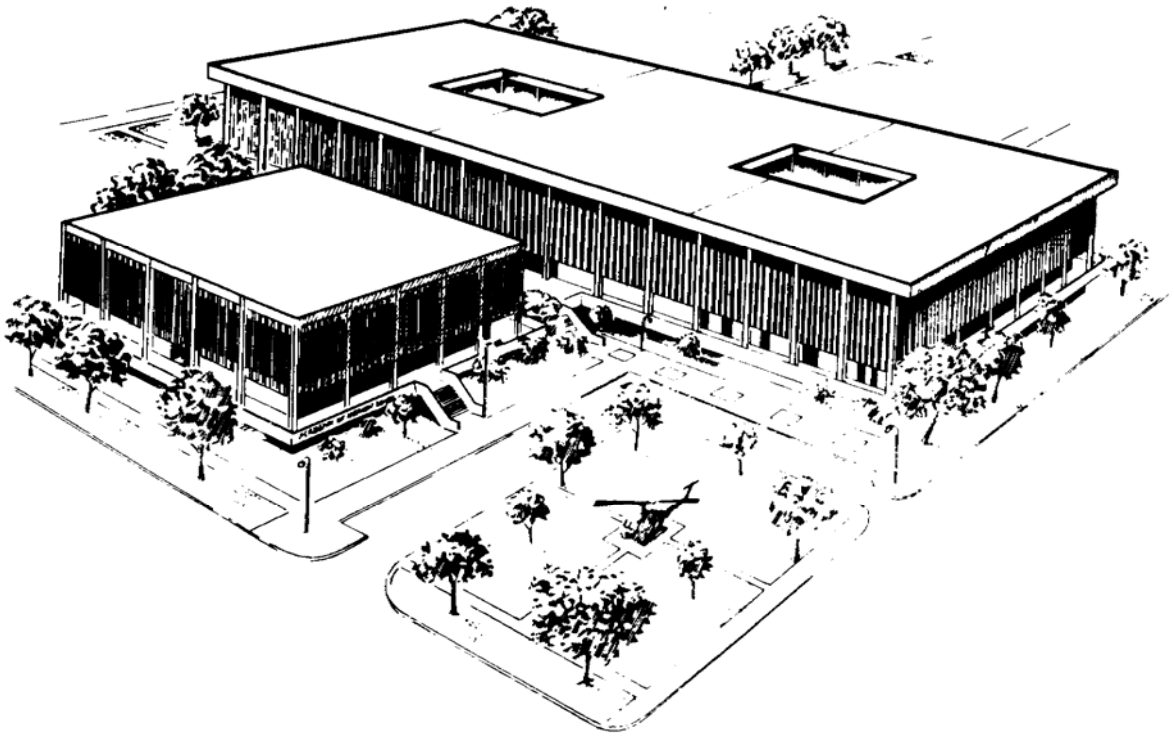

HEMATOLOGY I

SUBCOURSE MD0853 EDITION 200

DEVELOPMENT

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CORRESPONDENCE COURSE OF THE
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL

SUBCOURSE MD0853

HEMATOLOGY I

INTRODUCTION

This subcourse is concerned with the blood tests performed in the hematology section of the laboratory. The purpose of these tests is to aid the physician in diagnosis. Thus, these tests are important and often essential to the health and life of the patient. Thorough study of this subcourse should enable you to better fulfill your role in health care.

Subcourse Components:

This subcourse consists of 6 lessons and an Appendix. The lessons are:

Lesson 1.	Blood
Lesson 2.	Material Employed in Hematology.
Lesson 3.	Collection of Blood and Preparation of Blood Smears.
Lesson 4.	Morphology of Blood Cells.
Lesson 5.	Manual Cell Counts.
Lesson 6.	Hematocrit, Erythrocyte Sedimentation Rate, and Hemoglobin.
Appendix.	Glossary of Terms.

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

Upon successful completion of the examination for this subcourse, you will be awarded 12 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Section at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

LESSON ASSIGNMENT

LESSON 1	Blood.
TEXT ASSIGNMENT	Paragraphs 1-1 through 1-16.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

1-1. Select the statement that best describes the composition of blood.

1-2. Select the statement that best describes the cellular and liquid constituent characteristics of blood.

1-3. Select the characteristic features of normal and abnormal blood cell formation and maturation.

1-4. Select the functions of blood cells.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

BLOOD

Section I. THE COMPOSITION OF BLOOD

1-1. INTRODUCTION

Blood is a complex and unique fluid of variable composition circulating through the heart, arteries, capillaries, and veins, known as the vascular system of the body. It is a tissue in which cellular constituents are suspended in a liquid medium performing specialized functions. The prime function of blood is to carry oxygen from the lungs to the body tissues and carbon dioxide from the tissues to the lungs. Blood carries fluid to and from the tissues, thus maintaining proper fluid balance throughout the body (explained later in this paragraph). The average pH value of blood is 7.40. Blood also carries nutrients or food supplies from the digestive system to the body cells or tissues and transports waste products for the tissues to the kidneys and bowel for excretion to prevent accumulation. In response to trauma or infection, blood cells and antibodies are carried in the blood to a point protection against the causative agents of disease, or to transport blood-clotting substances to a break in a blood vessel to promote the clotting process when injury is caused by bleeding or hemorrhage. The blood also carries hormones from the endrocrine glands to the target organs, and it assists in the regulation of the body temperature by carrying excess heat from the interior of the body to the surface layers of the skin, where the heat is dissipated to the surrounding air. To perform these complex functions, blood is complex and is composed of two main parts. One part is composed of blood cells, the particles suspended in the plasma, making up approximately 45 percent of total blood volume and including erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). The red and white blood cells are known as corpuscles. The other part is plasma, the fluid portion of the blood, which consists primarily of water in which are dissolved proteins and many inorganic and organic substances carried by the blood to and from the tissues. Plasma makes up 55 percent of the blood.

1-2. GENERAL CELLULAR STRUCTURE

a. A typical cell is composed of a single nucleus embedded in cytoplasm. The living substance of the cell is grayish, viscous liquid called protoplasm. Protoplasm is enclosed in the cell interfaces by a cell membrane that selectively regulates the interchange of materials between the cell and its environment. A typical cell is shown in figure 1-1; however, some cells have different forms.

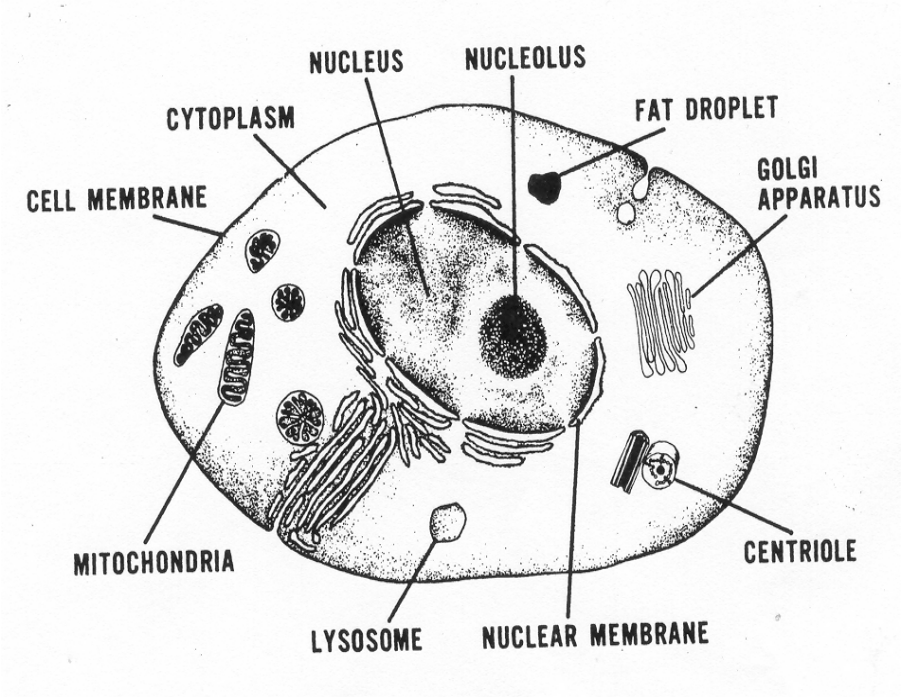


Figure 1-1. Typical cell structure.

b. The nucleus is a spherical oval body surrounded by a thin membrane (nuclear membrane). Contained in the nucleus is a sphere called the nucleolus. The nucleus is thought to be an organizing center for the cell and can have the capacity for cell production. The absence of nuclei signifies the end of cell development. Also found within the nucleus is a network of nuclear fibrils made up of DNA (deoxyribonucleic acid) and protein called chromatin. It is thought that the decreasing growth activity of a cell during maturation is regulated by chromatin.

c. Surrounding the nucleus is a mass of protoplasm called cytoplasm. Contained within the cytoplasm are numerous granules, filaments, and globules. These structures are divided into two groups known as organoids (organelles) and inclusions. The organoids are thought to perform most of the metabolic functions of the cell. Mitochondria, Golgi apparatus, fibrils, centrioles, and the chromatin substance are classified as orgnanelles. Cytoplasm inclusions are usually seen as granulation. The granulation is an accumulation of proteins, lipids, carbohydrates, pigments, and secretory granules.

1-3. CELLULAR CONSTITUENTS

a. **Erythrocytes**. An erythrocyte (red blood cell) is an elastic, non-nucleated, biconcave disc having a diameter of approximately 7.2 microns. The mature red cell contains about 34 percent hemoglobin (a complex iron-bearing pigment that transports oxygen). Hemoglobin is contained in the interior of the cell, and the outer surface of the cell is surrounded by a cell membrane. When unstained, the cell has a pale, greenish-yellowish appearance. It is buff pink with an accented central zone of pallor when stained with Wright's stain. The production of erythrocytes or erythropoiesis, occur primarily in the red marrow of the spongy bones. Erythrocytes make up the great majority of cells found in the peripheral blood. Their vast surface area is important in the transport of oxygen from the lungs to the tissues because of quick exchange of oxygen in both sites that occurs across the red cell surface. Erythrocytes are subject to many alterations in shape, size, staining properties, and structure in different disease processes. An adult female has approximately 4.8 million/cu mm red cells, and an adult male has approximately 5.4 million/cu mm red cells. The erythrocytes have an average life span of 80 to 120 days. See table 1-1 for purposes of the blood cell tree as described in this subcourse.

Leukocytes	Plasmocytic series
Granulocytes series	Plasmoblast
Myeloblast	Proplasmocyte
Promycelocyte	Plasmocyte
Neutrophils	
Neutrophilic myelocyte	Thrombocytes
Neutrophilic metayelocyte	Megakarycotic series
Neutrophilic band	Megakaryoblast
Neutrophil segmented cell	Megakaryocyte
Eosinophils	Thrombocyte
Basophilic myelocytes	
Metamyelocyte bands	Aragraulocytes
Segmented forms	
Basophils	Erythrocyte series
Lymphocyte series	Rubriblast
Lymphoblasts	Prorubricyte
Prolymphocytes	Rubricyte
Lymphocytes	Metarubricyte
Monocyte series	Reticulocyte
Monoblasts	Erythrocyte
Promonocytes	
Monocytes	

Table 1-1. Blood cell tree.

b. **Leukocytes**. Leukocytes are commonly known as white blood cells because of their lack of color in unstained preparations. They are nucleated cells that have an average diameter of 8 to 12 microns. There are 5,000-10,000 leukocytes per cubic millimeter of blood. Leukocytes are divided into three groups: (1) granulocytes (neutrophils, eosinophils, and basohils) that can phagocytize or ingest bacteria or other particles; (2) lymphocytes which participate in humoral (B lymphocyte) and cell mediated (T lymphocyte) immunity and (3) monocytes that phagocytose bacteria cellular debris and interact with lymphocytes in the processing of antigens in the immune reaction. In humans, there are 3 types of phagocytes: macrophages, granulocytes, and monocytes. All three types are attracted to bacteria and certain other particles by substances released by the particles (the process of chemotaxis). They then pin the particle against a surface and engulf it. They are differentiated by the specific nuclear and cytoplasmic staining properties.

c. **Thrombocytes**. Thrombocytes are found commonly known as platelets. They are the morphologically recognizable precursors of platelets and are detached fragments of the cytoplasm-megakaryocytes that are found in the bone marrow (others coming from red bone marrow include: promegakarycocytes and megakaryoblasts). Unstained platelets appear as small hyaline structures with a diameter of approximately two microns. When stained with Wright's stain, they have a pale blue cytoplasm with a dark granular center. Platelets are very fragile and live for a period of about 3 to 5 days.

1-4. LIQUID CONSTITUENTS

a. **Plasma**. Blood plasma is a clear, yellowish fluid that accounts for about 55 percent of the total volume of the blood. The chemical nature of plasma is very complex. It consists of 92 percent water, 7 percent proteins (albumin, globulin, and fibrinogen), carbohydrates (glucose), lipids (fats, lecithin, and cholesterol), dissolved gases (oxygen, carbon dioxide, and nitrogen), non-protein nitrogenous substances, and less than 1 percent of inorganic salts. Blood plasma is the fluid portion of the blood before clotting occurs. Put another way, plasma from which fibrinogen has been removed is called serum.

b. **Serum**. Serum is the fluid portion of blood after the clotting process is complete. Fibrinogen, the precursor of fibrin, is removed from plasma to form the framework of the blood clot.

Section II. FORMATION OF BLOOD CELLS

1-5. EMBRYONIC HEMATOPOIESIS

a. The primary source of blood cells is the mesenchyme connective tissue in the embryo. Three phases of embryonic hematopoiesis merge, resulting in the formation of blood (see figure 1-2).

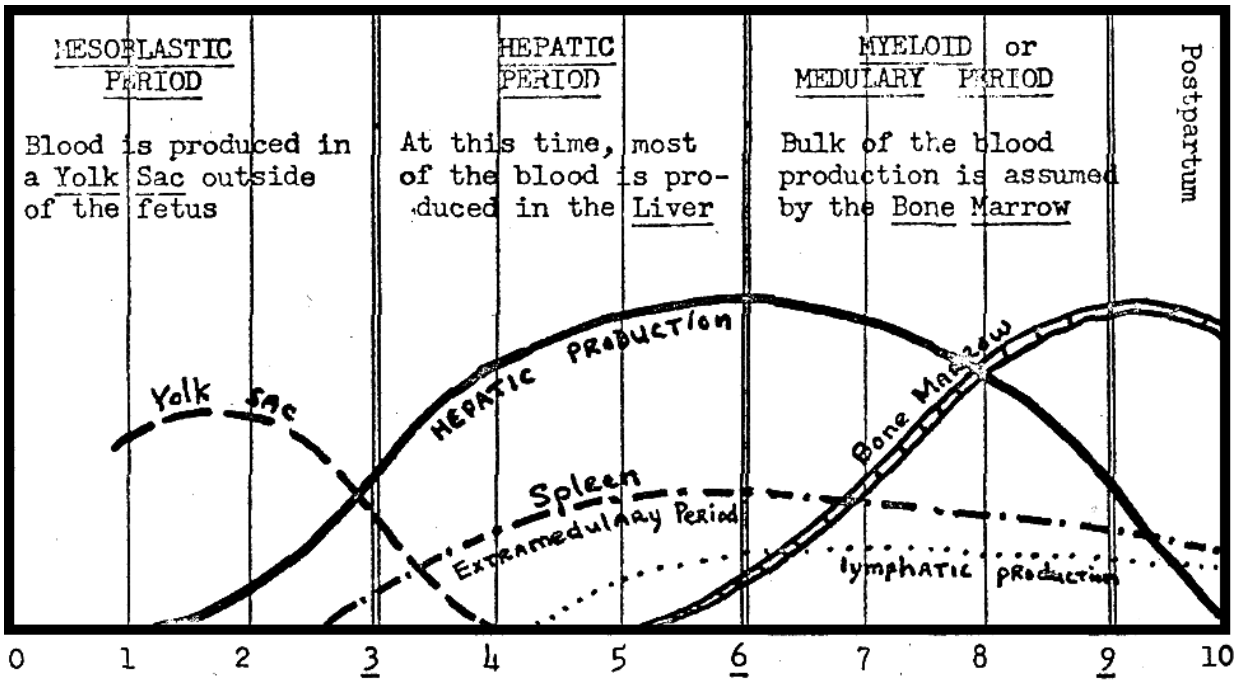


Figure 1-2. Embryonic hematophoiesis.

b. During the first two months of embryonic development, the mesoblastic phase occurs. The blood cells are formed in the blood islands of the yolk sac. Immature blood cells develop, having large nuclei containing a vesicular chromatin meshwork. The nuclei are surrounded by a thin rim of cytoplasm. The cells gradually develop hemoglobin to become a nucleated red blood cell. Mitosis occurs and daughter cells contain more hemoglobin. Finally the nucleus is lost and the nature erythrocyte is produced.

c. At two months, the hepatic phase begins. Blood cell development shifts to the body of the fetus as the organs of the reticuloendothelial system (liver, spleen, thymus, etc.) are formed. During this phase, red cells begin their normal development. Granulocytes and thrombocytes are formed in the liver while lymphocytes and monocytes are formed in the spleen and thymus.

d. The myeloid phase begins during the fifth month of gestation. Blood cells are formed in the bone marrow and lymphatic system that at the time of birth constitute the total sources of hematopoiesis. The bone marrow is the principle source of production of erythrocytes, granulocytes, and thrombocytes. The lymph nodes are the primary sites of production of lymphocytes, monocytes, and plasmacytes.

1-6. POSTNATAL HEMATOPOIESIS

a. Blood formation at birth is confined primarily to the bone marrow (central medullary structure of the bone). Blood cells multiply by mitosis and then mature to a specific cell type. The mature cells lose the ability to reproduce and develop a definite life span. Regeneration of blood cells after birth involves multiplication of precursor cells, evolution of the definitive characteristics of each type, and release of mature cells.

b. Myelopoiesis is the production of blood cells and bone marrow by the bone marrow (medullary site of production). The red bone marrow is the principle source of production of red cells and white cells of the granulocytic series. At birth, the central medullary structure of bones is red bone marrow and it is actively engaged in hematopoiesis. At about 5 years of age non-hematopoietic type of marrow (fatty yellow bone marrow) that is a reserve potential tends to replace most of the red bone marrow. This partial replacement of red marrow is complete when the individual reaches maturity (about 18 years of age) at which time, active hematopoietic centers in bone tissue are limited almost exclusively to the sternum, pelvic area, vertebrae, skull, ribs, clavicle, scapuli, and the epiphyses of the long bones.

c. Extramedullary hematopoiesis is blood production that occurs in sites other than the bone marrow. Active sites are the spleen, thymus, lymph nodes, and other lymphoid tissues. Cell production is largely limited to lymphopoiesis. The lymph nodes are the primary source of lymphocytes and plasmacytes (see figure 1-3).

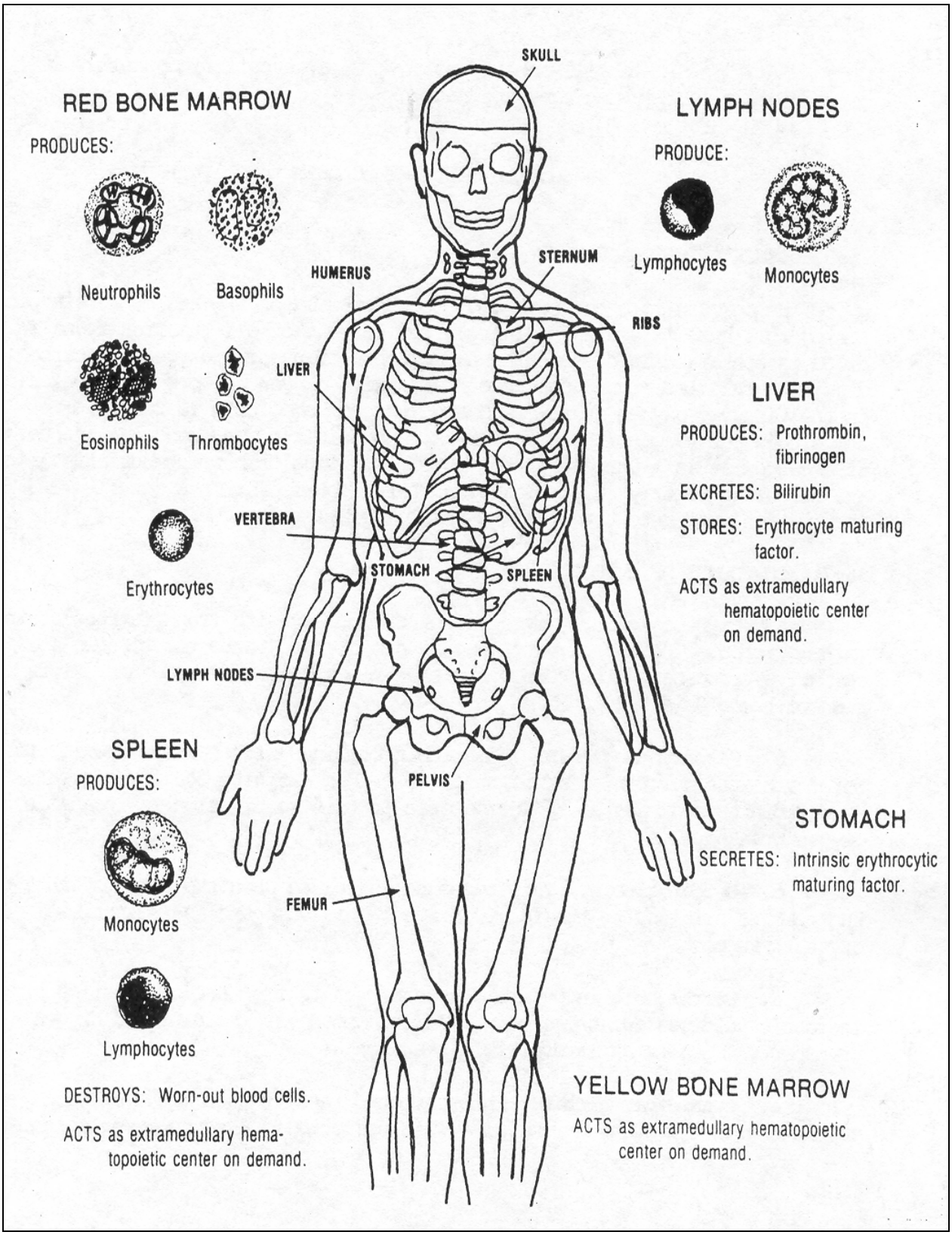


Figure 1-3. Hematopoietic system in an adult 18-20 years of age.

Section III. NORMAL CELL MATURATION

1-7. GENERAL FEATURES

In the course of blood cell maturation, certain specific features are developed (see figure 1-4). Each of the component parts of the cell undergoes a transformation during maturation. The immature cell or blast cell contains a large nucleus, a small amount of cytoplasm, and no granules. As the cell ages, the cytoplasm becomes less basophilic and nuclear chromatin becomes heavier (darker stain). Reduction in size and loss of nucleoli occurs as the cell becomes older. The three types of granulation (neutrophilic, basophilic, and eosinophilic) become more specific and smaller as the cell ages. Maturation, in general, involves: (1) cytoplasmic differentiation, (2) nuclear maturation, and (3) reduction in cell size.

1-8. CYTOPLASM

The basophilia of a blast cell is proportional to the ribonucleic acid (RNA) content. As the cell matures, the RNA content decreases and the cell becomes a paler blue. In the myeloid cells, a specific type of granulation occurs. When granules appear they are pinkish-red and few in number. The granules increase in number and differentiate into three types upon maturation. As the cell matures, it develops an affinity for the acid or basic portion of the stain (Wright's stain). Basophilic granules stain blue-black, eosinophilic granules stain red-orange, and neutrophilic granules stain pinkish-purple. Lymphocytes are usually devoid of cytoplasmic granulation but they can possess non-specific azurophilic (dark-purple) granules, usually characteristic of monocytes and plasmocytes. Upon maturation, the erythrocyte develops a light orange respiratory pigment called hemoglobin.

1-9. NUCLEUS

The nucleus of the young cell is large, round, and occupies most of the cell. As the cell matures, the size of the nucleus decreases. Nuclei of early or primitive cells usually have one or more nucleoli. The latter are small, round, homogeneous areas that usually stain light blue with a darker boundary. In appearance nucleoli are somewhat like craters in the nucleus. They are surrounded by strands of chromatin. These nucleoli, plus a delicate reticular network of chromatin, are the principle indicators of blood cell immaturity. As the cells mature, the nucleus gradually becomes smaller, stains darker, and chromatin meshwork become "coarse" with the strands of chromatin less fine and lacelike. In the course of cell development, the nucleus changes its shape, particularly in the granulocytic series, where it becomes indented, lobulated, segmented, or fragmented. As maturation or development progresses, the nucleus, if still intact, becomes small, compact, usually dark and structureless, and can completely disappear. The loss or shrinking of the nucleus is accompanied by a decrease in cell size.

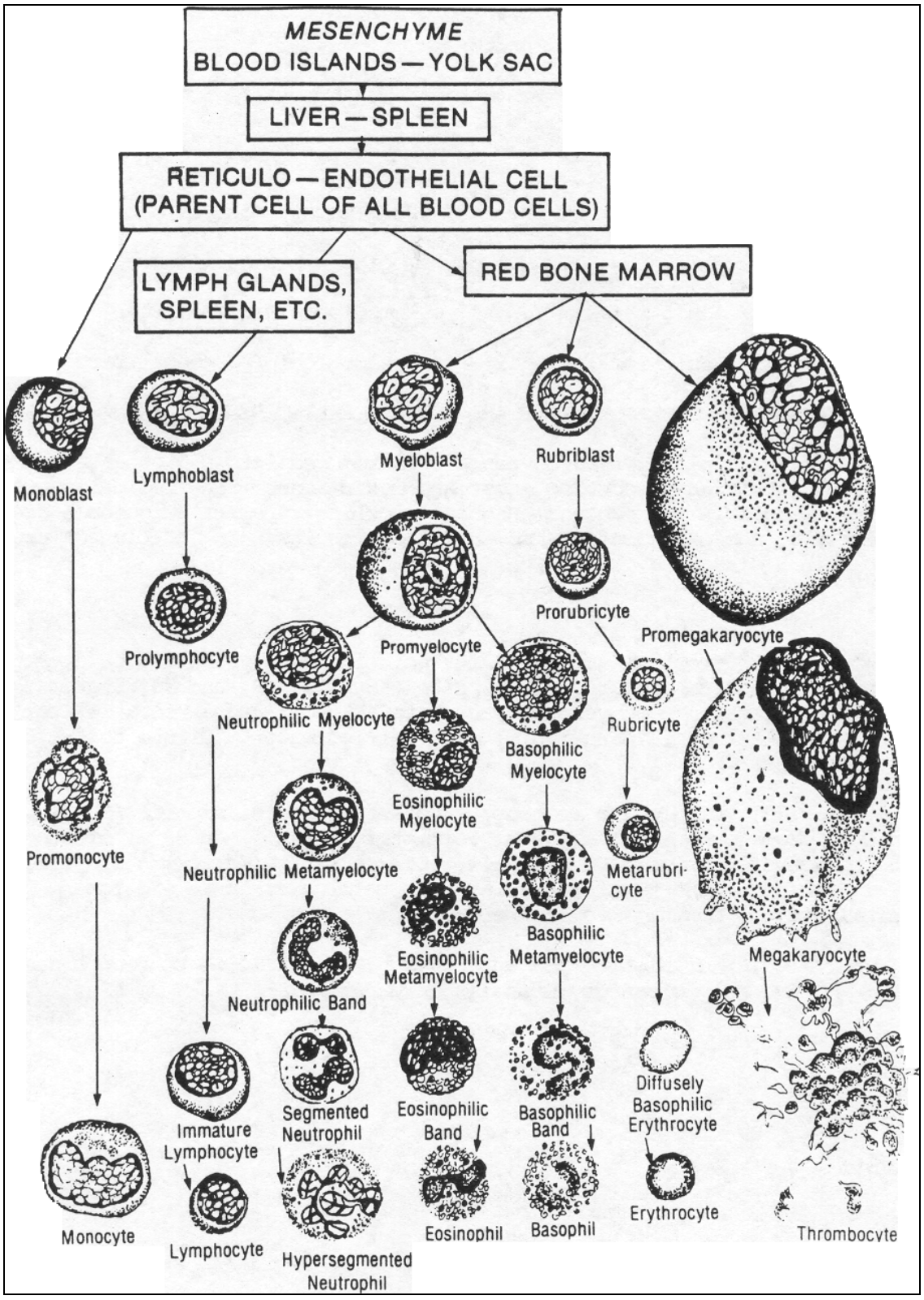


Figure 1-4. Development of blood cells.

Section IV. ABNORMAL CELL MATURATION

1-10. GENERAL FEATURES

Abnormal cell maturation is asynchronous development as opposed to normal cell maturation or synchronous development. Since the normal sequence of cell maturation is upset, atypical cells will be present. Abnormal cells can be recognized by: (1) abnormal cytoplasmic maturation, (2) abnormal nuclear maturation, and (3) abnormal size.

1-11. CYTOPLASM

Asynchronism of the cytoplasm is most commonly seen in the granulocytes. Granulation can be primitive or absent. In some instances, the granules fail to differentiate. Erythrocytes show basophilia late in the series and retarded hemoglobination. Inclusions in the cytoplasm, such as Dohle bodies (infectious diseases), Auer rods (leukemia), and toxic granulation (infection affecting the marrow) are seen in the abnormal white cells.

1-12. NECLEUS

Abnormal cells often show two nuclei in severe disturbances, such as leukemia. Nucleoli have a retarded reduction. The nucleus can have an irregular outline or indentation (Rieder cells). Hypersegmented nuclei occur in the neutrophils in sepsis and in pernicious anemia. Abnormal maturation of the nucleus often results in variation in cell size.

Section V. FUNCTIONS OF BLOOD CELLS

1-13. INTRODUCTION

Each component of blood is uniquely capable of performing one or more functions. Together, these components provide the maintenance of a relatively stable environment of the body by a variety of mechanism. This maintenance of a relative biological constancy or integrity is known as homeostasis. Once the blood cells reach full maturity, they enter the bloodstream and begin fulfilling their functions.

1-14. ERYTHROCYTES

Hemoglobin is the main functioning component of the erutjrpcyte (red blood cell). It carries out the transportation of oxygen to the tissues and the removal of carbon dioxide. Hemoglobin also aids in the maintenance of the delicate acid-based buffer system of the body. The erythrocyte must also supply energy to accomplish the active transport of glucose and ions against a gradient across the red cell membrane.

1-15. LEUKOCYTES

Leukocytes remove invading antigens (for example, bacteria) and to some extent transport and distribute antibodies. Monocytes and all of the granulocytes have been shown to demonstrate directional movement. Their movement is subject to chemotaxis or the response of living protoplasm to a chemical stimulus. As mentioned previously, the attraction of the leukocytes to bacteria and certain other particles by substances released by the particles is called chemotaxis. They either transport the particles or engulf them. The chemotaxis process of engulfing and destroying bacteria, or phagocytosis, is a prime function of leukocytes.

a. **Monocytes**. These cells will engulf bacteria and larger materials, including even protozoa and red cells, and are transformed into and/or are called macrophages. In this regard, monocytes are perhaps the most efficient phagocytes of all the cells. Monocytes contain many of the lytic enzymes that are found in microphages (granulocytes). In addition, monocytes contain lipases that enable them to dissolve the lipoid capsules of certain bacteria.

b. **Neutrophils**. Neutrophilic leukocytes are excellent microphages. That is, they engulf bacteria and other microscopic particles. The particles are first surrounded by cellular pseudopodia and then incorporated into a cell vacuole. There the foreign bodies mix with substances released from the cytoplasm of leukocytes. In this way the leukocyte is not injured by whatever "combat activity" is taking place in the vacuole. Neutrophils are fully developed (mature) cells that are incapable of mitotic division. They carry on active metabolism. Eventually the granulocytes disintegrate and in inflammatory processes are succeeded by monocytes.

c. **Eosinophils**. The eosinophils are easily distinguishable by their large cytoplasmic granules. They have some similarities with the neutrophil: they arise from a common stem cell and share same morphologic features. Unlike the neutrophil, however, eosinophils initially develop in the bone marrow, then are released into the circulation, and undergo further maturation in the spleen. Their emergence from the marrow seems to depend upon a stimulus provided by lymphocytes. Eosinophils are found in tissue fluid as well as in peripheral blood, especially in areas where there is an allergic reaction. Current thinking holds that eosinophils are involved in antigen-antibody reactions, and have been shown to phagocytize antigen-antibody reactants. Eosinophils are also thought to transport, or at least contain, lysins that act on fibrin. It is suggested that eosinophils limit the action of substances such as histamine. How this is accomplished is not yet clear. The mobilization of eosinophils from their reserve in the bone marrow is at least in part under hormonal control. If the adrenal cortex is functioning properly, an injection of adrenocorticotropic hormone (ACTH) results in a marked decrease in the number of circulating eosinophils and in the number of circulating lymphocytes. On the other hand, there is an increase in the number of circulating neutrophils.

d. **Basophils**. The function of basophils in man has not been ascertained. They quite possibly represent a vestige of evolution. Their granules have been found to contain heparin and these cells frequently appear during the clot dissolution phase of an injury. Hence, it has been suggested they may be involved in clot absorption.

e. **Lymphocytes**. The lymphocyte is now believed to be directly connected with antibody production. Undoubtedly, the lymphocyte performs important immunologic functions. According to very recent studies, many *of* the activities previously thought to take place in the reticuloendothelial (RE) system actually take place in lymphocytic tissue.

1-16. PLATELETS

Platelets possess metabolic system, expend energy, and respond to stimulus. They contain many enzymes and undergo respiratory activity and glycolysis. They possess coagulation factors usually designated as PF-1, PF-2, and on through PF-7. The cells contain fibrinogen and vasoconstrictor substances, calcium, and many other components that are either known or presumed to participate in the clotting mechanism. Clot-promoting lipoproteins are also found in platelets. In addition, well-defined antigens have been found in platelets. The role of platelets in the blood coagulation mechanism will be described in more detail in Subcourse MD0857 Lesson 2.

NOTE: See table 1-2 for a summary of blood cell functions.

BLOOD CELL NAME	FUNCTION
Erthrocytes	Contain hemoglobin, transport O_2 and CO_2, acid-base buffer, and supply energy and ions.
Leukocytes	Remove invading antigens and transport and distribute antibodies.
Monocytes	Phagocyte cells surround, engulf, and digest bacteria or other particles. Disolve lipoid capsules of certain bacteria.
Neutrophils	Engulf bacteria and other microscopic particles and perform "combat activity" in vacuoles. Carry on active metabolism.
Esoinophils	Involved in and phagocytize antigen-antibody reactants. Possibly transport or contain lysins and may limit action of substances like histamines.
Basophils	Involved with possible clot absorption.
Lymphocytes	Directly connected with antibody production and perform immunologic functions.
Platelets	Expend energy, respond to stimuli, undergo respiratory activity and glycolysis, and participate in the clotting mechanism.

Table 1-2. Blood cell functions.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise. Some exercises may require you to refer to the appendices located at the back of the subcourse.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Plasma constitutes approximately _____ percentage of the blood and is composed mainly of _____.

   a. 45 percent; water.

   b. 50 percent; protoplasm.

   c. 55 percent; water.

   d. 70 percent; cytoplasm.

2. What does chromatin regulate in a cell during maturation?

   a. Movement.

   b. Growth.

   c. Granulation.

   d. Waste products.

3. Cytoplasmic granulation is usually due to cytoplasmic:

   a. Fibrils.

   b. Inclusions.

   c. Organelles.

   d. Parachromatin.

4. When stained with Wright's stain, what is the color of the normal erythrocyte and how is it accented?

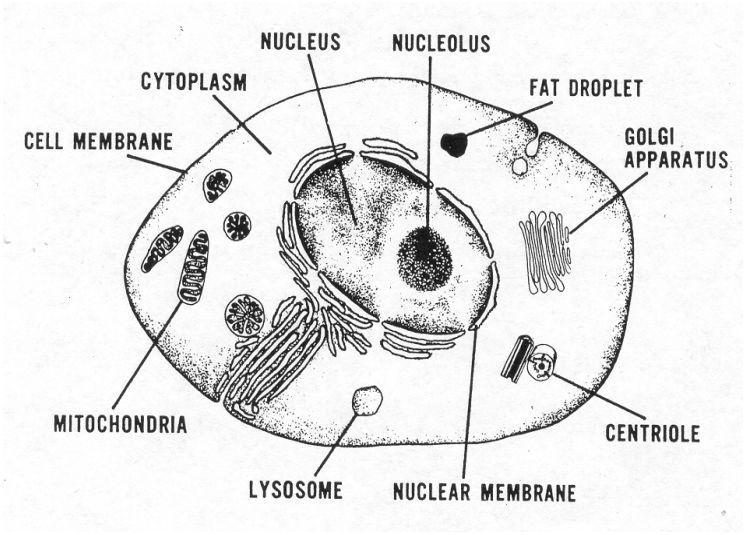
    a. Light orange granular zone.

    b. Pale green, central pale zone.

    c. Pale, greenish-yellowish, central pale zone.

    d. Buff pink, central zone of pallor.

INSTRUCTIONS: Exercise items 5 through 10 require you to select the name of the particle, activity or definition of the typical cell structure as shown below.



Typical cell structure.

5. A grayish, viscous liquid living substance within the cell that regulates the interchange of materials between the cell and the environment is called:

    a. Cytoplasm.

    b. Protoplasm.

    c. Centriole.

    d. Mitochondria.

6. The nuclear fibrils that possibly regulate growth activity during maturation and are made up of DNA (deoxyribonucleic acid) and protein are called:

    a. Lysosome.

    b. Golgi apparatus.

    c. Chromatin.

    d. Mitochondria.

7. Which chromatin substances are contained within cytoplasm and are classified as organelles?

    a. All granules, filaments, and globules inside the nucleus.

    b. Mitochondria, Golgi apparatus, fibrils, and centrioles are some of them.

    c. Some granules, filaments, and globules inside the nucleus.

8. Which organoid substance appears like a cork-screw cigar?

    a. Golgi apparatus.

    b. Fibrils.

    c. Centrioles.

    d. Mitochondria.

9. Mitochondria is defined in this subcourse as:

    a. A meshwork of lipid containing fibrils within the cytoplasmic portion of a cell.

    b. A spherical or oval body surrounded by a thin membrane (nuclear membrane).

    c. Granular components of a cell cytoplasm active in oxidative processes.

    d. A minute cell organoid within the centrosome.

10. Centriole is defined in this subcourse as:

    a. A meshwork of lipid containing fibrils within the cytoplasmic portion of a cell.

    b. A spherical or oval body surrounded by a thin membrane (nuclear membrane).

    c. Granular components of a cell cytoplasm active in oxidative processes.

    d. A minute cell organoid within the centrosome.

11. Only nucleated red blood cells are formed in the islands of the yolk sac during the _____ of embryonic development.

    a. First two months.

    b. Third to fifth months.

    c. Fifth to final months.

    d. Entire duration.

12. The bone marrow is the principal organ of red blood cell production during the myeloid phase as well as at birth and during the remainder of life.

    a. First two months.

    b. Third to fifth months.

    c. Fifth to final months.

    d. Entire duration.

13. As the child matures, myelopoiesis is increasingly confined to the:

    a. Bones.

    b. Medullary sites.

    c. Ligaments.

    d. Muscles.

14. Which definition of myelopoeisis is correct?

    a. It is the production of blood cells and bone marrow by the kidney.

    b. It is the production of blood cells and bone marrow by the liver.

    c. It is the production of blood cells and bone marrow by the bone marrow (medullary site of production).

    d. A decrease in the number of neutrophils in the blood.

15. During the formation of blood cells, which tissue is their primary source?

    a. Striated tissue.

    b. Mesenchyme connective tissue in the embryo.

    c. Liver.

    d. Tissue thromboplastin.

16. The three types of granulation occurring during normal cell maturation that become more specific and smaller as the cell matures are:

    a. Basophilic, hematopoietic, and eosinophilic.

    b. Non-hematopietic, neutrophilic, and eosinophilic.

    c. Netrophilic, basophilic, an eosinophilic.

    d. Basophllic, neutrophilic, and idiopathic.

17. Immature blood cells that are so young that they can hardly be distinguished morphologically from each other are called:

    a. First cells.

    b. Blast cells.

    c. Band cells.

    d. Monocytes.

18. What occurs to the RNA content of the blast cell as it matures?

    a. Increases.

    b. Decreases.

    c. All of the above.

19. Generally speaking, as normal cells maturate:

    a. The nucleus decreases in size.

    b. The cytoplasm differentiates.

    c. The cell reduces in size.

    d. All of the above.

20. What color does the blast cell develop into as it matures?

    a. Green.

    b. Dark purple.

    c. Blue.

    d. Pinkish-purple.

21. Lymphocytes during normal cell maturation are devoid of cytoplasmic granulation but can possess nonspecific _____ granules.

    a. Azurophilic.

    b. Dark green.

    c. Light pink.

    d. Rich orange.

22. What colors do basophilic and neutrophilic granules appear when Wright's stain is applied?

    a. Pink or brown-orange.

    b. Red-orange; blue-black.

    c. Blue-black; pinkish-purple.

    d. Reddish-purple; blue-black.

23. With Wright’s stain, eosinophilic granules appear large and:

    a. Pink or brown.

    b. Red-orange.

    c. Blue-black.

    d. Reddish-purple.

24. With Wright’s stain, the nonspecific azurophilic granule is:

    a. Pink or brown.

    b. Red-orange.

    c. Colorless.

    d. Red/dark purple.

25. The presence of _____ shaped nucleoli is generally a sign of a cell _____.

    a. Oblong; maturity.

    b. Crater; immaturity.

    c. Lacelike; cytoplasmic change.

    d. Coarse; granulocytic change.

26. Abnormal cytoplasmic maturation is seen most frequently in:

    a. Monocytes.

    b. Lymphocytes.

    c. Granulocytes.

    d. Erythrocytes.

27. What are Dohle bodies?

    a. Immture erythrocytes.

    b. Atypical monocytes.

    c. Abnormal lymphocytes.

    d. Inclusions in the cytoplasm.

28. What is another term for normal cell maturation?

    a. Polychromatic development.

    b. Synchronous development.

    c. Asynchronous development.

    d. Rieder development.

29. What term is used to describe abnormal cell maturation or uncoordinated cell development?

    a. abnormal mitosis.

    b. Asynchronous development.

    c. Binary fission.

    d. Synchronous development.

30. If asynchronism occurs in the cytoplasm, where would it most commonly be seen?

    a. Granulocytes.

    b. Nucleolus.

    c. Lysosome.

    d. Mitochondria.

31. When there is abnormal cell maturation, where do inclusions occur?

    a. Cell membrane.

    b. Nucleus.

    c. Cytoplasm.

    d. Fat droplet.

32 Which cytoplasmic cells show basophilia late in the series and hemoglobinization retardation during abnormal cells maturation?

    a. Monocytes.

    b. Lymphocytes.

    c. Granulocytes.

    d. Erythrocytes.

33. What type of inclusions are seen in leukemia?

    a. Auer rods.

    b. Rieder cells.

    c. Juvenile cells.

    d. Macrocytosis.

    e. Aplastic anemia.

34. When a cell is maturing abnormally, how many nuclei might it have if it has a severe disturbance such as leukemia?

    a. 0.

    b. 1.

    c. 2.

    d. 3.

35. The nuclei of abnormal cells often have:

    a. Two nuclei, in severe disturbances, as in leukemia.

    b. Reduced reduction of nucleoli.

    c. Undifferentiating granules.

    d. All of the above.

36. Hyposegmented nuclei occur in the neutrophils in _____ and in pernicious _____.

    a. Sepsis; leukemia.

    b. Sepsis; anemia.

    c. Sepsis; hemotoma.

    d. Sepsis; sickle cell anemia.

37. Homeostasis is defined as:

    a. Biological constancy or integrity of blood or the checking of the flow of blood, especially from a vessel.

    b. The spitting of blood; coughing up blood.

    c. Infection of the marrow; infectious disease.

    d. Blood cells reaching full maturity and entering the mainstream to begin their functions.

38. Which statement is correct concerning the function of the blood cell?

    a. Individually, each component of blood provides the maintenance for a relatively stable environment of the body by a variety of mechanisms.

    b. Together, the blood components provide the maintenance for a relatively stable environment of the body by a variety of mechanisms.

39. One function of the erythrocyte is to aid in the maintenance of the delicate acid-base buffer system of the body. Which part of the cell performs this function?

    a. Hemoglobin.

    b. Membrane.

    c. Lysosome.

    d. Glycoproteins.

40. Which are the oxygen-carrying cells of the body?

    a. Monocytes.

    b. Granulocytes.

    c. Lymphocytes.

    d. Erythrocytes.

41. _____ mainly controls the level of oxygen in the tissues.

    a. Hemoglobin.

    b. Chemotaxis.

    c. Phagocytosis.

    d. Adrenocorticotropic hormone.

42. Erythrocytes, leukocytes, and platelets are considered to be the _____ of (in) the blood.

a. Coagulation factors.

b. Immunologic functions.

c. Components.

d. Antibodies.

43. Which cells possess the powers of locomotion and phagocytosis?

a. Erythrocytes.

b. Leukocytes.

c. Thrombocytes.

d. Platelets.

44. Which cell is able to phagocytize the largest particles and because of this is called macrophage?

a. Neutrophils.

b. Eosinophils.

c. Basophils.

d. Monocytes.

45. Which cell has the main function of transporting O_2 to the tissues and removing CO_2?

a. Neutrophils.

b. Eosinophils.

c. Basophils.

d. Erythrocyte.

46. Which cell is surrounded by cellular pseudopodia and is not injured by “combat activity” taking place in the vacuole?

    a. Eosinophils.

    b. Neutrophils.

    c. Monocytes.

    d. Basophils.

47. Which cell is incapable of mitotic division?

    a. Eosinophils.

    b. Neutrophils.

    c. Monocytes.

    d. Basophils.

48. Which statement is correct?

    a. Monocytes are cells containing foreign bodies with substances released from the cytoplasm.

    b. Lymphocytes are now believed to contain heparin.

    c. Easinophils are involved with antigen-antibody reactions and eat antigen-antibody reactants.

    d. Basophils may be involved in attracting cells to substances and then either transport or engulf them.

49. Cellular particles associated with the clotting of blood are:

    a. Thrombocytes (platelets).

    b. Hemoglobin particles.

    c. White blood cells.

    d. Red blood cells.

50. Which statement is correct?

    a. Platelets possess metabolic systems, expend energy, respond to stimuli, and then disintegrate.

    b. Platelets possess metabolic systems, mobilize the bone marrow, expend energy, and respond to stimuli.

    c. Platelets possess carbon dioxide and metabolic systems, expend energy, and respond to stimuli.

    d. Platelets possess metabolic systems, expend energy, respond to stimuli, and undergo glycolysis.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. c (para 1-1)
2. b (para 1-2b)
3. b (para 1-2c)
4. d (para 1-3a)
5. b (para 1-2a)
6. c (para 1-2b)
7. b (para 1-2c)
8. d (figure 1-1)
9. c (appendix)
10. d (appendix)
11. a (para 1-5b)
12. c (para 1-5d)
13. a (para 1-6b)
14. c (para 1-6b)
15. b (para 1-5a)
16. c (para 1-7)
17. b (para 1-7)
18. b (para 1-8)
19. d (para 1-7)
20. c (para 1-8)
21. a (para 1-8)
22. c (para 1-8; figure 1-4; appendix)

23. b (para 1-8; figure 1-4; appendix)

24. d (para 1-8; appendix)

25. b (para 1-9)

26. c (para 1-11)

27. d (para 1-11)

28. b (para 1-10)

29. b (para 1-10)

30. a (para 1-11)

31. c (para 1-11)

32. d (para 1-11)

33. a (para 1-11)

34. c (para 1-12)

35. d (para 1-11, 1-12)

36. b (para 1-12)

37. a (para 1-13)

38. b (para 1-13)

39. a (para 1-14)

40. d (para 1-14)

41. a (para 1-14)

42. c (paras 1-13 to 1-16)

43. b (para 1-15)

44. d (para 1-15a)

45. d (para 1-14)

46. b (para 1-15b)

47. b (para 1-15b)

48. c (para 1-15c)

49. a (para 1-16)

50. d (para 1-16)

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2	Material Employed in Hematology.
TEXT ASSIGNMENT	Paragraphs 2-1 through 2-7.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

2-1. Correctly list the procedures in preparing laboratory reagents.

2-2. Select the statement that correctly describe how to properly label reagent containers

2-3. Select the safety precautions necessary to ensure that reagents and equipment are handled carefully and appropriately.

2-4. Correctly select the components and functions of the unopette system.

2-5. Select the correct procedure for using a blood counting chamber.

2-6. Correctly identify the parts and select the function of the compound microscope.

2-7. Select the statement that correctly describes he important characteristics and functions of centrifuges used in the laboratory.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

MATERIAL EMPLOYED IN HEMATOLOGY

Section I. LABORATORY REAGENTS

2-1. PREPARATION

Various stains and solutions are utilized in routine hematological examinations. These stains and solutions must be prepared with the utmost care and precisely according to formulations. Detailed directions for the preparation of all reagents that are required for performing procedures outlined throughout this subcourse are contained in the respective procedure. Careful attention should be given to precise measurements order in which reagents are added, control of temperature where indicated, filtration, and aging. Particular attention must be given to storage of reagents particularly with reference to requirements for refrigeration, incubation, and protection from intense light.

2-2. LABELING REAGENT CONTAINERS

Proper labeling of reagents is an extremely important detail. Labels should be complete, securely attached, and neatly and legibly written or preferably typewritten. Items recorded on the label should include all constituents and quantities utilized, date of preparation, initials of the individual who prepared the reagent, and expiration date if the solution deteriorates with age. Labels should be protected against damage from water or other fluids by covering with a protective coating of cellophane tape over the surface of the label.

2-3. SAFETY PRECAUTIONS

There are various precautions that must be taken in handling reagents in the hematology laboratory. Among the most important are the following:

a. Once a portion of a reagent has been removed from the original container, it should never be poured back because it can contaminate the remaining reagent.

b. Reagents are preferably stored in alphabetical order on shelving protected from dust, moisture, and direct sunlight.

c. Never use a reagent that cannot be clearly identified from the label on the container. Discard all reagents that cannot be accurately identified.

d. Always read the label before dispensing a reagent.

e. When working with newly prepared reagents, especially stains, ascertain whether desired results are being obtained. Unsatisfactory solutions should be discarded and replaced.

f. All mixing containers, stirring rods, and containers used for storage of reagents should be chemically cleaned prior to use.

g. During mixing and preparation, as well as in storage, it is good practice to avoid contact of reagents with metals. Many reagents contain substances that will react chemically with metals and produce changes that will render them unusable for laboratory work.

h. Do not allow inexperienced personnel to prepare reagents without close supervision.

i. Certain reagents are poisonous and adequate precautions should be taken to prevent accidental poisoning. All highly toxic reagents should be conspicuously labeled "POISON" and should be stored in a separate cabinet in the laboratory.

j. Commercial reagents should be checked with standards for purity. Record all lot numbers in case a reagent is not pure.

k. Test all new reagents to assure that proper results are attainable.

Section II. LABORATORY GLASSWARE

2-4. UNOPETTE SYSTEM

a. The Unopette System (Becton-Dickinson and Co.) consists of a disposable uniform-bore glass capillary pipet with an attached plastic tab for handling. The pipet (see figure 2-1) is attached to a plastic reservoir in which predetermined amounts of diluting fluids, depending on their purpose, can be placed. The blood aspirated into the diluting fluid can be mixed and dispensed with great ease and speed.

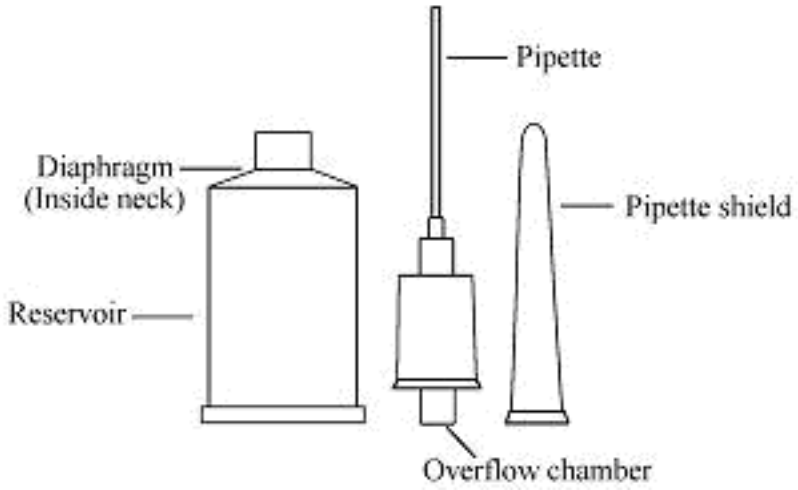


Figure 2-1. Unopette System.

b. The Unopette System can be used for a variety of hematological procedures. In general, the Unopette System is used as follows.

(1) Puncture diaphragm. Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir as follows.

(a) Place reservoir on a flat surface. Grasping reservoir in one hand, take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then removed (figure 2-2).

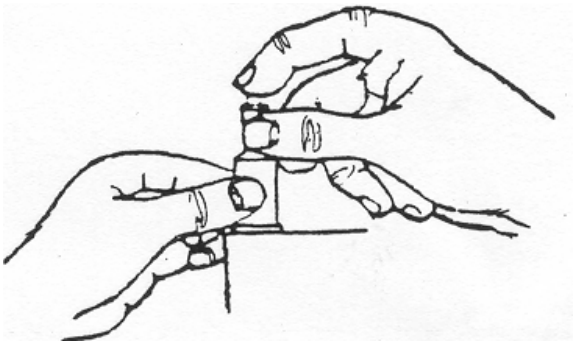


Figure 2-2. Unopetted filling procedure (puncturing diaphragm of reservoir).

(b) Remove shield from pipette assembly with a twist (figure 2-3).

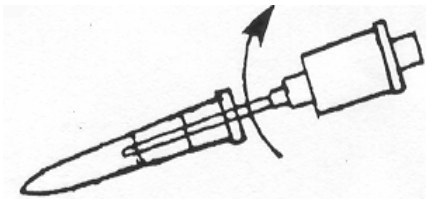


Figure 2-3. Unopetted filling procedure (removing shield).

(2) Add sample. Fill capillary with whole blood and transfer to reservoir as follows:

(a) Holding pipette almost horizontally, touch tip of pipette to blood. Pipette will fill by capillary action. Filling is complete and will stop automatically when blood reaches end of capillary bore in neck of pipette.

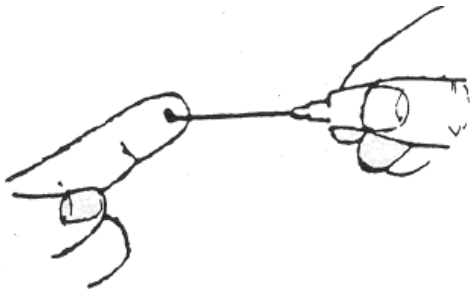


Figure 2-4. Unopetted filling procedure (filling pipette).

(b) Wipe excess blood from outside of capillary pipette making certain that no sample is removed from capillary bore.

(c) Squeeze reservoir slightly to force out some air. Do not expel any liquid. Maintain pressure on reservoir (figure 2-5).

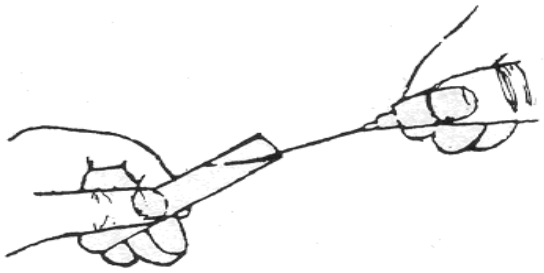


Figure 2-5. Unopetted filling procedure (forcing out air)

(d) Cover opening of overflow chamber of pipette with index finger and seat pipette securely in reservoir neck (figure 2-6).

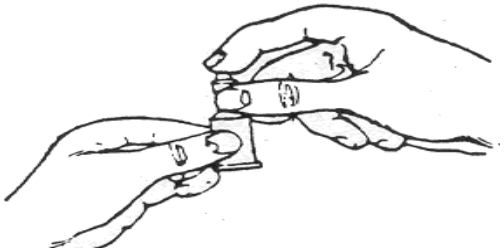


Figure 2-6. Unopetted filling procedure (seating pipette).

(e) Release pressure on reservoir. Then remove finger from pipette opening. Negative pressure will draw blood into diluent.

(f) Squeeze reservoir gently two or three times to raise capillary bore, forcing diluent up into, but not out of, overflow chamber, releasing pressure each time to return mixture of reservoir (figure 2-7).

Figure 2-7. Unopetted filling procedure (squeezing reservoir).

(g) Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent (figure 2-8).

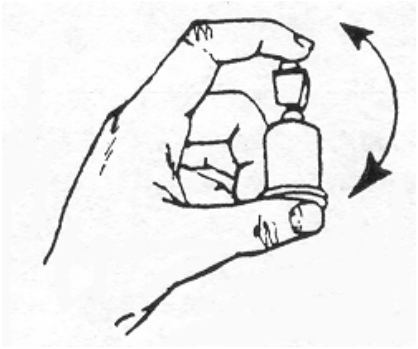


Figure 2-8. Unopetted filling procedure (mixing).

(h) Let stand for ten (10) minutes to allow red cells to hemolyze.

2-5. BLOOD CELL COUNTING CHAMBERS

a. The most common type of hemacytometer consists of two counting chambers separated by grooves or canals. On the smooth glass surface of the counting chambers are straight lines etched into glass in a gridwork pattern. The Neubauer ruling, preferred for hematological work, consists of a gridwork with dimensions of 3 mm by 3 mm. It is further divided into 9 smaller squares with dimensions of 1 mm by 1 mm; 4 of these squares are used for the white count. The 8 outer squares are further subdivided into 16 squares 0.25 mm on a side. The central square is divided into 25 squares, 0.20 mm on a side, which are used for the platelet count. Thus the large squares are 1 square mm, the 16 small squares in the outer large squares are 1/16 square mm and the 25 central squares are 1/25 square mm (see figure 2-9).

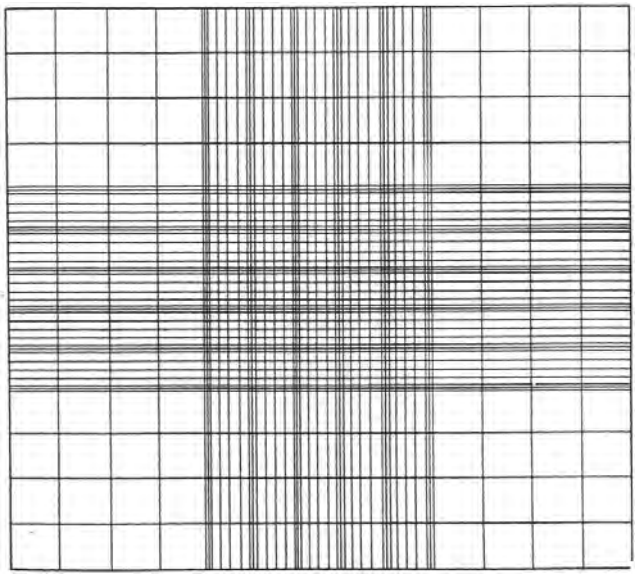


Figure 2-9. Rulings on a hemacytometer.

b. The cover glass must be free of visible defects and must be optically plane on both sides within ± 0.002 mm according to the United States (US) Bureau of Standards. When the cover glass is placed on the platform, the space between it and the ruled platform should be 0.1 mm.

Section III. LABORATORY EQUIPMENT

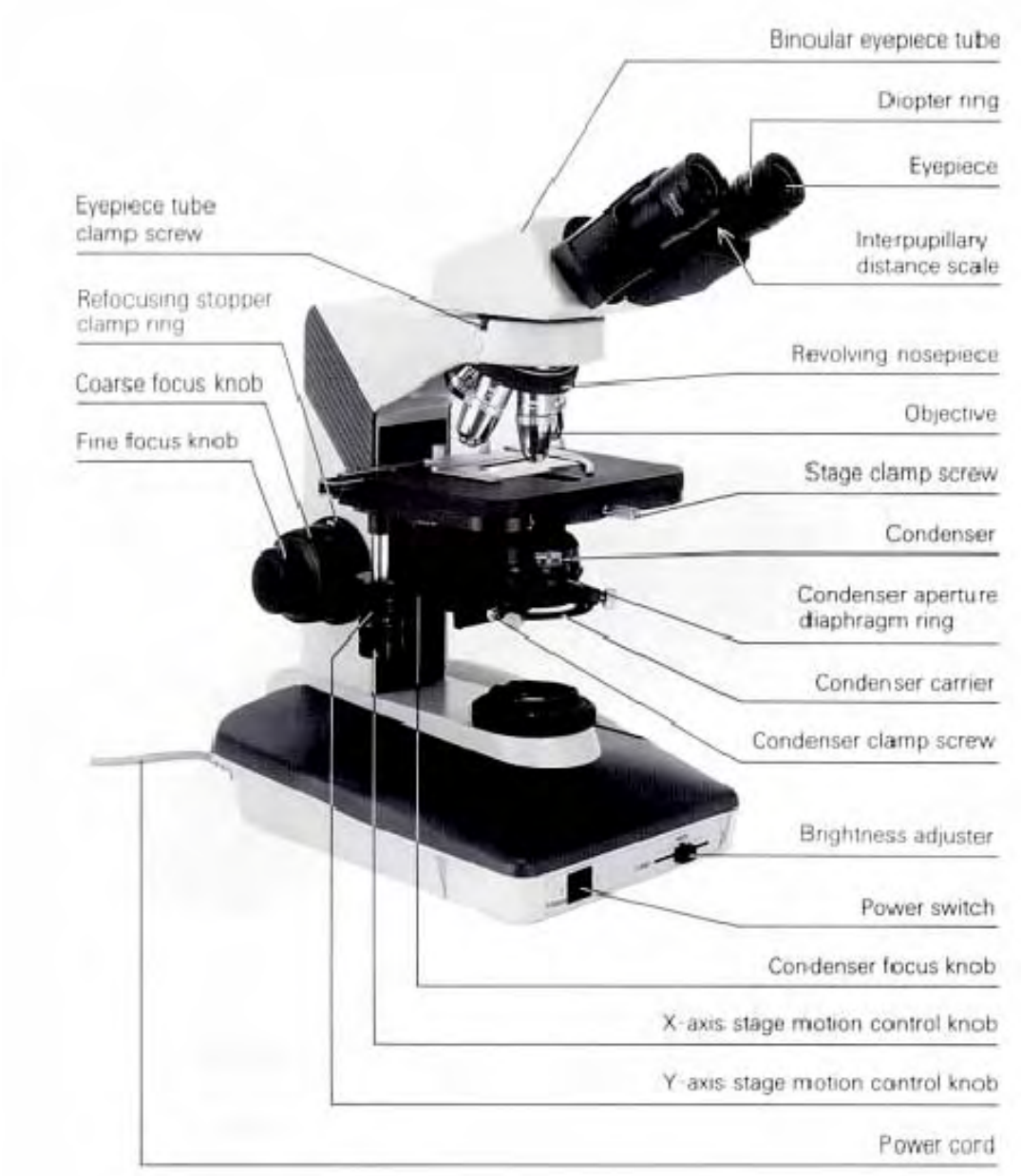
2-6. MICROSCOPES

a. **Introduction**. A modern microscope for use in the hematology laboratory is equipped with an illuminator system, a substage condenser system, an objective system, a projector (eyepiece or ocular system), an iris diaphragm, nicol prisms, a tubular barrel (monocular or binocular bodies), and a mechanical stage (figure 2-10). A compound microscope or bright field microscope uses a combination of lenses, the objective lens (lens closer to the object) and the ocular lens (lens closer to the eye) to project the image to the retina of the eye. The objective lens acts much like a small projection lens which projects an enlarged primary image near the top of the tubular barrel. This image, formed in air, is known as an "aerial image". This object is viewed through the projector or eyepiece that acts like a magnifier except that it magnifies an aerial object instead of an actual object. The final image projected on the retina of the eye is called a "virtual image" because the light rays appear to come from the image. The rays are actually created by an increase in magnification by the lens system.

b. **Magnification**.

(1) Magnification in a microscope is limited to the useful magnification that can be achieved, that is, the ability to obtain fine detail of the object being examined. This ability to render visible the fine detail is the resolving power of the microscope. The resolving power of a microscope is dependent on the numerical aperture (N.A.) of the objective lens and condenser lens. Therefore, proper adjustment of these lenses is essential in order to obtain useful magnification.

(2) Microscopes in general use in medical laboratories are provided with three objectives with focal lengths of 10x, 40x, and 100x, respectively. Microscopes are usually provided with 10X (most common) ocular. Multiplying the power of the ocular by the power of the objective gives the degree of magnification of the object under observation. The degree of magnification is expressed in diameters (refers to an increase in diameter). The ocular magnification, the millimeter length of the objective, its magnification power, and the total apparent increase obtained using oculars and objectives of the powers shown are given:



Ocular	**Objective**	**Magnification**
10X	10x	100 diameters
10X	40x	400 diameters
10X	100x	100 diameters

Magnification is increased in practice by using a higher power objective. Most microscopes are equipped with a revolving nosepiece, and selection of an objective lens is done with ease.

Figure 2-10. Compound microscope.

c. **Illumination**.

(1) Compound microscopes are dependent on electricity as the primary source for illumination power. Correct illumination of the object under study is an extremely important detail. Incorrect lighting of the object can lead to inaccurate results and conclusions. Correct illumination can be obtained from an iris diaphragm or substage light (abbe condenser).

(2) Illumination entering the microscope in which the light source is imaged at the specimen resulting in increased but uneven brightness is considered to be critical illumination. The Koehler illumination is a field of evenly distributed brightness across the specimen

(3) Regulation of the amount of light admitted is accomplished by the abbe condenser on the substage. The size of the opening in the diaphragm is controlled by a lever on the side of the condenser. The lever of the abbe condenser should never be forced to the full limit in either direction. Generally, when observing liquid preparations under low power, the condenser opening should be partially closed. Under the high dry objective, the condenser is generally opened to a greater degree to allow more light to pass through the material. When observing stained preparations under the oil immersion objective, the abbe condenser is usually opened wide.

(4) The substage condenser functions to direct a light beam of the desired numerical aperture (N.A.) and field size onto the specimen. The size of the opening in the condenser together with its position up or down controls the light entering the system. When the condenser is close to the stage, concentration of light is greater; as the condenser is moved downward, less light passes upward through the object under observation.

(5) Improper illumination is indicated when: (1) dark points or shadows appear in the field; (2) the outline of an object is bright on one side and dark on the other; or (3) the object appears to be in a glare of light. This can usually be corrected by changing the position of the iris diaphragm , by reducing the amount of light by adjusting the size of the opening in the iris diaphragm, or by raising or lowering the condenser.

d. **Focusing**. Focusing can be defined as the adjustment of the relationship between the optical system and the object so that a clear image is obtained. Several important rules to be observed when focusing the microscope on the preparation are:

(1) After the object is mounted on the stage, the objective to be used is turned into line with the eyepiece.

(2) Movement of the objective is accomplished by revolving the nosepiece. The nosepiece is provided in order to enable rapid, convenient substitution of one objective for another. This change is effected by grasping two of the objectives between the thumb and forefinger of the right hand and rotating them until the desired objective is brought into line with the axis of the body tube. It is very important that exact alignment be obtained. The correct setting is indicated by a slight "click" as the objective comes into position.

(3) Whenever the nosepiece is revolved, its movements should be observed to make certain that the objectives do not come into contact with the object. Some microscopes are not parfocal; that is, objects in focus under low power will not be in focus when the nosepiece is rotated to a higher power of magnification. It may, therefore, be necessary to refocus when changing to higher magnification. In microscopes that are parfocal, it is possible to swing other objectives into place without touching the coarse adjustment and with only a slight turn of the fine adjustment knob required to restore perfect focusing.

(4) To bring an object into focus, watch from the side and use the coarse adjustment to lower the objective until it is below the point at which the object would normally be expected to come into view.

NOTE: To avoid damage to slide or microscope, view from side for preliminary focusing. Then, using the coarse adjustment and at the same time looking through the ocular, raise the objective very slowly until the field comes into view. Further adjust to the best image, using only the fine adjustment.

(5) In focusing upward with the fine adjustment, the object will first appear in faint outline, then gradually more distinctly, and finally, sharply defined. If the adjustment goes beyond the point of sharp definition, return to the point of greatest clarity by using the fine adjustment.

(6) Never move an objective downward while looking through the eyepiece. When the objective is moved downward, always observe the downward motion with the eye held level with the microscope stage. Failure to observe these precautions can result in damage to the lens of the objective or the object under study.

e. **Care of the Microscope**. The microscope is an instrument of precision with many delicate parts, and it must be handled with the utmost care. Care should not be confined to the optical elements alone. The microscope is a combination of optical and mechanical excellence, one complementing the other. The following precautions should always be observed in the care of the microscope:

(1) No unauthorized person should manipulate the microscope.

(2) Keep the microscope as free from dirt and dust as possible. Dusty lenses produce foggy images, while dust in the focusing mechanisms causes excessive wear of those parts.

(3) The microscope should be always covered when not in use.

(4) Care should be taken to prevent all parts of the microscope from coming into contact with acid, alkali, chloroform, alcohol, or other substances that corrode metal or dissolve the cementing substance by means of which the lenses are secured into the objectives and oculars.

(5) Always carry the microscope with two hands by the arm and base.

(6) Avoid sudden jars */* such as placing the microscope on the table with undue force.

(7) No dust should be permitted to settle on the lenses nor should the finger come in contact with any of the surfaces.

(8) The lens system should never be separated, as the lenses are liable to become decentered and dust can enter.

(9) Avoid all violent contact of the objective lens and the cover glass.

(10) Keep eyepieces in the microscope at all times to keep free of dust.

(11) To remove dust, brush the lenses with a soft brush, or a burst of air. Avoid hard wiping, as dust is often hard and abrasive.

(12) Ethanol or methanol can be used in cleaning lenses or removing oil from objectives. Only a small amount is necessary and should be used with lens paper.

(13) The microscope should be protected against direct sunlight and moisture.

(14) In very warm, humid climates, microscopes should be stored in dry cabinets when not in use. Such cabinets should be reasonably airtight, equipped with a light bulb to supply heat, and several cloth bags containing a hygroscopic salt, such as calcium chloride, to absorb moisture. In warm, humid climates, the lenses of unprotected microscopes can be attacked by certain fungi that etch glass and ruin the lenses.

(15) After use, always turn the nosepiece to a position, which brings the low power objective into direct line with the opening in the substage condenser. If this precaution is not taken, the longer, higher-powered objectives can accidentally come into contact with the condenser lens

(16) The entire microscope should be cleaned frequently to remove dust, finger marks, oil, grease, and remnants of specimens. All parts of the microscope should be kept scrupulously clean at all times.

(17) Never tamper with any of the parts of the microscope. If the instrument does not seem to be functioning properly, immediately call the matter to the attention of the laboratory supervisor.

(18) Maintenance of the microscope should be done in accordance with the manufacturer's booklet of instruction.

(19) Immediately after use, the oil immersion objective must be wiped clean of oil with a soft, absorbent lens paper.

f. **Types**.

(1) Oil immersion. This type of microscope is extensively for Erythrocyte morophology, estimated platelet counts, and differentiate leukocytes.

(2). Phase microscopy. Phase microscopy is becoming increasingly prevalent in platelet counting. In bright-field illumination, a completely transparent specimen is difficult to see in any detail. By using phase contrast, transparent living objects can be studied. Phase microscopy operates on the principle that if a portion of light is treated differently from the rest, and caused to interfere with the rest, it produces a visible image of an otherwise invisible transparent specimen. Phase contrast accessories are available from the standard optical companies.

(3) Fluorescence microscopy. In hematology, used primarily for antinuclear antibody, T-cell and B-cell studies.

2-7. CENTRIFUGES

These are laboratory devices or units that apply a relatively high centrifugal force (up to 25,000 g) to a specimen, causing its separation into different fractions according to their specific gravities. Centrifugation is the process of separating components of a mixture (away from a center as in centrifugal force) on the basis of differences in densities of the different components using a centrifuge.

a. **Table Top Models**. These units are mounted on rubber feet that absorb vibration. The speed is controlled by means of a rheostat on the front panel. Top speeds of centrifuges will vary and the top speed of a particular instrument should be known in order to use the speed control device. Those centrifuges have adapters to hold 6 tubes and adapters for 12 tubes.

b. **Floor-Mounted Models**. The heavier floor-mounted models accommodate a large number of tubes at one time. The top speed of these instruments is higher than that of table models. Because of their increased inertia, they are equipped with a brake to facilitate stopping. In these units, the tubes are placed in balanced receptacles that are mounted on spokes emanating from a central hub.

c. **Microhematocrit Centrifuge**. This centrifuge is a special type of high-speed centrifuge employed to spin capillary tubes. The circular tube holder on this centrifuge is flat and surrounded by a rubber ring. It has a capacity of 24 capillary tubes. After a capillary tube is filled with blood, it is closed with a commercial clay sealing material. During centrifugation the sealed end is always placed in position facing toward the outside of the holder plate. Most centrifuges of this type spin the tubes at 10,000 rpm.

d. **Precautions**. In all instances where centrifugation is required, careful attention must be given to balancing the units. This means that tubes must be placed exactly opposite each other, they must be of identical weight, and they must contain the same amount of fluid. If at all possible, centrifuges should be equipped with tachometers so that speed nay be checked and controlled. Certain procedures, such as hematocrits, require a critical relative centrifugal force (RCF or g). Relative centrifugal force is the weight of a particle in a centrifuge relative to its normal weight, the centrifugal force per unit mass in gravities (g). To determine the RCF (or g) for these procedures, consult the serology manual or a monograph. The inside of the centrifuges should occasionally be cleaned to prevent dust particles from being blown into specimens. The lid on the centrifuge should be closed and locked before and during operation. Only open the lid when the centrifuge has stopped rotating.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. When using laboratory reagents, what routine hematological care should be taken in their preparation?

   a. Follow detailed procedural directions.

   b. Measure regents exactly.

   c. Control the temperature where indicated.

   d. All of the above.

2. Particular attention must be given to storage of laboratory reagents, particularly with reference to requirements for:

   a. Refrigeration.

   b. Ultra violet light.

   c. Protection from intense cold.

   d. All of the above.

3. A reagent container is correctly labeled if the label contains the:

    a. Constituents, initials of the individual who prepared the reagent in alphabetical order, expiration date, quantity used, and date prepared.

    b. Expiration date, initials of the individual who prepared the reagent, constituents quantity used, temperature control, and date prepared.

    c. Initials of the individual who prepared the reagent, stains, constituents, expiration date, quantity used, and date prepared.

    d. Constituents, expiration date, initials of the individual who prepared the reagent, quantity used, and date prepared.

4. A reagent's container label is properly labeled and protected if the label is:

    a. Complete and securely attached.

    b. Neatly and legibly written or preferably typewritten.

    c. Covered with a protective coating of cellophane tape over the surface of the label.

    d. All of the above.

5. Why must an unused portion of a reagent never be poured back into tile original container?

    a. Possible explosions.

    b. Inefficiency.

    c. Contamination.

    d. Improper labeling.

6. When storing reagents on shelving, protect them from:

    a. Darkness.

    b. Moisture.

    c. Direct heat.

    d. All of the above.

7. What should you do if you cannot clearly read the reagent's label and/or identify the contents of the container?

    a. Use the contents.

    b. Check with your colleague.

    c. Discard it properly and use a reagent's container with a label that you can read and the contents you can identify.

    d. Save it for next time.

8. When preparing laboratory reagents for storage, which items should be chemically cleaned prior to use?

    a. Mixing containers.

    b. Stirring rods.

    c. Storage containers.

    d. All of the above.

9. At what point is it is a good, safe, practice to avoid contact of reagents with metals since metals may become unusable for laboratory work?

    a. Labeling and heating.

    b. Refrigeration and labeling.

    c. Preparation and mixing.

    d. Preparation and heating.

10. Highly toxic reagents should be conspicuously labeled:

    a. Reagent.

    b. "POISON."

    c. Poison.

    d. “REAGENT."

11. The most common type of hemacytometer consists of _____counting chambers separated by grooves or canal?

    a. Four.

    b. Two.

    c. Sixteen.

    d. None of the above.

12. From the list, which is **NOT** a proper procedure for puncturing the diaphragm using the Unopette System?

    a. Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir.

    b. Grasping the reservoir in one hand, take pipette assembly in other hand and pull tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.

    c. Grasping the reservoir in one hand, take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.

    d. Remove shield from pipette assembly with a twist.

13. What phenomenon is used to fill the Unopette capillary with blood?

    a. Gravity.

    b. Capillary action.

    c. Brownian movement.

    d. Osmosis.

14. When using the Unopette system, at what point will the pipette automatically stop filling and be complete?

    a. When the blood reaches the top reservoir.

    b. When negative pressure is applied.

    c. When the blood reaches the end of the capillary bore.

    d. When the overflow chamber is filled.

15. How many times should the reservoir be squeezed and with what pressure to raise the capillary bore and force the diluent up into, but not out of, the overflow chamber using the Unopette system?

    a. 4 to 6; hard.

    b. 3 to 5; even.

    c. 2 to 3; gentle.

    d. 1 to 6; steady.

16. Using the Unopette System, after the blood is thoroughly mixed with the diluent and left to stand for 10 minutes, the red cells will:

    a. Overflow.

    b. Settle out.

    c. Hemolyze.

    d. Shrink.

17. What are the outer dimensions of the Neubauer ruling?

    a. 0.20 by 0.20 mm.

    b. 0.25 by 0.25 mm.

    c. 1 by 1 mm.

    d. 3 by 3 mm.

18. What configuration does the most common type of hemacytometer look like for counting blood cells?

    a. Two counting chambers separated by grooves or canals.

    b. Three counting chambers separated by grooves or canals.

    c. Two counting chambers separated by rough indentations.

    d. Four counting chambers that are not separated.

19. How many squares are used to count white cells, when the dimensions of the Neubauer ruling are further divided into 9 smaller squares, with dimensions of 1 mm by 1 mm?

    a. 2.

    b. 3.

    c. 4.

    d. 5.

20. Using the Neubauer ruling for the platelet count, which portion and how many mms are used?

    a. 25 middle squares; 0.30 mm on a side.

    b. Outer 8 squares; 0.15 mm on a side.

    c. 10 outer squares; 0.05mm on a side.

    d. 25 middle squares; 0.20 mm on a side.

21. When taking a blood cell count, the cover glass must be free of visible _____ and optically _____ on both sides.

    a. Defects; plane.

    b. Outer squares; plane.

    c. Stains; rough.

    d. Blood; clean.

22. The central square is divided into25 squares, 0.20 mm on a side, and used for the _____count.

    a. Neutrophil.

    b. White blood cell.

    c. Platelet.

    d. All of the above.

23. The cover glass must be free of visible defects and must be optically plane on both sides within $\pm$ 0.002 mm according to the:

    a. United Glass Bureau.

    b. United Blood Association.

    c. United States (US) Bureau of Standards.

    d. Neubauer Standard Bureau.

24**.** Regulating the amount of light admitted on a microscope is accomplished by:

    a. Objectives.

    b. Power sourse.

    c. Abbe condensor.

    d. Diaphragm condenser.

25. The compound microscopes are provided with what three common objectives:

    a. 250x, 1.9 x, 35x.

    b. 10x, 40x, 150x.

    c. 20x, 100x, 40x.

    d. 10x, 100x, 40x.

    e. 43x, 90x, 5x.

26. Select the correct items normally found on a modern microscope used in a hematology laboratory.

    a. A darkening system, a substage condenser system, an objective system, a projector (eyepiece or ocular system), an iris diaphragm, nicol prisms, a tubular barrel (monocular or binocular bodies), and a mechanical stage.

    b. An illuminator system, a substage condenser system, an objective system, a projector (eyepiece or ocular system), an iris diaphragm, nicol prisms, a tubular barrel (monocular or binocular bodies), and a mechanical stage.

    c. An illuminator system, a substage condenser system, an objective system, a projector (eyepiece or ocular system), a round barrel (monocular or binocular bodies), and a mechanical stage.

    d. An illuminator system, a substage condenser system, an objective system, a projector (eyepiece or ocular system), an iris diaphragm, nicol prisms, a tubular barrel (monocular or binocular bodies), and a mechanical stage.

27. What combination of lenses does a compound microscope use?

    a. Objective lens.

    b. Ocular lens.

    c. Aerial image magnifier.

    d. a and b.

28. Select the best explanation of an "aerial image".

    a. An image formed in the air.

    b. An image formed in the air. The object is viewed through the projector or eyepiece that acts like a magnifier except that it magnifies an aerial object instead of an actual object.

    c. An image formed in the air. The object is viewed through the magnifying glass except that it magnifies an aerial object instead of an actual object.

    d. An image formed on a surface. The object is viewed through the projector or eyepiece that acts like a magnifier except that it magnifies an aerial object instead of an actual object.

29. The ability of a microscope to render fine detail is dependent upon the numerical aperture and proper adjustment of which lens(es)?

    a. Ocular and objective.

    b. Ocular and condenser.

    c. Objective and condenser.

    d. Objective only.

30. When rotation of a microscope's fine adjustment causes an object in the center of the field to sway from side to side, the lighting is:

    a. Central.

    b. Oblique.

    c. Too dim.

    d. Too intense.

31. Name one item the resolving power of the microscope is dependent upon during magnification?

    a. Focal lengths.

    b. Binocular bodies.

    c. Arial image.

    d. N.A. of the objective.

32. Preliminary focusing of a microscope should be observed from the:

    a. Ocular.

    b. Objective.

    c. Top of the microscope.

    d. Side of the microscope.

33. Since correct illumination of an object under study is an extremely important detail, what can incorrect lighting cause?

    a. Inaccurate results and conclusions.

    b. Inaccurate steps and timings.

    c. Faulty conclusions and recommendations.

    d. Changing positions and recommendations.

34. What is the function of the substage condenser when illuminating slides under the microscopic?

    a. Indicates dark spots.

    b. Reduces glare.

    c. Directs a light beam.

    d. Correct inconsistencies by changing the position left or right.

35. Which statement about the substage condenser is true?

    a. The substage condenser functions to direct a hair beam of the desired numerical aperture (N.A.) and field size onto the specimen.

    b. The size of the opening in the condenser together with its position up or down controls the light entering the system.

    c. When the condenser is open to the stage, concentration of light is greater.

    d. As the condenser is moved upward, less light passes downward through the object under observation.

36. Improper illumination is indicated when:

    a. Light points appear on the outer edges of the slide.

    b. The center of an object is bright on one side and dark on the other.

    c. The object appears to be in dull light.

    d. Shadows appear in the field.

37. Which three parts of a microscope may be adjusted to control the illumination?

    a. Light switch, iris diaphragm, and ocular.

    b. Binocular, iris diaphragm, and objective.

    c. objective, iris diaphragm, and condenser.

    d. Ocular, objective, and condenser.

38. When using a microscope the "virtual image" projected on the retina of the eye is _____ the image.

    a. Initial.

    b. Intermediate.

    c. Final.

    d. Aperture.

39. Which of the following is used to clean microscope lenses?

    a. Methanol.

    b. Acetone.

    c. Household bleach.

    d. Saturated sodium hydroxide.

40. The rays from a microscope are actually created by an increase in magnification by the:

    a. Monocular.

    b. Lens system.

    c. Fluorescence microscopy.

    d. Phase microscopy.

41. Because of dust, the lens system should:

    a. Be separated.

    b. Never be separated.

    c. Be covered with gauze.

    d. Be cleaned with bleach.

42. After a capillary centrifuge tube is filled with blood, it is sealed with:

    a. Clay.

    b. Paper.

    c. Glass.

    d. Wax.

43. Most microhematocrit centrifuges have a speed of about:

    a. 500 rpm.

    b. 1,000 rpm.

    c. 5, 000 rpm.

    d. 10,000 rpm.

44. Which centrifuge has the higher type of top speed for instruments?

    a. Table top model.

    b. Floor-mounted model.

    c. Both a and b.

    d. None of the above.

45. Whenever centrifugation is required, which precaution must always be followed?

    a. Careful attention must be given to balancing the units. This means that tubes must be placed exactly opposite each other, they must be of identical weight, and they must contain the same amount of fluid.

    b. Centrifuges should be equipped with a microhematocrit so that speed may be checked and controlled.

    c. Hematocrits require a regular force and can adapt for 6 to 19 tubes.

    d. The heavier, floor-mounted models require rubber feet to absorb vibrations so as to accommodate a large number of tubes, which are housed in mounted receptacles on spokes from a distance hub.

SOLUTIONS TO EXERCISES, LESSON 2

1. d (para 2-1)
2. a (para 2-1)
3. d (para 2-2)
4. d (para 2-2)
5. c (para 2-3a)
6. b (para 2-3b)
7. c (para 2-3c)
8. d (para 2-3f)
9. c (para 2-3g)
10. b (para 2-3i)
11. b (para 2-5a)
12. b (para 2-4)
13. b (para 2-4b(a))
14. c (para 2-4b(2)(a))
15. c (para 2-4b(2)(f))
16. c (para 2-4b(2)(h))
17. d (para 2-5a)
18. a (para 2-5a)
19. c (para 2-5a)
20. d (para 2-5a)
21. a (para 2-5b)
22. e (para 2-5a)

23. a (para 2-5b)

24. c (para 2-6)(3)

25. c (para 2-6b)(2)

26. d (para 2-6b)

27. d (para 2-6a)

28. b (para 2-6a)

29. c (para 2-6b(1))

30. b (para 2-7)

31. d (para 2-6b)(1)

32. d (para 2-6d(4) NOTE)

33. a (para 2-6c(1))

34. c (para 2-6ac(4))

35. b (para 2-6c(4))

36. d (para 2-6c(5))

37. c (para 2-6c(5))

38. c (para 2-6a)

39. a (para 2-6e(12))

40. b (para 2-6a

41 b (para 2-6e(8))

42. a (para 2-7c)

43. d (para 2-7c)

44. b (para 2-7b)

45. a (para 2-7d)

End of Lesson 2

LESSON ASSIGNMENT

LESSON 3	Collection of Blood and Preparation of Blood Smears.
TEXT ASSIGNMENT	Paragraphs 3-1 through 3-8.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

3-1. Select the statement which best describes the requirements for selection and care of blood collection equipment.

3-2. Select the correct steps in collecting, processing, and recording blood specimens.

3-3. Select the names and functions of commonly used anticoagulants

3-4. Select the correct method and use the proper procedures in staining blood films for the type of blood cells found.

3-5. Select the correct function for each of Wright's component stain and buffer solutions.

3-6. Select the correct steps to prepare a blood smears.

3-7. Select the factors that affect the quality of stained blood smears.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 3

COLLECTION OF BLOOD AND PREPARATION OF BLOOD SMEARS

Section I. COLLECTION OF BLOOD SPECIMENS

3-1. INTRODUCTION

a. Hematological laboratory procedures are based upon the examination of blood specimens. To obtain valid test results, specimens must be properly collected, processed, and recorded. Blood specimens are usually obtained by either venous or capillary puncture. The source of the specimen is determined chiefly by the quantity of blood required to perform the laboratory procedures and the age and condition of the patient.

b. There is generally little difference in blood counts performed on venous or capillary blood if a free-flowing capillary blood specimen is obtained. Valid blood counts cannot be made when capillary specimens are not taken from a free-flowing sample or when they are obtained from cyanotic or calloused areas or areas of local stasis. White blood cell counts made on blood obtained from such sources can vary as much as 1000 to 1500 cells per cu mm from their real value. For general purposes, however, venous samples are preferable since they allow for multiple and repeated hematological examinations and provide a sufficient quantity of blood for performing any other required laboratory procedure. Further, with venous blood the chances of error are reduced because operations are made under ideal conditions and repeat operations are possible. In situations where there are limitations on the quantity of blood that can be obtained, that is, in small infants or extensive burn cases, microquantitative methods are satisfactory for performing an analysis on a specimen obtained by capillary puncture.

3-2. VENIPUNCTURE

a. **Site**. To obtain blood by venipuncture, draw the specimen directly from a patient's vein with a sterile hypodermic needle and syringe or a vacuum blood sample device.

(1) In adults use the veins located in the proximal forearm or antecubital space as illustrated in figure 3-1. In infants employ the jugular or femoral vein for the venipuncture. The vein selected should be large, readily accessible, and sufficiently close to the surface to be seen and palpated. If venipuncture poses a problem due to the age of the patient, sclerotization due to repeated venipuncture, or any other unusual circumstance, the technician should consult a physician concerning the procedure. UNDER NO CIRCUMSTANCES SHOULD A TECHNICIAN WITHDRAW BLOOD FROM A SAGITTAL SINUS, JUGULAR VEIN, OR FEMORAL VEIN. This should be left to the discretion of the physician in charge.

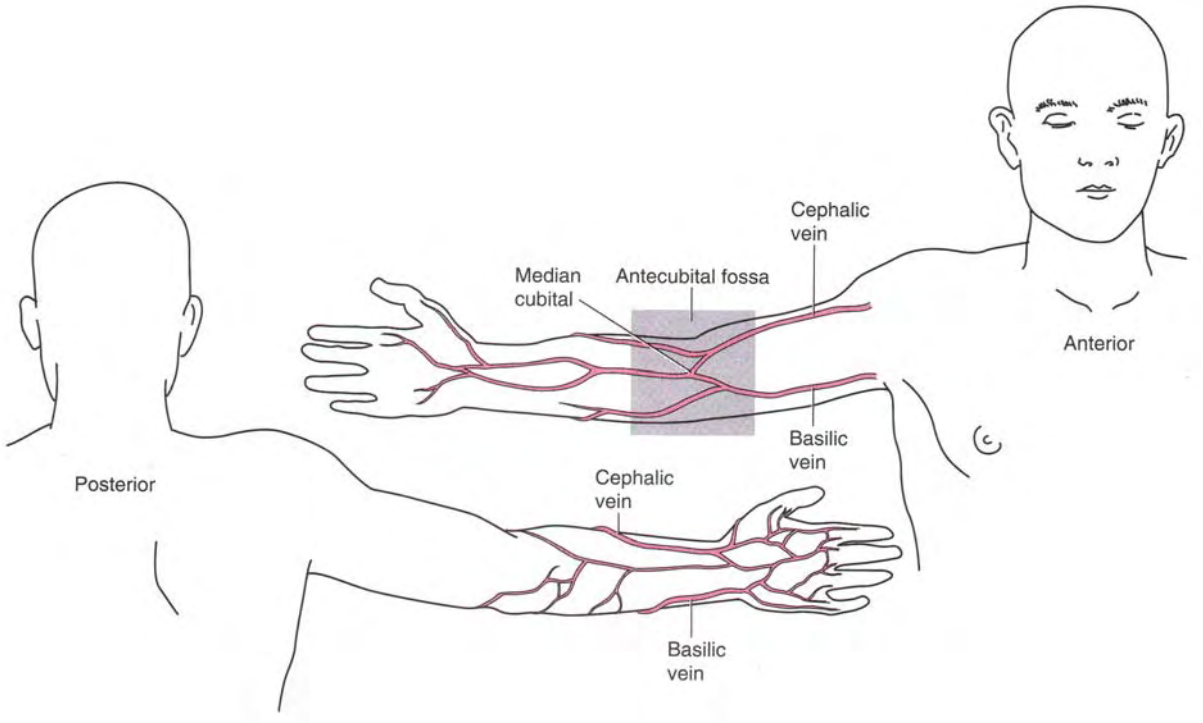


Figure 3-1. Site of venipuncture.

(2) Occasionally, the best vein is found on the hand, leg, or foot. These areas are more sensitive, and the veins are not as firmly anchored as those of the arm. Veins can become distended and easier to enter by allowing the arm to hang down for 2 or 3 minutes, by massaging the blood vessel toward the body, or by gently slapping the site of puncture. Young and vigorous persons usually have elastic veins well filled with blood. Elderly or debilitated persons can have sclerosed or fragile veins, which are hard to enter or which collapse easily.

b. **Equipment**. All syringes, needles, lancets, and other instruments used for the collection of blood specimens must be sterile. Disposable syringes or blood collection sets with vacuum tubes are available through normal supply channels. These should be used whenever possible. Aseptic technique is necessary to prevent the possible transmission of homologous serum hepatitis. The following are necessary to perform a venipuncture.

(1) Isopropyl alcohol, 70 percent, prep pads.

(2) Tourniquet.

(3) Sterile syringes or vacuum blood sample devices.

(4) Gauze pad, 2 x 2 inches.

(5) Needle, 1 to 1 ½ inches long, 19–23 gauge.

(6) Suitable blood collection tubes and labels.

(7) Gloves, latex.

c. **Preparation**.

(1) Cleanse hands thoroughly with soap and water.

(2) Place an identifying label on the blood collecting tube.

(3) Assemble the sterile needle and syringe. If a vacuum system is used, screw the needle into the plastic holder. Always leave the cap over the needle when not in use.

(4) Check to make sure that the syringe works smoothly. The syringe must be dry to avoid hemolysis of the red cells. The plunger must match the syringe and must be pushed firmly to the bottom of the cylinder to prevent injection of air into the vein. This can be fatal.

d. **Syringe Procedure**.

(1) Place a tourniquet around the patient's arm above the elbow tightly enough to check venous circulation, but not so tightly as to stop arterial flow. (If latex tubing is used, place it approximately 2 inches above he proposed venipuncture site). Form a loop with the longer end and draw the loop under the shorter end so that the tails of the tubing are turned away from the proposed site (see figure 3-2a).

CAUTION: Do not allow the tourniquet to remain in place for more than 1 minute. Check the pulse at the wrist to make sure that arterial circulation is not cut off.

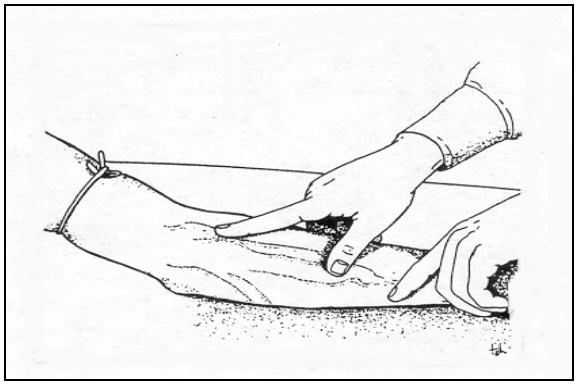


Figure 3-2a. Venipuncture procedure: Locate the vein.

(2) Instruct the patient to make a tight fist.

(3) By inspection and palpation locate the desired vein, determine the direction of its course, and estimate its size and depth (see figure 3-2a venipuncture procedure, a through h).

(4) Release tourniquet.

(5) Cleanse the skin over the selected vein with prep pads in 70 percent isopropyl alcohol in a circular motion starting from the center and working your way out. Allow the area to air dry for 30 seconds to 1 minute. Do not contaminate the area after cleaning (see figure 3-2b).

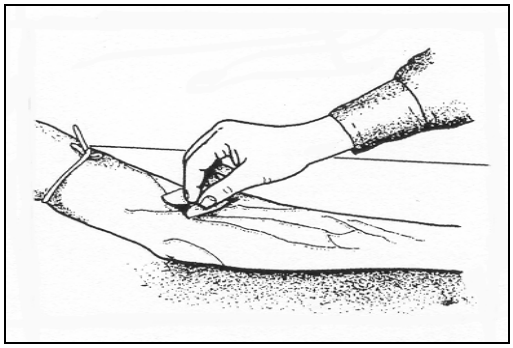


Figure 3-2b. Venipuncture procedure: Clean the puncture site.

(6) Put on gloves.

(7) Replace tourniquet on arm and have the patient straighten out the arm and make a fist.

(8) Grasp the syringe in the right hand and place forefinger on the hub of the needle to guide it. Grasp the forearm with the left hand about 2 inches below the area to be punctured and hold the skin taut with the thumb (see figure 3-2c).

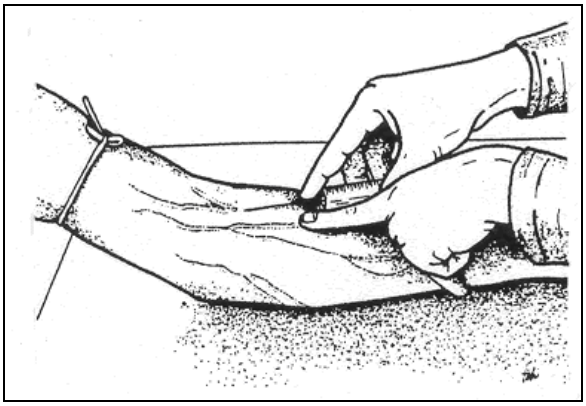


Figure 3-2c. Venipuncture procedure: Guide needle toward the vein.

(9) With the needle bevel up, parallel to, and alongside the vein, insert the needle quickly under the skin and then into the vein. The insertion into the skin and vein can be performed in one complete motion (see figure 3-2d). After entry into the vein, blood will appear in the needle hub. Do not probe or move the needle horizontally, as discomfort and possible nerve damage may result.

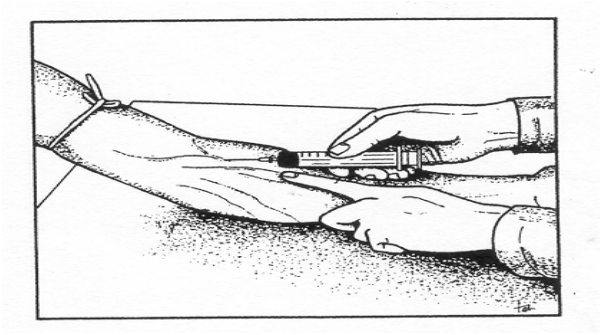


Figure 3-2d. Venipuncture procedure: Insert needle into the vein.

(10) Aspiration of the blood is accomplished by gently pulling upon the syringe plunger (see figure 3-2e). The syringe barrel should be held steady during this process. Withdraw the desired quantity.

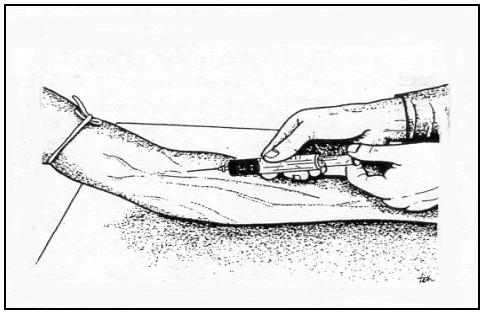


Figure 3-2e. Venipuncture procedure: Aspirate the blood.

(11) Remove the tourniquet by pulling on the long, looped end of the tubing only after blood is drawn into the syringe (see figure 3-2f).

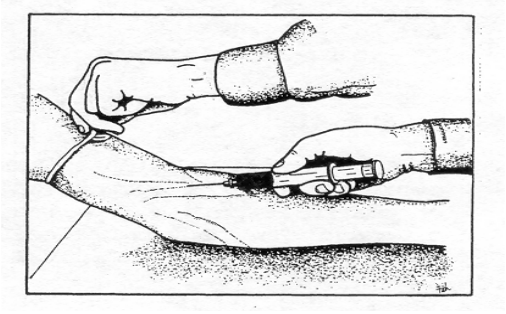


Figure 3-2f. Venipuncture procedure: Remove the tourniquet.

CAUTION: Do not remove the needle now. If the needle were remove prior to the Tourniquet being removed, blood would be forced out of the venipuncture site, resulting in blood loss and/or hematoma formation (Pooling of blood under the skin).

(12) Place a sterile gauze pad over the point where the needle entered the skin and deftly withdraw the needle simultaneously putting pressure on the site (see figure 3-2g).

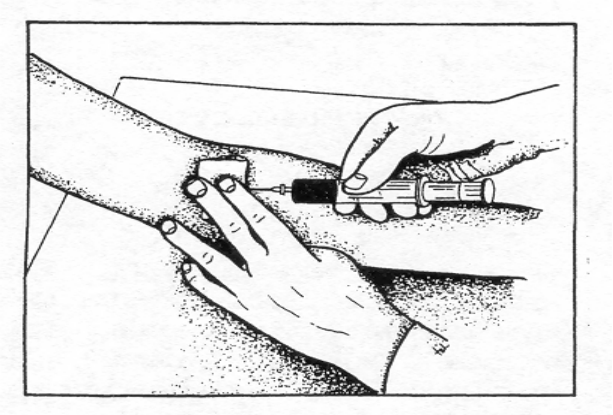


Figure 3-2g. Venipuncture procedure: Place a sterile pad over the site and withdraw the needle.

(13) Have the patient extend the arm and maintain light pressure on the gauze pad over venipuncture site (see figure 3-2h).

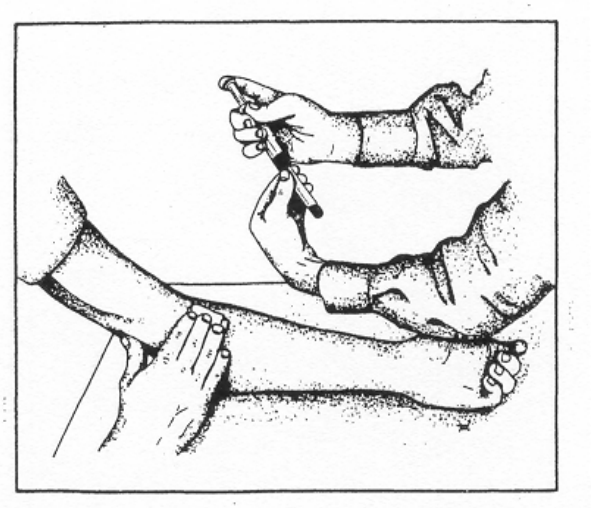


Figure 3-2h. Venipuncture procedure: Have the patient extend the arm and maintain light pressure on the site.

e. **Vacutainer Procedure**.

(1) Place the Vacutainer tube in the holder until the rubber stopper reaches the guideline. The short needle should be embedded in the stopper, but the needle must not break the vacuum (see figure 3-3).

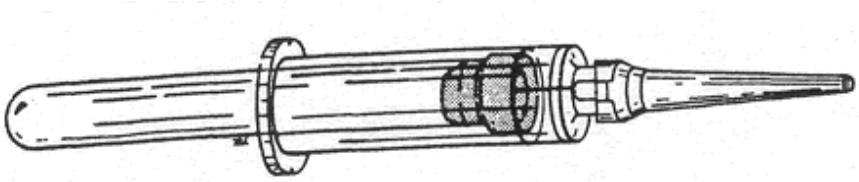


Figure 3-3. Vacutainer system.

(2) Follow steps 1-8 in syringe procedure.

(3) Enter the vein with the needle parallel to and alongside the vein. Probing or horizontal movement of the needle while under the skin must be avoided.

(4) After entry into the vein push the tube all the way into the holder; vacuum is broken, and blood flows freely into the tube. Release the tourniquet at this time by pulling the long, looped end of the tube.

(5) If the multiple needle is used or more than one tube is required, release the tourniquet after the first tube is filled; remove the filled tube and insert the next one.

CAUTION: Ensure the needle is not moved while tubes are being changed.

(6) Place a sterile gauze pad over the point where the needle enters the skin and deftly withdraw the needle, placing pressure on the site.

(7) Have the patient extend the arm and maintain light pressure on the gauze pad over the venipuncture site.

f. **Discussion**.

(1) Cleanliness is essential when performing a venipuncture.

(2) It is most important that correct technique be practiced in order to avoid unnecessary pain to the patient, prevent tissue damage, secure a good representative blood specimen, and prevent contamination of the specimen or infection of the patient.

(3) Syringes and needles must be thoroughly inspected for damage or malfunction.

(4) If difficulty is experienced in entering the vein or a hematoma begins to form, release the tourniquet and promptly withdraw the needle and apply pressure to the wound.

(5) Vigorous pulling on the plunger of the syringe can collapse the vein, produce hemolysis of the blood specimen, or cause air to enter the syringe.

(6) When repeated venipunctures have to be performed on one patient, it is advisable to select different sites for blood withdrawal.

(7) Remove the tourniquet as early as possible once a good flow of blood has been established. Prolonged application of the tourniquet results in partial stasis of blood and changes many quantitative values of blood components.

(8) Blood drawn by venipuncture is often stored for a period of time before it is analyzed. For this reason, certain general precautions must be followed in order to ensure a valid analysis. Before withdrawing blood from its container, make sure the anticoagulated blood sample is thoroughly but gently mixed. Blood containers should be tightly stoppered at all times to prevent drying or contamination. Store the blood specimen in the refrigerator. Blood count must be done within 3 hours of collection. Under no circumstances should blood taken for hematological examinations be stored overnight.

3-3. CAPILLARY PUNCTURE

a. **Site**. Several different sites are suitable for capillary puncture. Because it is the most accessible, the palmer or lateral surface of the tip of the finger (preferably ring finger) is the most common site in adults. However, certain problems can be encountered such as heavy calloused areas or excessive tissue fluids (edema) that tend to result in non-representative samples. The lobe of the ear can be used for capillary puncture. However, differences in cell concentration do occur when blood is obtained from this site, primarily because of higher lymphocyte concentrations in the ear lobe. Because of the small amount of tissue on the fingers of infants, preferred site is the heel or big toe. A modification of the normal technique that has proven quite satisfactory when working with the heel of infants is to make two incisions in a crisscross fashion or “T”.

NOTE: To be a valid report, work done on capillary blood must be from a FREE-FLOWING puncture wound.

b. **Equipment**.

(1) Gauze pads 2 x 2 inches.

(2) Blood lancet.

(3) Glass slides, heparinized capillary tubes, and other devices to receive the specimen.

(4) Isopropyl alcohol, 70 percent, prep pads.

c. **Procedure**.

(1) The puncture site should be warm to assure good circulation of blood. If it is cold, apply warm water (38º to 40º C) for a few minutes. If blood is to be drawn from the ear, the edge of the lobe, not the flat side, should be punctured.

(2) The site to be punctured is first rubbed with alcohol prep pads to remove dirt and epithelial debris, increase circulation, and render the area reasonably disinfected (see figure 3-4 capillary puncture procedure, a through d).

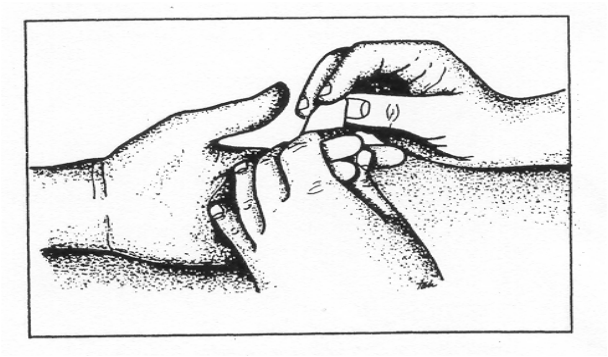


Figure 3-4a. Capillary puncture procedure: Clean the puncture site.

(3) Allow sufficient time for the circulation to equalize.

(4) While making a finger puncture, apply gentle pressure to the finger to hold the skin taut. Hold the finger in one hand and the lancet in the other. The puncture is made perpendicular to the lines of the fingerprints, which results in a more free-flowing wound (see figure 3-4b).

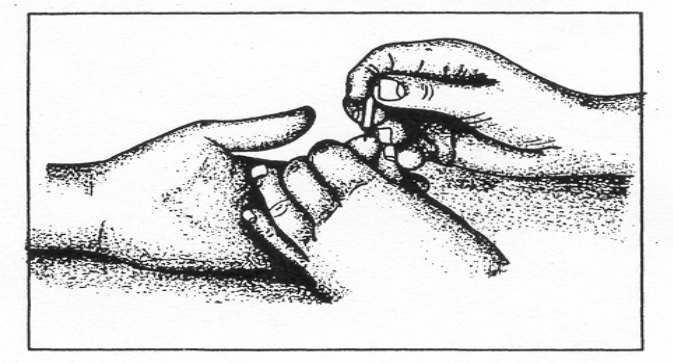


Figure 3-4b. Capillary puncture procedure: Puncture the finger.

(5) The first drop of blood that appears is wiped away before specimens are taken (see figure 3-4c).

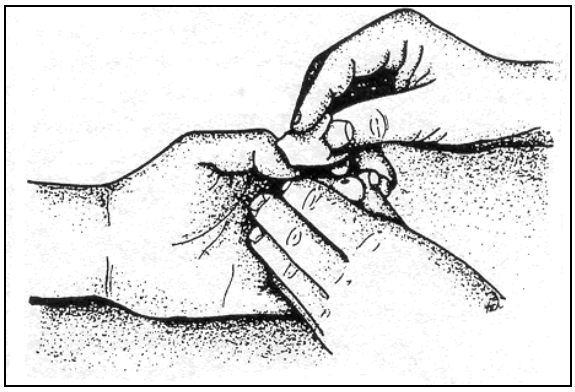


Figure 3-4c. Capillary puncture procedure: Wipe away first drop of blood.

(6) The blood must not be squeezed out since this dilutes it with fluid from the tissues, thus altering the ratio of cellular elements to fluid, as well as the ratio of cellular elements to each other.

(7) After the desired specimens have been collected, have the patient hold a sterile dry gauze pad over the wound until bleeding stops (see figure 3-4d).

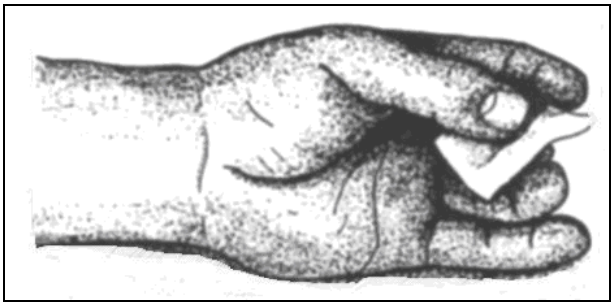


Figure 3-4d. Capillary puncture procedure: Apply pressure to the site.

d. **Discussion**.

(1) Us a disposable lancet for puncture of the skin.

(2) Do not use the finger on a hand which has been hanging over the side of the bed as it is likely to be congested. Edematous or cyanotic areas should not be used.

(3) The finger should be thoroughly dry prior to puncture; blood will not well up on a finger that is moist. Furthermore, the alcohol or other antiseptic used can coagulate the blood proteins causing cell clumping and erroneous values as well as dilute cell volumes. This will result in incorrect counts and differentials.

(4) Finger punctures should be made along the lateral aspect of the fingertip. More nerve endings are located on the fingerprint area of the fingers; therefore, more pain results from punctures in this area. Scars can also form in these sensitive areas, and difficulty may be encountered in puncturing a callous. All of these difficulties are eliminated by drawing the blood from the lateral rather than the ventral aspect of the finger.

3-4. ANTICOAGULANTS

a. Anticoagulants are used to prevent the clotting of the blood specimens and the reagent employed should not bring about alteration of blood components. Unfortunately, many anticoagulants can alter cell structures as well as coagulation. The anticoagulants most often used are ethylene-diamine-tetra-acetate (EDTA), ammonium-potassium oxalate and heparin.

b. The choice of anticoagulant will depend on the analysis to be made. Ethylene-diamine-tetra-acetate (EDTA) is the anticoagulant of choice for most hematological analyses. This anticoagulant causes a minimum of distortion to the cells and platelets. It does not dissolve quickly in blood, however, so the tube must be inverted four or five times after blood is added. The dipotassium salt is prepared as a 1 percent solution in distilled water, and a final concentration of 0.5 ml of anticoagulant for each 5 ml blood is used. Another common anticoagulant is arnmonium-potassium oxalate. This combination of oxalates does not shrink or enlarge the red blood cells appreciably. It is essential, however, to add an optimal volume of blood to the oxalate, no less than 3.5 nor more than 6.0 mI.

c. Heparin does not alter the size of cellular components. It is, in fact, the standard for comparison of anticoagulant distortion. Heparin is more expensive and dissolves less readily than double oxalate salts. Approximately 0.5 to 1.0 mg is required to anticoagulate 5 ml of blood for 72 hours. The quantity of anticoagulant noted above in each case is sufficient to prevent clotting of the blood specimen. On the other hand, an excess of anticoagulant should be avoided because too much will result in distortion of cells and hemolysis. Ideally, differential blood smears should not be prepared from blood that contains an anticoagulant.

d. If oxalate is added to vials and dried in an oven, take great care to avoid temperatures above 80^{o}C. Oxalates are converted to carbonates by prolonged exposure to elevated temperatures. Under normal circumstances, it should not be necessary to prepare your own oxalate solutions since prepared anticoagulant vacuum tubes are available from Federal medical supply sources.

e. Sodium citrate is the anticoagulant of choice for coagulation studies. It is used in a concentration of 1 part 0.11 M sodium citrate to 9 parts whole blood. It prevents coagulation by binding the calcium of the blood in a soluble complex.

f. Sodium oxalate is another anticoagulant widely used in coagulation studies. It is used in a concentration of 1 part 0.1 M sodium oxalate to 9 parts whole blood. The sodium oxalate combines with calcium in the blood to form insoluble calcium oxalate, thereby, preventing coagulation.

NOTE: Under normal circumstances, it should not be necessary to prepare an anticoagulant since prepared anticoagulant tubes are available through the Federal supply system.

g. A correctly anticoagulated blood sample is essential to the proper performance of a blood cell count. The cellular constituents must remain free in the plasma and should be as similar as is possible to those remaining in the patient's circulation.

Section II. PREPARATION AND STAINING OF BLOOD SMEARS

3-5. INTRODUCTION

a. The type of blood cells found in the peripheral blood smears may be of diagnostic and prognostic importance. For this reason proper preparation and staining of blood films is essential for the identification and study of different kinds of leukocytes. The appearance of erythrocytes and thrombocytes will often give important clues that help distinguish between different types of diseases or other physical changes.

b. There are two basic methods for the preparation of blood smears: the cover slip and the slide methods. The cover slip method has certain advantages over the slide method; distribution of cells is like that of the in vivo circulation. The principal disadvantage of the latter method is that cover slips are very fragile and easily broken during processing.

c. The slides and cover glasses must be chemically clean and dry.

d. The foundation for the morphological study of blood was based on Ehrlich's investigations of the aniline dyes, dating back to 1877, while he was still a student. Originally, simple dyes were used in the clinical laboratory and tissues were stained successively if more than one color was desired. The majority of the aniline dyes are in the form of salts of acids and bases. During the process of staining, compounds are probably formed between the basic dyes and the acid nuclear substances of cells and between the acid dyes (so called "neutral" dyes). In this way, the staining principles of the original components were preserved; and, in addition, new staining properties dependent upon the union of the component dyes were developed. These were, therefore, termed polychromic dyes.

e. One modification of these polychromic stains is Wright's stain. This is the stain most used in Army laboratories today. Wright's stain is a methyl alcohol solution of an acid dye and a basic dye. The acid dye is known as eosin, which is red in color. The basic dye, methylene blue, is blue in color. The white cells are mostly identified by their preference for these dyes. In some cases the cells are even named for the dye that they prefer. For example, cells that prefer a mixture of the acid and basic dye are called neutrophils. In the staining process, a buffer solution is used to control the acid-base balance of the stain. This is a most important function. If the buffer solution is too acid it makes the acid dye too bright and the basic dye too faint. On the other hand, if the buffer solution is too basic it makes the basic dye too bright and the acid dye too faint. In either case, the result is a poorly stained slide. The acid-base balance of a solution is measured by its pH value. A buffer solution should have a pH value between 6.4 and 6.8. This allows the best color contrast between acid and basic dyes.

f. When optimal staining conditions exist, Wright’s stain is very satisfactory and easily differentiates cells. The eosin component stains cell cytoplasm, and the methylene blue component stains nuclear material, granules, and inclusions. Both stains oxidize rapidly because they are in alkaline solution. Giemsa, a purified polychrome stain, is added to compensate for this defect by maintaining the azurophilic staining property of the mixture.

3-6. SLIDE METHOD FOR PREPARING BLOOD SMEARS

a. **Principle**. A small drop of blood is placed near one end of a clean glass slide. Using a second slide as the spreader, the blood is streaked into a thin film and allowed to dry. It is then fixed and stained with modified Wright's stain.

b. **Equipment**.

(1) Venipuncture or finger puncture material.

(2) Clean glass slides.

c. **Reagents**.

(1) Methanol Fixative.

(2) Eosinate stain (orange).

(3) Polychrome stain (purple).

(4) Water, deionized.

3-7. SLIDE PREPARATION

a. Make a finger puncture or venipuncture in the usual manner.

b. Touch a drop of blood to a clean glass slide at a point midway between the sides of the slide and a short distance from one end. If a venipuncture is made, use a capillary tube to transfer a drop of blood from the tube to the slide. If a finger puncture is made, dispense the drop of blood from the puncture site after discarding the first drop.

NOTE: The drop of blood should be no larger than 1/8 to 3/16 inch in diameter (see figure 3-5, side method for preparation of blood films, a through c.

c. Lay the specimen slide on a flat surface and hold it securely. Place a smooth, clean edge of the spreader slide on the specimen slide at an angle of about 30^0 from the horizontal (see figure 3-5a).

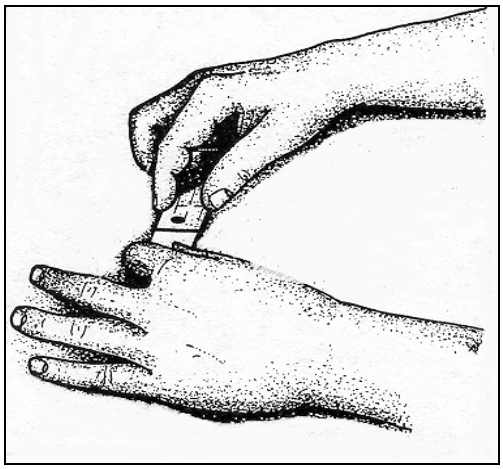


Figure 3-5a. Side method for preparation of blood films: Place spreader slide at an angle of about 30^0 from the horizontal.

d. Pull the spreader slide toward the drop of blood until contact is made within the acute angle formed by the two slides as shown in figure 3-5b.

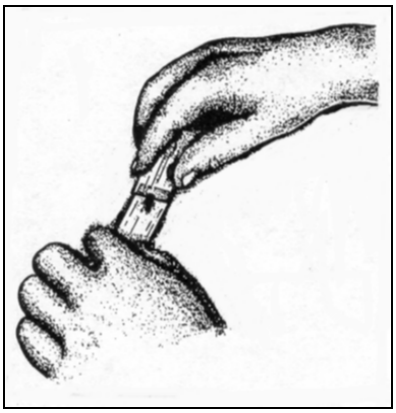


Figure 3-5b. Side method for preparation of blood films: Contact blood with spreader.

e. Allow the blood to spread toward the sides of the slide.

f. Push the spreader slide smoothly and lightly toward the opposite end of the specimen slide, drawing the blood behind it into a thin film (see figure 3-5c).

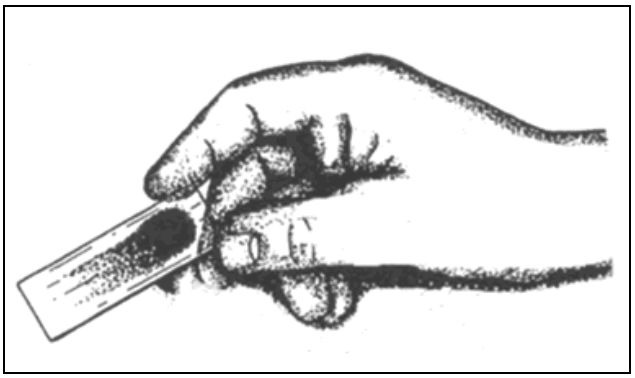


Figure 3-5c, Side method for preparation of blood films: Finished slide.

g. Allow the blood film to air-dry completely. Do not blow on the slide in an effort to enhance drying.

h. Using a lead pencil, write the name (or identification) of the patient on the frosted end of the slide. Do not use a wax pencil or marker as it dissolves during the staining process.

3-8. SLIDE STAINING

a. Place stains in four separate containers in the following order:

(1) Methanol fixative.

(2) Eosinate stain (orange).

(3) Polychrome stain (purple).

(4) Water, deionized.

b. Dip the air dried blood smear in Methanol Fixative (up and down motion) for 30 seconds.

c. Dip the smear in orange Eosin stain (up and down motion) for 30 seconds.

d. Rinse slide with distilled or deionized water (tap water has chlorine which bleaches the stain.

e. Dip the smear in purple Polychrome stain (up and down motion) for 30 seconds.

f. Let slide air dry in the vertical position – do not blot.

g. Cover solutions to prevent evaporation.

NOTE: Touch off excess liquid at the container edge to reduce carryover from one solution to another. - Stain smears within one hour of collection – WBC's degrade in stored samples. Staining time vary between manufacturers

h. Discussion.

(1) A properly prepared blood smear is margin-free; has no lines, ridges, or holes; is placed centrally on the slide; has an adequate thin area; and has a uniform distribution of leukocytes.

(2) It is preferable that blood smears not be made from blood containing anticoagulants since the leukocytes change their staining characteristics, develop vacuoles, engulf oxalate crystals, and show nuclear deformities. However, satisfactory slides are made with blood anticoagulated with EDTA.

(3) Avoid the following errors:

(a) Thick films made from an excess amount of blood placed on the slide.

(b) Delay in transferring the blood to the slide.

(c) A spreader slide that has damaged or unpolished ends.

(d) The use of dirty, dusty, greasy, or scratched slides.

(4) All slides most by fixed in methanol 30 minutes before staining.

(5) In cases of marked leukopenia, smears can be prepared from the white cell layer ("huffy coat") obtained by centrifuging the blood slowly in a Wintrobe hematocrit tube at 500-800 rpm for 5 minutes.

(6) It is important that the blood film be completely dried before staining; otherwise the wet areas will wash off the slide.

(7) Protect blood slides from insects such as flies, cockroaches, etc. They can "clean" raw blood slides very rapidly.

(8) Protect slides from areas of high humidity. Excessive moisture tends to hemolyze red blood cells.

(9) Slides should be stained as soon as possible after preparation. White cells tend to become distorted and disintegrate very rapidly, thus causing considerable difficulty in identification.

(10) After the staining is complete, do not blot the smear but air-dry it. To speed up the drying process, the smear can be placed in the heat of the substage light. It is important that the slide not be heated too intensely or too long since overheating tends to darken the staining reaction.

(11) A good quality smear should macroscopically pinkish-gray in hue. It should not be blue, green, or red. Microscopically, the red blood cells should be pink to orange and the white blood cells bluish if they display their true staining color.

(12) If the RBCs are bluish or green, this indicates that the stain is too alkaline. With an alkaline stain, the WBCs stain heavily and generally display fair distinguishing characteristics. However, the heavy stain masks any abnormalities of the RBCs. Heavy staining can be caused by:

(a) Blood smears which are too thick.

(b) Over-staining (prolonged buffer action).

(c) Evaporation of the methanol in the stain.

(d) Stain or diluent which is alkaline.

(e) Alkaline fumes.

(13) If the red blood cells are bright red, the stain is too acid. In this condition they stain well but the white blood cells (except eosinophilic granules) stain very poorly if at all. Thus, the stain is of no value for differential studies. "Tendency toward acid staining is caused by:

(a) Incomplete drying before staining.

(b) Insufficient staining (insufficient buffer action).

(c) Overdilution of the stain with buffer.

(d) Prolonged washing of the slide after staining.

(e) Stain or buffer which is acid.

(f) Acid tunes.

(14) The staining reactions of blood are as given in table 3-1.

Type of blood cell or component	**Good stain**	**Acid stain**	**Alkaline stain**
Erythrocytes All nuclei Eosinophilic granules	Pink to orange Purple-blue Granules red	Bright red Pale blue Brilliant red, distinct	Blue or green Dark blue Deep gray or blue
Neutrophilic Granules Lymphocyte Cytoplasm	Violet-pink Blue	Pale Pale blue	Dark, prominent

Table 3-1. Staining reactions.

(15) A poorly stained smear can sometimes be saved by washing rapidly with 95 percent alcohol, washing quickly in water, then restraining.

Continue with Exercises

EXERCISES, LESSON 3

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of these items, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution. Some questions have more than one answer, so read them carefully.

1. To obtain valid blood test results, specimens must be properly:

   a. Collected.

   b. Processed.

   c. Recorded.

   d. a and c.

   e. a, b, and c.

2. Blood counts on venous and capillary blood are nearly the same if the capillary puncture is:

   a. Shallow.

   b. Sterile.

   c. Free-flowing.

   d. Located on the finger.

3. Valid blood counts cannot be made when:

   a. Capillary specimens are not taken from a free-flowing sample.

   b. When capillary specimens are obtained from cyanotic or calloused areas.

   c. When sources vary as much as 150 to 1550 cells per cu mm from the real value.

   d. a and b.

4. Venous samples are preferred over capillary samples because they:

    a. Allow for several and repeated hematological examinations.

    b. Provide a sufficient amount of blood to perform the various laboratory tests needed.

    c. Provide for less chance of error because operations are made under better conditions and repeated operations are possible.

    d. a, b, and c.

5. Which blood count method would be performed if blood from an extensive burn victim was needed?

    a. Venous vacutainer collection.

    b. Capillary (micro quantitative) collection method.

    c. Syringe method.

    d. Arterial blood collection.

6. When collecting blood for white blood cell counts, blood obtained from free-flowing areas or areas of local stasis sources can vary as much as:

    a. 400-1000 cells per cu mm from their real value.

    b. 800-1200 cells per cu mm from their real value.

    c. 1000-1300 cells per cu mm from their real value.

    d. 1000-1500 cells per cu mm from their real value.

7. For adults, which veins should be used for venipuncture?

    a. Veins located in the distal forearm or antecubital space.

    b. Veins located in the proximal forearm or antecubital space.

    c. The jugular vein.

    d. The femoral vein or antecubital space.

8. For the elderly or debilitated persons, or those who may have sclerosed or fragile veins, what should you do for the venipuncture?

    a. Consult with a physician concerning the procedure.

    b. Take blood from the veins located in the proximal forearm or antecubital space.

    c. Use the jugular vein.

    d. Select the femoral vein.

9. If blood is needed from infants, which veins should be used for the venipuncture?

    a. Sagittal sinus area.

    b. Veins located in the proximal forearm or antecubital space.

    c. The collapsed vein.

    d. The jugular or femoral vein. The vein selected should be large, readily accessible, and sufficiently close to the surface to be seen and palpated.

10. If venipuncture poses a problem due to the age of the patient, sclerotization due to repeated venipuncture, or any other unusual circumstance, what procedure is to be followed?

    a. Under some circumstances the technician should withdraw blood from a sagittal sinus, jugular vein, or femoral vein.

    b. Under no circumstances should a technician withdraw blood from a sagittal sinus, jugular vein, or femoral vein.

    c. The technician may withdraw blood from a sagittal sinus, jugular vein, or femoral vein.

    d. Withdraw blood from a sagittal sinus, jugular vein, or femoral vein.

11. Which of the following is normally used for the collection of blood specimens?

    a. Isopropyl alcohol, 40 percent.

    b. Gauze pads, 6 x 6 inches.

    c. Needle, 1 to 1 1/2 inches, 19-23 gauge.

    d. Needle, large bevel.

12. Veins are made easier to enter if:

    a. The site of puncture is gently slapped.

    b. The vein is massaged toward the heart.

    c. a and b.

    d. The arm hangs down for 4 to 6 minutes.

13. Generally speaking, veins from which group of people tend to collapse more easily; and, therefore, greater care may be needed to select and puncture the vein?

    a. Children.

    b. Middle-aged adult.

    c. Elderly.

    d. Hypertensive people.

14. Blood collection instruments should be:

    a. Glass and disposable.

    b. Plastic and calibrated.

    c. Sterile and disposable.

    d. Aseptic and anticoagulated.

15. The syringe and needle for venipuncture must be dry to avoid _____ of the red blood cells.

    a. Hemolysis.

    b. Coagulation.

    c. Contamination.

    d. Hemoglobin reduction.

16. What must the technician do to prevent an possibly fatal injection of air into the vein,?

    a. Use a longer plunger than the syringe.

    b. Use a shorter plunger than the syringe.

    c. It makes no difference.

    d. The plunger must match the syringe.

17. If latex tubing is used as a tourniquet, how far above the venipuncture site should it be secured?

    a. 1 inch.

    b. 2 inches.

    c. 3 inches.

    d. 4 inches.

18. Prolonged application of a tourniquet may change the concentration of many blood components. The maximum period over which a tourniquet should be applied for a venipuncture is:

    a. 1 minute.

    b. 2 minutes.

    c. 4 minutes.

    d. 6 minutes.

19. Besides inspecting and palpating to locate the desired vein for venipuncture, on what other items should you focus?

    a. Direction of vein course and estimate its size and depth.

    b. Direction of vein course and estimate its length and color.

    c. Direction of vein course and estimate its position and elasticity.

    d. The vein's thickness, length, and size.

20. When preparing for the venipuncture, what should be done with the needle?

    a. Keep the cap on until ready to stick.

    b. Place it on a sterile pad.

    c. Dispose in sharps container.

    d. Sterilize it with alcohol.

21. What are the reasons for inspecting a possible puncture site?

    a. Estimate the size and depth of the vein (some may be too small or shallow).

    b. Determine the direction of the vein's course (puncture with the grain, so to speak).

    c. Palpate the vein (for resiliency).

    d. All of the above.

22. What may NOT be done once the puncture area is cleansed and excess alcohol wiped off?

    a. Grasp the forearm with the left hand.

    b. Straighten the arm.

    c. Contaminate the area.

    d. Have the patient make a clenched fist.

23. Puncture of the Vacutainer stopper is completed immediately:

    a. Before the needle enters the vein.

    b. After the needle enters the vein.

    c. Before withdrawal of the needl.

    d. After withdrawal of the needle.

24. Which way is the needle bevel supposed to be and how is it to be situated at time of entry?

    a. Bevel side down; parallel with and alongside the vein.

    b. Bevel side up; adjunct with and alongside the vein.

    c. Bevel side perpendicular; perpendicular to and close to the vein.

    d. Bevel side up; parallel with, and alongside the vein.

25. After the needle for a venipuncture is withdrawn, what must the patient do?

    a. Take an iron compound.

    b. Lie down for 10 minutes.

    c. Keep his fist clenched for 5 minutes.

    d. Maintain light pressure on the gauze pad over the site.

26. Which of the following is an important vacutainer procedure?

    a. The short needle should be embedded in the stopper, but the needle must not break the vacuum.

    b. Any needle should be embedded in the stopper, but the needle must not break the vacuum.

    c. The long needle should be embedded in the stopper, but the needle must not break the vacuum.

    d. The first needle should be embedded in the stopper, but the needle must not break the vacuum.

27. If the multiple needle is used or more than one tube is required for venipuncture, which of the following is to be followed?

    a. Tighten the tourniquet after the first tube is filled; remove the filled tube and insert the next one.

    b. Loosen the tourniquet after the first tube is filled; remove the filled tube.

    c. Release the tourniquet after the first tube is filled; remove the filled tube and insert the next one.

    d. Tighten the tourniquet after the first tube is filled and insert the next one.

28. Why must you be careful not to remove the needle while tubes are being changed?

    a. The blood will continue to flow.

    b. The skin may rip.

    c. a and b may occur separately or at one tine.

    d. All the above.

29. Why is it most important that correct venipuncture technique be practiced?

    a. Avoid unnecessary pain to the patient.

    b. Prevent tissue damage.

    c. Secure a good representative blood specimen.

    d. Prevent contamination of the specimen or infection of the patient.

    e. All of the above.

30. What may occur to the donor if the tourniquet is not removed as early as possible once the blood starts flowing well?

    a. Coagulation of the blood.

    b. Changes in the quantitative values of the blood components.

    c. Hemolysis of the blood specimen.

    d. All of the above.

31. The blood count should be performed within _____ once the blood is collected.

    a. 30 minutes.

    b. 3 hours.

    c. 24 hours.

    d. 48 hours.

32. The site of a capillary puncture should be:

    a. Warm.

    b. Cold.

    c. Hot.

    d. 24º C.

33. When is the tourniquet released and removed?

    a. When a hematoma begins to form, the first drop of blood appears, or when it is hard to enter the vein.

    b. When it is hard to enter the vein, a hematoma begins to form, or the first drop of blood appears, aspiration occurs.

    c. When a hematoma begins to form, or it is hard to enter the vein.

    d. All of the above.

34. What will occur if blood is squeezed from a capillary puncture?

    a. Infections.

    b. Unnecessary pain.

    c. Free- flowing punctures.

    d. Inaccurate test results.

35. Which aspect of the fingertip should be used as the site for a capillary puncture?

    a. Dorsal.

    b. Ventral.

    c. Frontal.

    d. Lateral.

36. Sodium citrate is a good anticoagulant for coagulation studies because:

    a. A concentration of one part 0.2 M sodium citrate is used to 9 parts of whole blood.

    b. It binds the calcium of the blood into a soluble complex to prevent coagulation.

    c. It combines cellular constituents in the plasma.

    d. A concentration of one part 0.1 M sodium citrate is used to 15 parts of whole blood.

37. Which of the following is true of heparin?

    a. It does not alter the size of cellular components.

    b. It dissolves more rapidly than double oxalate salts.

    c. It is least expensive.

    d. It can be used in excessive amounts.

38. EDTA ammonium-potassium oxalate, and heparin are commonly used:

    a. Stains.

    b. Buffers.

    c. Fixatives.

    d. Anticoagulants.

39. What are the two basic methods for the preparation of blood smears?

    a. Cover slip and polychromic stains.

    b. Slide and acid dye.

    c. Slide and cover slip.

    d. Methylene blue and slide.

40. How many solutions are needed to perform a Wright's stain buffer?

    a. 1.

    b. 2.

    c. 3.

    d. 4.

41. Before staining, what should be done?

    a. Dip in Esoin stain for 30 seconds.

    b. Fix in methanol for 30 seconds.

    c. Rinse in deionized water.

    d. Dip in Polychrome stain.

42. On a dried blood smear, where is the name or identification of the patient written?

    a. Side.

    b. Middle.

    c. Thin area.

    d. Frosted end.

43. When smears for a differential leukocyte count contain a low concentration of white blood cells, but marked leukopenia, they can be prepared from the _____ layer by slowly centrifuging the blood specimen in a _____ tube.

    a. Top; volumetric.

    b. Buffy coat; Wintrobe hematocrit.

    c. Plasma layer; test.

    d. Red blood cell layer; Vacutainer.

44. If areas of a blood smear are still wet when staining is to begin, they will:

    a. Hemolyze.

    b. Wash away.

    c. Stain well.

    d. Stain too heavily.

45. What is indicated if, when staining the slide, the RBCs are bluish or green?

    a. The stain is too acidic.

    b. The WOC stains very lightly.

    c. Insufficient staining.

    d. The film is too thick.

46. Why should slides be stained quickly after preparation?

    a. So buffers will appear unequal.

    b. WBC distort and disintegrate quickly.

    c. Lines and ridges will appear.

    d. Acid fumes will develop.

47. Which is an error that should be avoided when staining slides?

    a. Routinely transferring of blood to the slide.

    b. Using an oil cover slide.

    c. Using clean, dust free, and smooth slides.

    d. Using thin films of blood and placing on slides.

Check Your Answers on Next Page

SOLUTIONS TO EXECISES, LESSON 3

1. e (para 3-1a)
2. c (para 3-1b)
3. d (para 3-1b)
4. d (para 3-1b)
5. b (para 3-1b)
6. d (para 3-1b)
7. b (para 3-2a(1))
8. a (para 3-2a(1))
9. d (para 3-2a(1))
10. b (para 3-2a(1))
11. c (para 3-2b)
12. c (para 3-2a(2))
13. c (para 3-2a(2))
14. c (para 3-2b)
15. a (para 3-2c(4))
16. d (para 3-2c(4))
17. b (para 3-2d(1))
18. b (para 3-2d(1), CAUTION}
19. a (para 3-2d(3))
20. a (para 3-2c(3))

21. d (para 3-2d(3))

22. c (para 3-2d(5)

23. b (para 3-2e(4))

24. d (para 3-2c(9))

25. d (paras 3-2d(11), e(7))

26. a (para 3-2e(1))

27. c (para 3-2e(5))

28. d (para 3-2d)

29. e (para 3-2f(2))

30. b (para 3-2f(7))

31. b (para 3-2f(8))

32. a (para 3-3c(1))

33. c (para 3-2f(4))

34. d (paras 3-3a, c(6), d(3)

35. d (para 3-3d(4))

36. b (para 3-4e)

37. a (para 3-4c)

38. d (para 3-4a)

39. c (para 3-5b)

40. d (para 3-6c(1)

41. b (para 3-6c(1)(c)

42. d (para 3-7h)

43. b (para 3-8e(5))

44. b (para 3-8e(6))

45. d (para 3-8e(14))

46. b (para *3-8*e(9))

47. b (para 3-8e(3))

End of Lesson 3

LESSON ASSIGNMENT

LESSON 4	Morphology of Blood Cells.
TEXT ASSIGNMENT	Paragraphs 4-1 through 4-13.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

4-1. Select the statement that best describes a general rule of cell identification.

4-2. Select the stages of erythrocyte development, the order of maturation and series, and characteristics of each stage with variation.

4-3. Select the stages of leukocyte development, the order of maturation and series, and characteristics of each stage with variation.

4-4. Select the stages of thrombocyte development, the order of maturation and series, and characteristics of each stage.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 4

MORPHOLOGY OF BLOOD CELLS

Section I. GENERAL INFORMATION

4-1. BASIC CONCEPTS OF CELL MORPHOLOGY

a. In this lesson, all normal and most commonly seen abnormal blood cells are morphologically described. Although general rules for identification are given along with representative photographs and drawings, it is important to realize that no biological entity fits the guidelines precisely.

b. The present classification of blood cells is man's attempt at identifying stages of maturation by assigning artificial steps to a continuing process. The process is a smooth, continuing one, and therefore no one cell ever precisely fits the criteria for a specific stage. These stages are artificial classifications that exist to simplify identification.

4-2. GENERAL RULES OF CELL IDENTIFICATION

Certain general rules are applied to all cell maturation (hemopoiesis) either in the erythrocyte, leukocyte, thrombocyte, or plasmocyte series. Although these rules are broken by individual cells, they are an aid to classifying cells.

a. Immature cells are larger than mature cells and become smaller as they mature.

b. The relative and absolute size of the nucleus decreases as the cell matures. In some cell series the nucleus disappears.

c. The cytoplasm in an immature cell is quite blue in color and lightens as the cell matures.

d. The young nucleus is reddish and becomes bluer as the cell ages.

e. Nuclear chromatin is fine and lacy (lacelike) in the immature cell It becomes coarse and clumped in the more mature cells.

f. If there is doubt in the identity of a cell, classify to the more mature form.

Section II. ERYTHROCYTES

4-3. INTRODUCTION

a. In the normal development and maturation (erythropoiesis) of the erythrocytic series, the red blood cell undergoes a graduation of morphological changes. This cell development is a gradual transition (as noted in ASCP terminology—they are listed from the most immature to mature cells) and six different stages can be identified. The nomenclature used to describe red blood cells is recommended by the American Society of Clinical Pathologists and the American Medical Association. The terms with some of their synonyms are as given in table 4-1.

ASCP Terminology	Synonyms
Rubriblast	Pronormoblast
Prorubricyte	Basophilic normoblast
Rubricyte	Polychromatophilic normoblast
Metarubricyte	Orthochromatic normoblast
Diffusely basophilic erythrocyte	Polychromatic erythrocyte (Retic)
Erythrocyte	Normocyte (Mature Red Blood Cell)

Table 4-1. Terminology.

b. Erythropoiesis is regulated by the intake of substances to build the cells, the storage of these substances, and their proper utilization. When normal erythropoiesis occurs, both the cytoplasm and the nuclei of the cells grow at a synchronized rate. Individual differences in physiology and physical structure of the erythrocyte account for minor morphological changes so often encountered. In certain diseases, these morphological changes may vary to a greater extent. These variations occur in size, shape, staining, and inclusions in the erythrocyte.

4-4. ERYTHROCYTIC SERIES

See figures 4-1 through 4-6.

a. **Rubriblast (Pronormoblast)**. See figure 4-1.

(1) Size. 12 to 19 microns in diameter. The nuclear to Cytoplasm Ratio (N:C ratio) is 4:1.

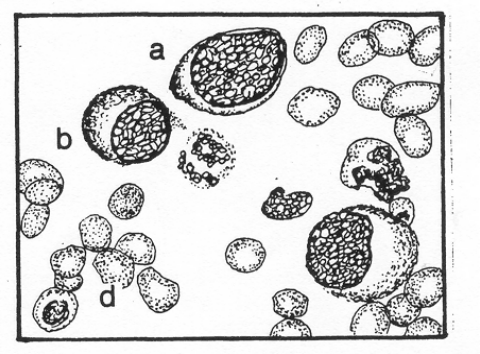


Figure 4-1. Erythrocytes series: Rubriblast.

(2) Nucleus. This cell has a large round-to-oval purple nucleus that occupies most of the cell. The nuclear chromatin is arranged in a close mesh network forming a reticular appearance. There are 0-2 light blue nucleoli present within the nucleus.

(3) Cytoplasm. The cytoplasm is dark blue (basophilic), granule-free, and limited to a thin rim (perinuclear halo) around the nucleus. There is no evidence of hemoglobin formation.

b. **Prorubricyte (Basophilic Normoblast)**. See figure 4-2.

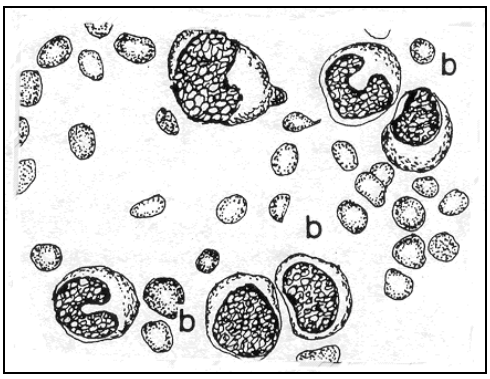


Figure 4-2. Erythrocytes series: Prorubricyte.

(1) Size. 12 to 17microns in diameter. N:C ratio 4:1.

(2) Nucleus. The nucleus is generally round, dark purple, am smaller than the nucleus of the rubriblast. The chromatin is coarse am clumped giving the nucleus a darker stain. Nucleoli are usually not present, but when they are, they appear more prominent than in the rubriblast.

(3) Cytoplasm. The cytoplasm is royal blue and more radiant than in the rubriblast. Cytoplasmic granules are not present.

c. **Rubricyte (Polychromatic Normoblast)**. See figure 4-3.

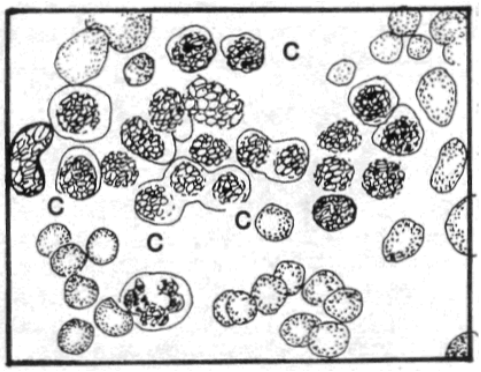


Figure 4-3. Erythrocytes series: Rubricyte.

(1) Size. 11 to 15 microns in diameter. N:C ratio 1:1.

(2) Nucleus. The nucleus is dark, round or oval, and smaller than the prorubricyte nucleus. The chromatin material is found in dense, irregular clumps. Nucleoli are not present.

(3) Cytoplasm. The cytoplasm is more abundant than in the precursor cells. It is blue-pink (polychromatic), the pink resulting from the first visible appearance of hemoglobin. Cytoplasmic granules are absent.

d. **Metarubricyte (Orthochromatic Normoblast)**. See figure 4-4.

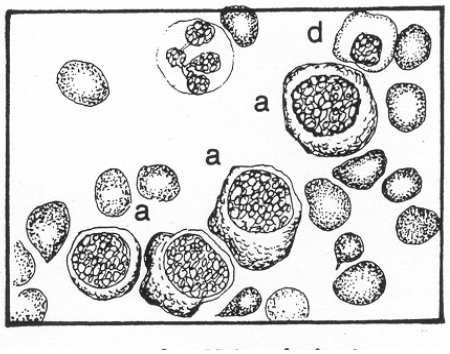


Figure 4-4. Erythrocytes series: Metrarubricyte.

(1) Size. 8 to 12 microns in diameter.

(2) Nucleus. This cell has a pyknotic nucleus (a homogeneous blue-black mass with no structure) that is round. The nucleus will be extruded from the cell in the later period of this stage. This is the main difference between the rubricyte and the metarubricyte.

(3) Cytoplasm. The cytoplasm is abundant, reddish to buff pink.

e. **Reticulocyte (Polychromatic Erythrocyte)**. See figure 4-5.

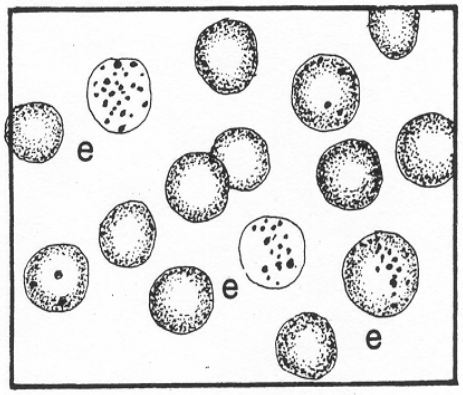


Figure 4-5. Erythrocytes series: Reticulocyte.

(1) Size. 7 to 10 microns in diameter.

(2) Nucleus. The nucleus is absent.

(3) Cytoplasm. The cytoplasm stains a bluish-buff with Wright's stain and there is no central light pallor as in the erythrocyte. With supravital staining, this cell will show light blue reticulum strands in the cytoplasm.

f. **Erythrocyte**. See figure 4-6.

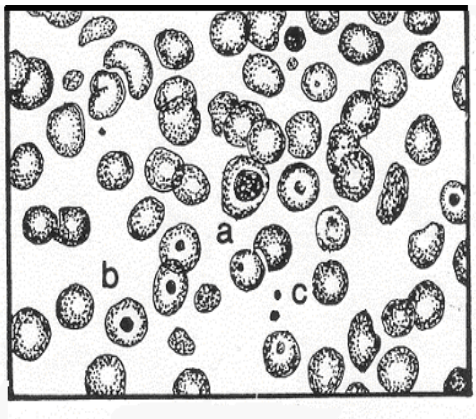


Figure 4-6. Erythrocytes series: Erythrocyte.

(1) Size. 6 to 8 microns in diameter.

(2) Nucleus. The nucleus is absent.

(3) Cytoplasm. The cytoplasm of the periphery is pinkish red with a central zone of pallor. The central pallor normally does not exceed one-third of the diameter of the cell and the color reflects the amount of hemoglobin present. Erythrocytes are able to change shape in order to transport oxygen. Erythrocytes can become flexible or pliable, and even deformable when traveling through microcirculation.

4-5. VARIATIONS IN ERYTHROCYTES

a. **Size**.

(1) Anisocytosis. Anisocytosis (see figure 4-7) is a variation in the size of erythrocytes beyond the normal limits. Cells of varying size are seen in the same fields.

(2) Macrocytes. Macrocytes are erythrocytes larger than 9 microns in diameter. These cells may be found in liver disease.

(3) Microcytes. These erythrocytes are smaller than 6 microns in diameter. These cells are found in thalassemia and other anemias.

b. **Shape**.

(1) Poikilocytosis. This term describes a marked variation in the shape of erythrocytes. Poikilocytes can be pear-shaped, comma-shaped, oval- shaped, or various other bizarre forms (see figure 4-7). These cells are encountered in pernicious anemia and many other types of anemia.

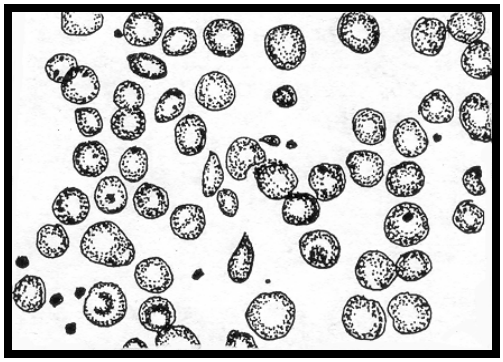


Figure 4-7. Variations in erythrocytes: Marked Poikilocytosis. Anisocytosis and target cells.

(2) Sickle cell (Drepanocytes). Sickle cells (figure 4-8) are abnormal erythrocytes that assume a crescent or sickle-shaped appearance under conditions of reduced oxygen tension. The presence of sickle cells is an inherited abnormality due to the presence of hemoglobin S. Sickle cell anemia is encountered primarily in Blacks.

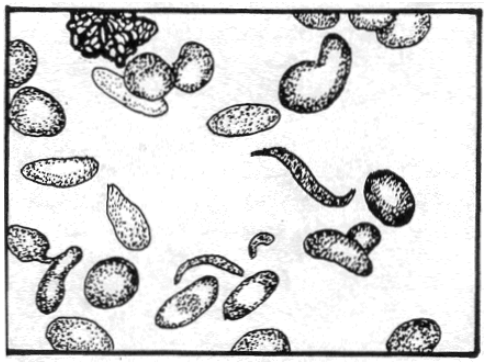


Figure 4-8. Variations in erythrocytes: Poikilocytosis: Sickle cells.

(3) Spherocytes. These are abnormal erythrocytes that are spherical in shape, having a diameter smaller than normal, and a darker stain (without central pallor) than normal erythrocytes. These cells are found in instances of hemolytic anemias and are particularly characteristic of congenital hemolytic jaundice, a hereditary disorder.

(4) Ovalocvtes (elliptocyte). These cells are abnormal erythrocytes that have an oval or “sausage” shape (see figure 4-9). They can be found in hereditary elliptocytosis.

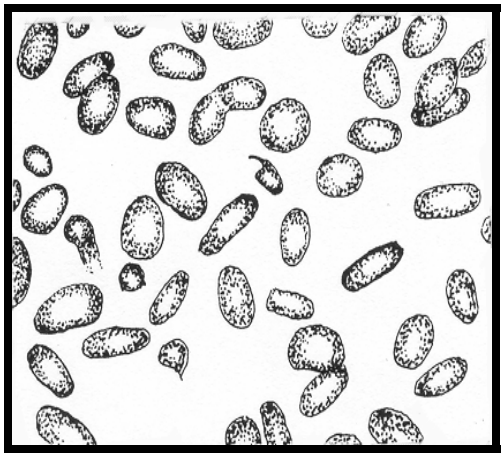


Figure 4-9. Variations in erythrocytes: Elliptocytes (oval erythrocytes).

(5) Target cells (codocyte). Target cells (figure 4-10) are erythrocytes that have deeply stained (pink) centers and borders, separated by a pale ring, giving them a target-like appearance. They are associated with liver disease and certain hemoglobinopathies.

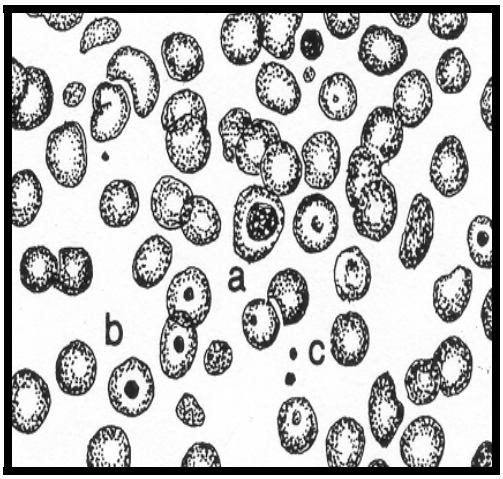


Figure 4-10. Variations in erythrocytes:
a. Metarubricyte, b. Target Cell, c. Crenated RBC

(6) Burr cells (echinocyte). Burr cells (figure 4-11) are triangular or crescent-shaped erythrocytes with one or more spiny projections on the periphery. These cells are seen in uremia, acute blood loss, cancer of the stomach and pyruvate kinase deficiency.

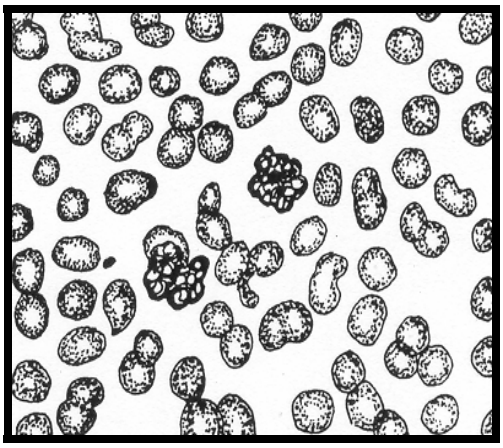


Figure 4-11. Variations in erythrocytes:
Crenated RBC burr cells. Acanthocytes 2 leukocytes.

(7) Acanthocytes (spur cells). Acanthocytes are irregularity-shaped erythrocytes with long spiny projections. They are seen in a congenital abnormality characterized by serum concentration of low density (beta) lipoproteins.

(8) Crenated erythrocytes. This condition occurs when blood films dry too slowly and the surrounding plasma becomes hypertonic. There is no pathological significance when they are found in blood smears.

(9) Schistocytes. These are red blood cell fragments. Frequently these cells have a hemispherical shape (helmet cells).

(10) Rouleaux formation. This phenomenon is adherence of erythrocytes to one another presenting a stack-of-coins appearance. It occurs in conditions characterized by increased amounts of fibrinogen and globulin.

c. **Staining.**

(1) Hypochramia. Hypochramia (figure 4-12) is a condition in which the normal central pallor is increased due to decreased hemoglobin content. This condition is characteristic of many anemias.

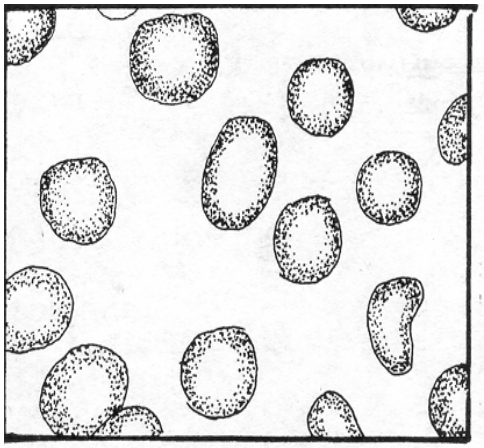


Figure 4-12. Variations in erythrocytes:
Hypochromic macrocytic erythrocytes.

(2) Polychromatophilia. This term describes non-nucleated erythrocytes that show bluish coloration instead of light pink. Polychromatophilia is due to the fact that the cytoplasm of these cells does not mature, resulting in the abnormal persistence of the basophilic cytoplasm of the earlier nucleated stages.

d. **Inclusions**.

(1) Howell-Jolly bodies. These are nuclear remnants found in the erythrocytes of the blood in various anemias. They are round, dark violet granules about one micron in diameter (see figure 4-13). Generally, only one Howell-Jolly body will be found in any one red cell. However, two or more may sometimes be present. Howell-Jolly bodies generally indicate absent or non-functioning spleen. They occur in megalobastic anemia and in other forms of nuclear maturation defects.

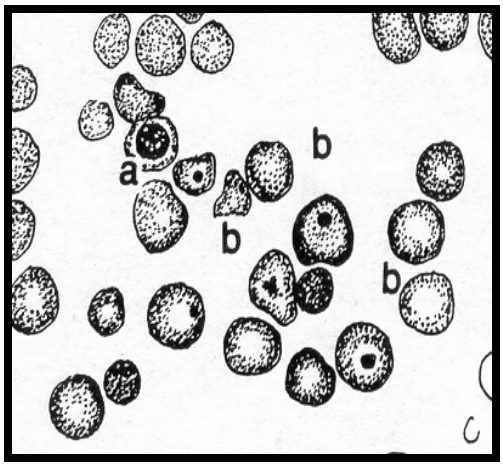


Figure 4-13. Variations in erythrocytes:
a. Metarubricyte. b. Howell-Jolly bodies.

(2) Cabot's rings (ring bodies). These are bluish threadlike rings found in the red cells in the blood of patients with severe anemias (figure 4-14). They are interpreted as remnants of the nuclear membrane and appear as ring or “figure-eight' structures. Usually only one such structure will be found in any one red cell.

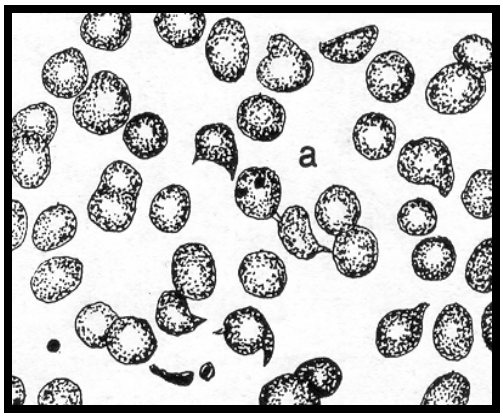


Figure 4-14. Variations in erythrocytes: (a) Cabot's ring.

(3) Basophilic stippling. Round, small, blue-purple granules of varying size in the cytoplasm of the red cell represent a condensation of the immature basophilic substance (see poly-chromatophilia) that normally disappears with maturity. This is known as basophilic stippling (figure 4-15). It can be demonstrated by standard staining techniques in contrast to reticulocyte filaments that require a special stain. Stippling occurs in anemias and heavy metal poisoning (lead, zinc, silver, mercury, bismuth) and denotes immaturity of the cell.

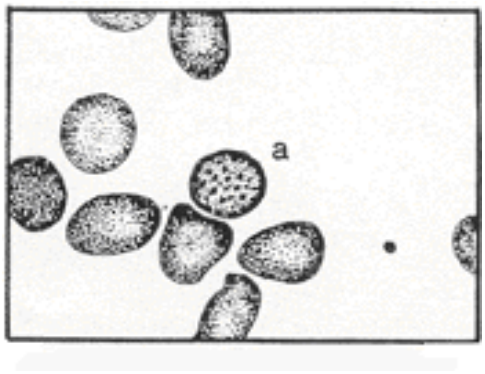


Figure 4-15. Variations in erythrocytes: (a) Basophilic stippled erythrocyte.

(4) Heinz-Ehrlich bodies. These are small inclusions found primarily in those hemolytic anemias induced by toxins. They are round, refractile bodies inside the erythrocyte and are visible only in unfixed smears. It is thought that they are proteins that have been dematured and that they are an indication of erythrocyte injury.

(5) Siderocytes. These are erythrocytes containing iron deposits. These deposits indicate an incomplete reduction of the iron from ferric to the ferrous state that is normally found in hemoglobin. Prussian blue stain must be used to readily demonstrate these cells.

e. **Megaloblastic Erythrocytes**. The development of megaloblastic cells is caused by a deficiency of vitamin B_{12} or folic acid. Pernicious anemia is a disease considered to be due to a deficiency in vitamin B_{12} and/or certain related growth factors. With this deficiency, the erythrocytes do not mature normally and are generally larger than normal. The most notable characteristic of this abnormal maturation is a difference in the rates of maturation of the cytoplasm and the nucleus. The development of the nucleus is slower than that of the cytoplasm, so that in the more mature of the nucleated forms a spongy nucleus as well as an exceptionally large size may be observed. Nuclear chromatin in the megaloblast is much finer and is without the clumps observed in the rubriblast. Such development is termed asynchronism. The mature cell is large (about 10 microns) and is termed a megalocyte. The younger cells of this series are named by adding the suffix “pernicious anemia type," that is rubricyte, pernicious anemia type, and so forth. See figures 4-16 and 4-17.

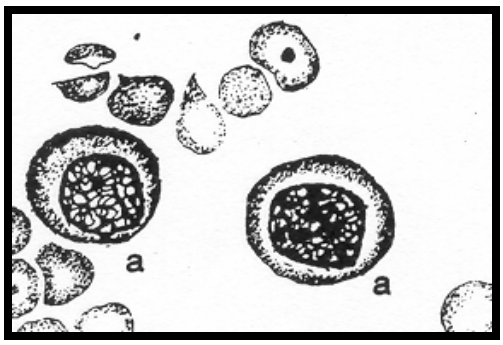


Figure 4-16. Variations in erythrocytes: (a) Rubricytes (pernicious anemia).

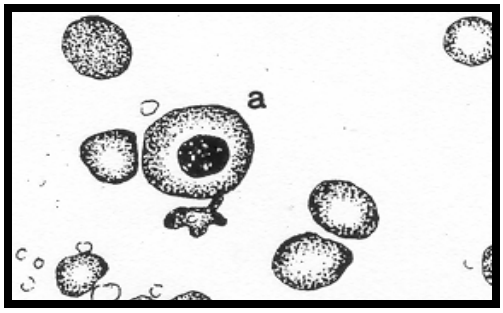


Figure 4-17. Variations in erythrocytes: (a) Metarubricyte (pernicious anemia).

Section III. LEUKOCYTES

4-6. GRANULOCYTIC SERIES

The stages in the normal maturation of the granulocytes are: myeloblast, promyelocyte, myelocyte (neutrophilic, eosinophilic, and basophilic), metamyelocyte (neutrophilic, eosinophilic, and basophilic), band cell (neutrophilic, eosinophilic, and basophilic), and segmented cell (neutrophilic, eosinophilic, and basophilic). As the granulocytes mature, the granules increase in number. These granules later become specific and differ in the affinity for various dyes. Neutrophilic granules do not stain intensely with either dye. Basophilic granules have an affinity for the basic or blue dye. Eosinophilic stain red with an affinity for the acid dye. The criteria for identification of the various stages of the granulocytic series are: size of cell, nucleus-cytoplasm ratio, nuclear shape, number of nucleoli, and the type and size of cytoplasmic granulation.

a. **Myeloblast**. See figure 4-18.

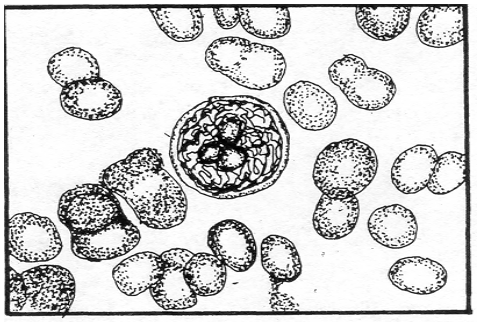


Figure 4-18. Granulocytic series: Myeloblast

(1) Size. 15 to 20 microns in diameter.

(2) Nucleus. The nucleus is round or ovoid and stains predominantly reddish-purple. The interlaced chromatin strands are delicate, well defined, and evenly stained. Two to five pale blue nucleoli are demonstrable. The nucleus occupies most of the cell with a nucleus-cytoplasm ratio of 4:1. It is separated from the cytoplasm by a definite nuclear membrane.

(3) Cytoplasm. The cytoplasm is a narrow, moderate blue and smooth, no granules, and a rim around the nucleus.

b. **Promyelocyte**. See figure 4-19.

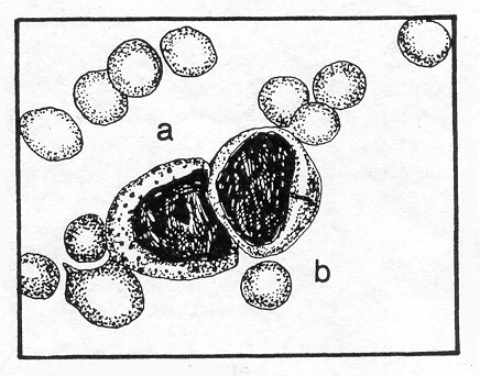


Figure 4-19. Granulocytic series: a. Promyelocyte. b. Promyelocyte with auer body.

(1) Size. 15 to 21 microns in diameter.

(2) Nucleus. The nucleus is round or ovoid with coarse-clumping, purple chromatin material. Two to three oval, light-blue nucleoli are usually present. The nucleoli are less distinct than in the myeloblast. This cell has a nucleus-cytoplasm ratio of 3:1.

(3) Cytoplasm. The cytoplasm is light purple and contains varying numbers and sizes of dark nonspecific granules that stain red to purplish-blue. The granules usually overlie the nucleus.

c. **Myelocyte**. See figure 4-20. In the myelocytic stage, the granules are definite and so numerous that frequently they obscure nuclear detail. While promyelocytes are sometimes distinguished as neutrophilic, eosinophilic, or basophilic, the differentiation is generally considered as first occurring in the myelocytic stage.

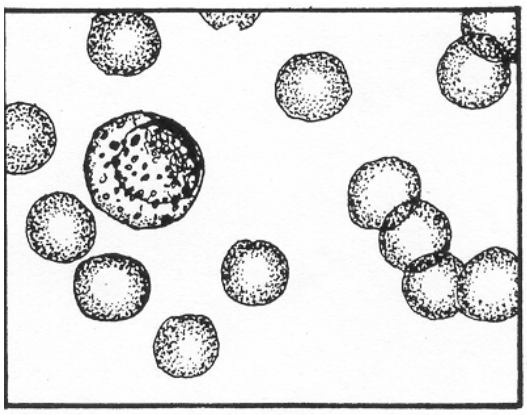


Figure 4-20. Granulocytic series: Myelocyte.

d. **Neutrophilic Myelocyte**.

(1) Size. 12 to 18 microns in diameter.

(2) Nucleus. The nucleus is round, oval, or flattened on one side. The chromatin strands are light purple, unevenly stained, and thickened. Nucleoli are usually absent. The nucleus is smaller than the earlier cells of this series with a nucleus-cytoplasm ratio of 2:1 to 1:1.

(3) Cytoplasm. The cytoplasm is pink with blue patches and contains a small relatively light area of ill-defined, pink granules, which develop among the dark, nonspecific, azurophilic granules of the promyelocyte. As the myelocyte ages, the dark granules become less prominent and the light-pink-colored neutrophilic granules predominate.

e. **Metamyelocyte**. See figure 4-21.

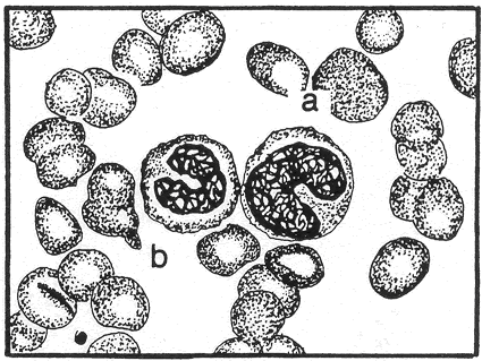


Figure 4-21. Granulocytic series: a. Metamyelocyte, b. Band neutrophil.

(1) Size. 10 to 15 microns in diameter.

(2) Nucleus. The nucleus is indented or kidney-shaped. The indention is less than half the width of an arbitrary round nucleus. The nucleus is eccentric or centrally located in the cell. The nuclear chromatin pattern is coarse and clumped. Nucleoli are absent. The nucleus-cytoplasm ratio is approximately 2:1 to 1:1.

(3) Cytoplasm. The cytoplasm is pinkish-blue and has moderate to abundant specific granules.

f. **Neutrophilic Band**.

(1) Size. 9 to 15 microns in diameter.

(2) Nucleus. The nucleus is shaped like a horseshoe with a dark pyknotic mass at each pole of the nucleus where the lobes develop. The nucleus is deeply indented from the metamyelocyte stage. The nucleus-cytoplasm ratio is approximately 1:2.

(3) Cytoplasm. The cytoplasm moderate to abundant pink and contains many small evenly distributed violet-pink granules.

g. **Neutrophilic Segmented Cell**.

(1) Size. 9 to 15 microns in diameter.

(2) Nucleus. The nucleus has two to five definite lobes separated by a very narrow filament or strand. The nucleus has dense, coarse, clumped, irregular chromatin pattern. The granules are small pinpointed pink to rose-violet and specific. The nucleus-cytoplasm ratio is approximately 1:3.

(3) Cytoplasm. The cytoplasm is light pink and the small, numerous, and evenly distributed neutrophilic granules have a light pink color.

h. **Development of the Eosinophilic Group**. Cells of the eosinophilic group are characterized by relatively large, spherical, cytoplasmic granules that have a particular affinity for the eosin stain. The earliest eosinophil (myelocyte) has a few dark spherical granules with reddish tints that develop among the dark, nonspecific granules. As the eosinophilic cells pass through their various developmental stages, these granules become less purplish-red and more reddish-orange. The dark blue, nonspecific granules, characteristic of the promyelocyte and the early myelocyte stages, disappear. Because the percentage of eosinophils is usually low in bone marrow peripheral blood smears, no useful clinical purpose is served by routinely separating the eosinophils into their various myelocyte, metamyelocyte, band, and segmented categories. On the other hand, in situations such as eosinophilic leukemia in which the eosinophils are greatly increased, an analysis of the incidence of the various stages would be useful in diagnosis.

i. **Eosinophil**. See figure 4-22.

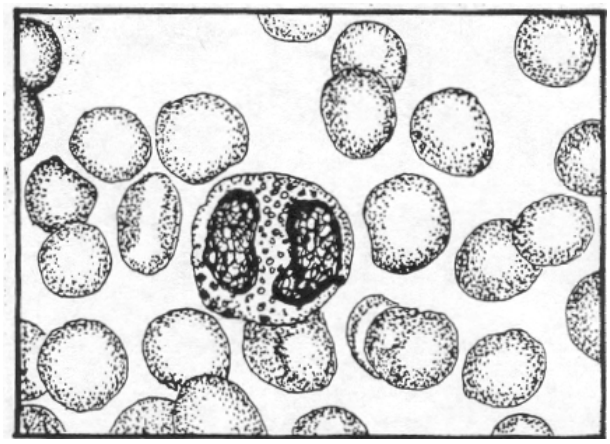


Figure 4-22. Granulocytic series: Eosinophil

(1) Size. 9 to 15 microns in diameter.

(2) Nucleus. The nucleus usually has two definite lobes separated by a very narrow filament or strand. Has clumped, coarse chromatin pattern. Seldom does an eosinophil have more than two lobes.

(3) Cytoplasm. The cytoplasm contains bright reddish-orange, distinct granules. The granules are spherical, uniform in size, and evenly distributed throughout the cytoplasm, but rarely overlie the nucleus.

j. **Development of the Basophilic Group**. These cells have round, indented, band, or lobulated nuclei and are classified according to the shape of the nuclei, as basophilic rnyelocytes, metamyelocytes, bands, and segmented forms. These cells are so few in peripheral blood and bone marrow that there is little clinical value in differentiation of the various maturation stages. See figure 4-23.

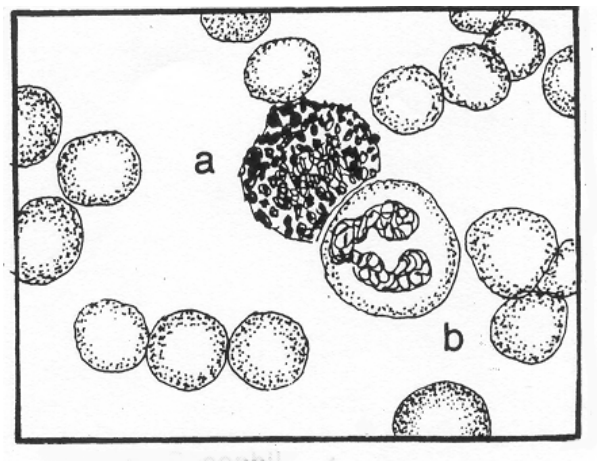


Figure 4-23. Granulocytic series: a. Basophil, b. Neutrophil: segmented.

k. **Mature Basophils**.

(1) Size. 10 to 16 microns in diameter.

(2) Nucleus. The nucleus has definite lobes and are separated by a very narrow filament or strand. The nuclear details are obscured by the large coarse cytoplasmic granulation.

(3) Cytoplasm. The cytoplasm is covered by many blue to black granules. These granules are unevenly distributed and vary in number, size, shape, and color usually blue-black.

4-7. GRANULOCYTES

Granulocytes are leukocytes devoid of specific granulation. These cells generally originate in the lymphatic system, but can be found in normal bone marrow. Granulocytes include the lymphocytic series, monocytic series, and plasmocytic series.

4-8. LYMPHOCYTIC SERIES

The stages in the development of the lymphocytic series are: lymphoblast, prolymphocyte, and lymphocyte. These cells are fragile and can show shape variants. Lymphocytes usually have round contours, blue cytoplasm, and eccentrically located round nuclei. Cells of this series are differentiated on the basis of the nuclear chromatin.

a. **Lymphoblast**. See figure 4-24.

(1) Size. 10 to 18 microns in diameter.

(2) Nucleus. The nucleus has an oval or round shape and stains reddish-purple. The nuclear chromatin is fine, well distributed, and coarser than in the myeloblast. Chromatin is condensed at the edges of the nucleus to form a definite nuclear membrane. One to two nucleoli are present. The nucleus is prominent with a nucleus-cytoplasm ratio of 6:1.

(3) Cytoplasm. The cytoplasm is moderate to dark blue and smooth with a frequent perinuclear clear zone.

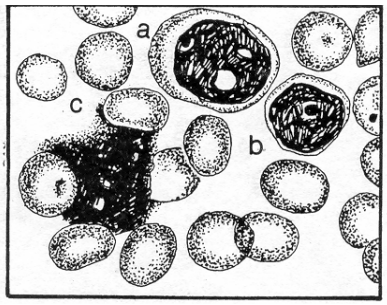


Figure 4-24. Lymphocytic series: a. Lymphoblast. b. Lymphocyte. c. Smudge cel.l

b. **Prolymphocyte**. See figure 4-25.

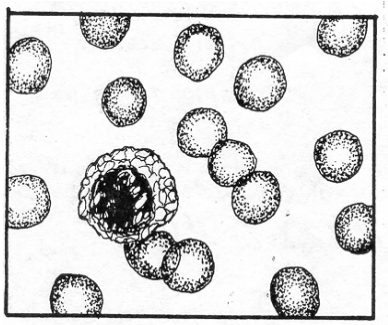


Figure 4-25. Lymphocytic series: .Prolymphocyte.

(1) Size. 10 to 18 microns in diameter.

(2) Nucleus. The nucleus is oval and slightly indented. The nuclear chromatin is coarse, slightly clumped, am dark purple. One light blue nucleolus is usually present. The nucleus- cytoplasm ratio is 5:1.

(3) Cytoplasm. The cytoplasm varies from moderate to dark blue and it can show a few red-purple (azurophilic) granules.

c. **Lymphocyte**. See figure 4-26.

(1) Size. The mature cell of this series varies greatly in size. Small lymphocytes are 7 to 9 microns in diameter. The large lymphocytes are 6-16 microns in diameter.

(2) Nucleus. The nucleus is round or oval and can be slightly indented. The nuclear chromatin is markedly condensed, dark purple-blue, and clumped. Nucleoli are absent and a definite nuclear membrane ids present. The nucleus-cytoplasm ratio is approximately 1.5:1.0.

(3) Cytoplasm. The cytoplasm is light blue to blue with a perinuclear clear zone around the nucleus. A few azurophilic granules can be seen in the cytoplasm of larger lymphocytes.

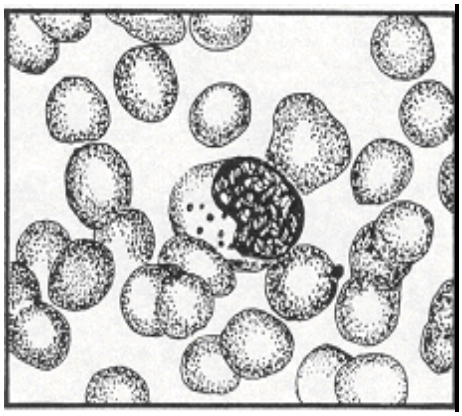


Figure 4-26. Lymphocytic series: Lymphomcyte, azurophilic granulation.

4-9. MONOCYTIC SERIES

The stages in the development of the monocytic series are monoblast, promonocyte, and monocyte. Cells of the series are slightly larger than granulocytes. They are round with smooth margins and seldom show shape variants. The mature monocyte is differentiated from the lymphocyte and metamyelocyte by the very fine, light staining nucleus.

a. **Monoblast**. See figure 4-27.

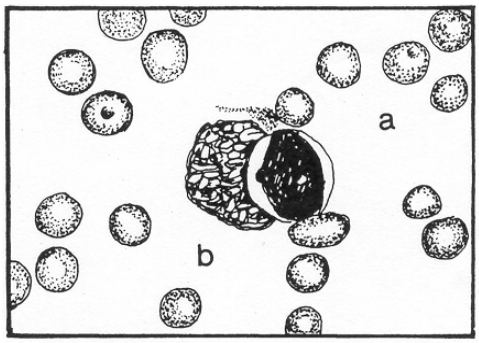


Figure 4-27. Monocytic series: a. Monoblast. b. Stem (ferrata cell).

(1) Size. 12 to 20 microns in diameter.

(2) Nucleus. The nucleus is round or oval fine lacy light blue- purple in color. The nuclear chromatin is moderately basophilic to blue-gray. One to two distinct nucleoli are present. The nucleus-cytoplasm ratio is 4:1 to3:1.

(3) Cytoplasm. The cytoplasm is a clear, deep blue and follow a thin rim around the nucleus.

b. **Promonocyte**. See figure 4-28.

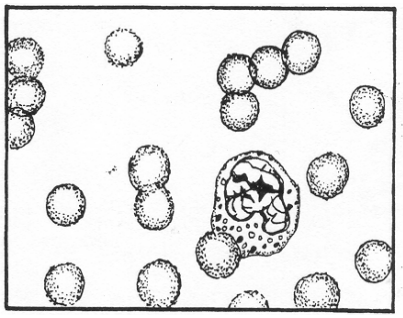


Figure 4-28. Monocytic series: Promonocyte.

(1) Size. 14 to 18 microns in diameter.

(2) Nucleus. The nucleus is oval or single fold. The nuclear chromatin is fine and spongy1 to 5 nucleoli may represent. The nucleus-cytoplasm ratio is 3:1 to 2:1.

(3) Cytoplasm. The cytoplasm is blue-gray with ground glass appearance with fine dust like azurophilic (red-purple) granules.

c. **Monocyte**. See figure 4-29.

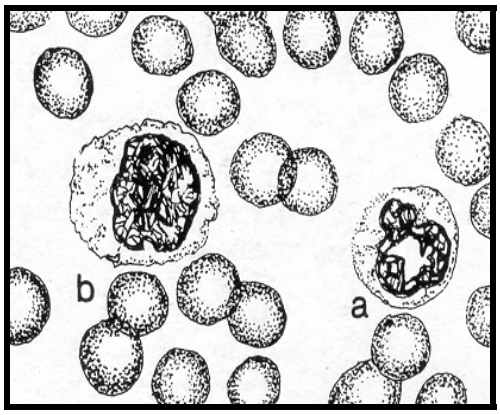


Figure 4-29. Monocytic series: a. Neutrophil (late band). b. Monocyte.

(1) Size. 14 to 20 microns in diameter.

(2) Nucleus. The nucleus is round or kidney-shaped, but can be deeply indented or slightly lobed. One of the most distinctive features of the monocyte is the presence of superimposed lobes, giving the nucleus the appearance of brain-like convolutions. Heavy lines marking the edges of the folds and grooves are features that are not seen in other cells. Another feature of the nucleus, which is of value in diagnosis, is the tendency for the nuclear chromatin to be loose with light spaces in between the chromatin strands, giving a coarse linear pattern in contrast to the lymphocyte that has clumped chromatin. Nucleoli are absent. The nucleus- cytoplasm ratio is approximately 2:1 to 1:1.

(3) Cytoplasm. The cytoplasm of the monocyte is dull gray-blue while the cytoplasm of the neutrophils in the adjacent fields is definitely lighter and is pink rather than gray-blue. The nonspecific granules of the monocyte are usually fine and evenly distributed, giving to the cell a dull, opaque or ground-glass appearance. In addition to the background of evenly distributed nonspecific granules, there may be a few unevenly distributed larger azurophilic granules. Vacuoles are often demonstrable in the cytoplasm.

4-10. PLASMOCYTIC SERIES

Plasmocytes constitute approximately one percent of the white cells in the normal bone marrow. These cells can represent in the peripheral blood in chronic infections, granulomatous and allergic diseases, and multiple myeloma. The stages of development are: plasmoblast, proplasmocyte, and plasmocyte.

a. **Plasmoblast**. See figure 4-30.

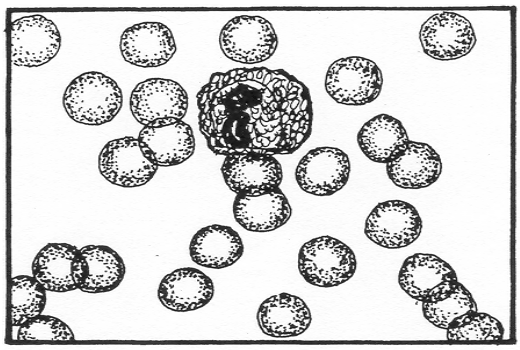


Figure 4-30. Plasmocytic series: Plasmoblast.

(1) Size. 18 to 25 microns in diameter.

(2) Nucleus. The nucleus is large, oval or round, and located off center in the cell. Nuclear chromatin is more clumped than in reactive lymphocyte. There may be lighter staining area near the nucleus (perinuclear halo). There are multiple nuclei that may or may not be visible. The cytoplasm is basophilic, abundant and non-granular. The nuclear cytoplasm ratio is 4:1.

b. **Proplasmocyte**. See figure 4-31.

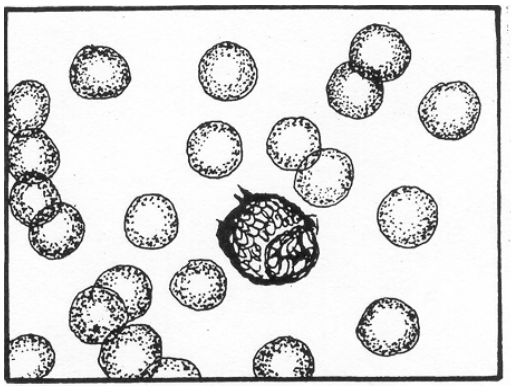


Figure 4-31. Plasmocytic series: Proplasmocyte.

(1) Size. 15 to 25 microns in diameter.

(2) Nucleus. The nucleus is ovoid and located eccentrically. The chromatin is purple, coarser, and more clumped. One to two nucleoli are present. The nucleus-cytoplasm ratio is 3:1.

(3) Cytoplasm. The cytoplasm is intensely basophilic, usually bluer than a blast and is nongranular. A lighter-staining area in the middle of the cell (in the cytoplasm, next to the nucleus) may become visible. This is termed a hof or perinuclear halo. May have occasional nucleoli.

c. **Plasmocyte**. See figure 4-32.

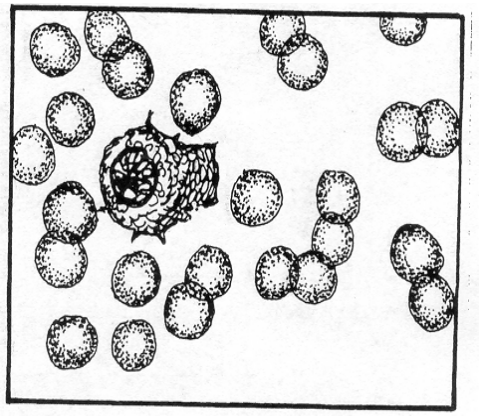


Figure 4-32. Plasmocytic series: Plasmocyte

(1) Size. 8 to 20 microns in diameter.

(2) Nucleus. The nucleus is round to ovoid, and eccentrically located (located near the edge of the cell). The chromatin is coarse, lumpy, and purple. Nucleoli are not usually present. The nucleus-cytoplasm ratio is 2:1 to 1:1.

(3) Cytoplasm. The cytoplasm adjacent to the nucleus is lightly stained in contrast to the periphery of the cell that has a high saturation of red and blue dyes. In same cells, the dark cytoplasm has a greenish or larkspur-blue color. The cytoplasm contains multiple small and relatively unstained globules embedded in a bluish-red filamentous matrix. It is the presence of these tapioca-like globules in the dark surrounding medium that gives the plasmocyte its characteristic mottled and foamy appearance and its brilliant translucency. In occasional cells, the globules can be quite prominent and take a red or bluish-red stain. Such globules are called Russell or fuchsin bodies, or eosinophilic globules. Vacuoles of various sizes are frequently demonstrable.

4-11. VARIATIONS OF LEUKOCYTES

Variations of leukocytes occur as a result of abnormal maturation of the nucleus and/or cytoplasm. These variations are induced by leukemic states, infectious diseases, and toxicity. Described below are the most frequently occurring variations.

a. **Dohle Bodies**. Dohle bodies are light blue or blue-gray, small, round inclusions found in the cytoplasm of neutrophilic leukocytes. Contains RNA and may represent localized failure of the cytoplasm of neutrophils. The variation may occur in toxic conditions such as severe infections, burns, poisoning, and following chemotherapy.

b. **Auer Rods**. Auer rods are rods or spindle-shaped, cytoplasmic inclusions. They stain reddish-purple and are 1 to 6 microns long and less than 1.5 microns thick. They are frequently found in myelogenous leukemia. Also, found in the cytoplasm of myeloblast, and monoblasts.

c. **Toxic Granullation**. Toxic granulation (figure 4-33) occurs in the neutrophilic metamyelocyte, band, and segmented cells. These granules are distinguished from the normal granulation because they are large, coarser and stain a dark purple. The variations occur in toxic states, severe infections, and burns. Believed to be primary granules which show increased alkaline phosphate activity.

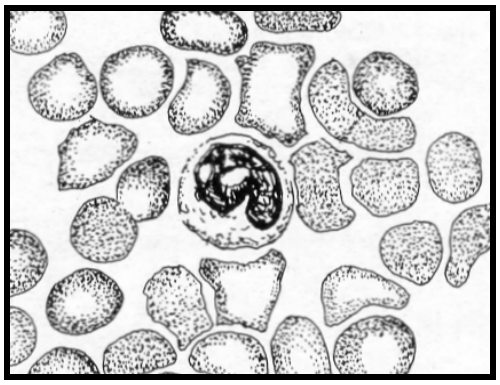


Figure 4-33. Variations in leukocytes:
Band Neutrophil: Toxic Granulation

d. **Basket Cell**. A basket cell is a ruptured leukocyte that has a network appearance. These cells result from a partial breakdown of the immature and fragile leukocytes. Basket cells are found predominantly in diseases with an acute shift toward immature forms, for example, leukemias.

e. **Vacuolated Cell**. A vacuolated cell is a degenerated cell with holes or vacuoles in the cytoplasm. Vacuolated cells can be seen in severe infections, poisoning, and leukemias, and in cells that have been in Heller & Paul oxalate too long.

f. **Hypersegmentation**. A normal neutrophilic segmented cell has a nucleus with an average of three lobes or segments. In a hypersegmented cell the nucleus is broken up into six or more lobes (see figure 4-34). This cell usually has a larger diameter than a normal neutrophilic segmented cell. Hypersegmentation is often seen in pernicious anemia and folic acid deficiency. They may also be found in chronic infections.

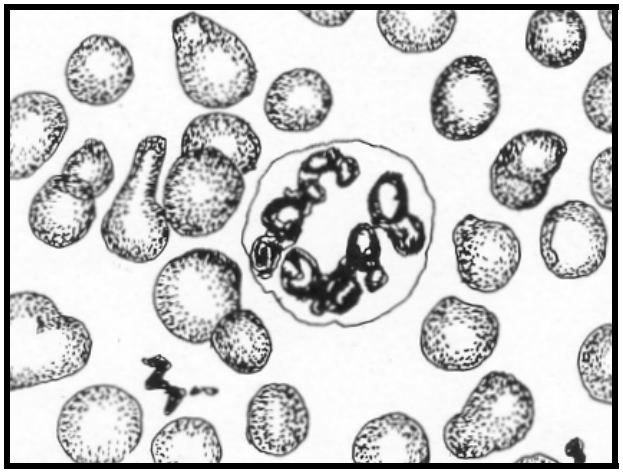


Figure 4-34. Variations in leukocytes: Neutrophili: hypersegmented.

g. **Atypical Lymphocytes**. See figure 4-35. These lymphocytes are characteristic of infectious mononucleosis but they may also be seen in apparently healthy individuals and those with certain other diseases. Atypical lymphocytes are larger than normal and vary in appearance. Downey and McKinley described three types of atypical lymphocytes, but this classification has no real clinical purpose.

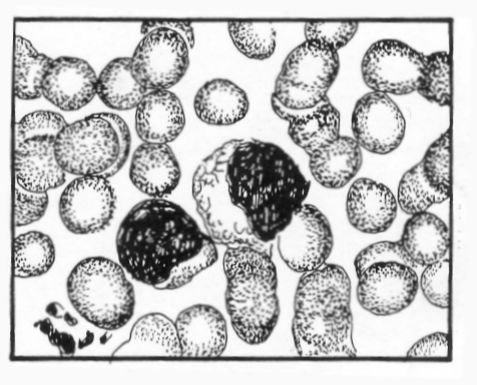


Figure 4-35. Variations in leukocytes: Atypical lymphocytes (infectious mononucleosis).

(1) Size. Large, up to 20 microns in diameter.

(2) Nucleus. The nucleus is oval or kidney shaped with very coarse chromatin strands not as lumpy as a normal lymphocyte.

(3) Cytoplasm. The cytoplasm is blue to dark blue. Often it is vacuolated which gives rise to a foamy appearance.

h. **L.E. Cells**. See figure 4-36.

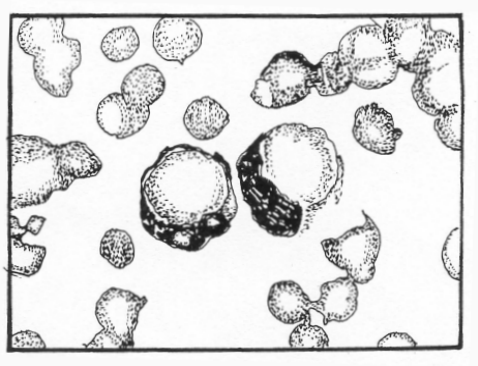


Figure 4-36. Variations in leukocytes: L.E. cells.

(1) Persons having lupus erythematosus, one of the “collagen” diseases, have an abnormal; plasma protein that causes swelling and breakdown of certain blood cell nuclei in vitro. This degenerated nuclear material attracts phagocytic cells, particularly segmented neutrophils, which engulf this nuclear mass. The resulting phagocyte and inclusion material is termed an "L.E.” cell.

(2) The nucleus of an L.E. cell is adjacent to the peripheral outline of the inclusion material. The inclusion is smooth and silky or light purple and has no visible chromatin network.

i. **Rosettes**. Rosette formation is the intermediate stage in the formation of an L.E. cell. A rosette formation consists of neutrophilic leukocytes surrounding free masses of lysed nuclear material.

j. **Tart cells**. A tart cell, which may be confused with the L.E. cell, contains lysed nuclear material within its cytoplasm. It differs from an L.E. cell because the inclusion retains characteristic nuclear structure. This inclusion is not smooth and has a darker staining periphery. The significance of tart cells is not known but their presence in an L.E. preparation does not signify a positive test for systemic lupus erythematosus.

Section IV. THROMBOCYTES

4-12. INTRODUCTION

a. The general pattern of thrombocyte maturation is slightly different from that of leukocyte maturation. The cells of the megakaryocytic series tend to grow larger as they mature until there is cytoplasmic fragmentation (or breaking off) to form the cytoplasmic thrombocytes seen in the peripheral blood.

b. Azurophilic granulation begins to appear in the second stage *of* development and continues until it almost obscures the nuclear lobes. The nucleus develops from a fine single lobe to multiple ill-defined lobes. The stages in the normal maturation of the megakaryocytic series are: megakaryoblast, promegakaryocyte, megakaryocyte, and thrombocyte.

4-13. MEGAKARYOCYTIC SERIES

a. **Megakaryoblast**. See figure 4-37.

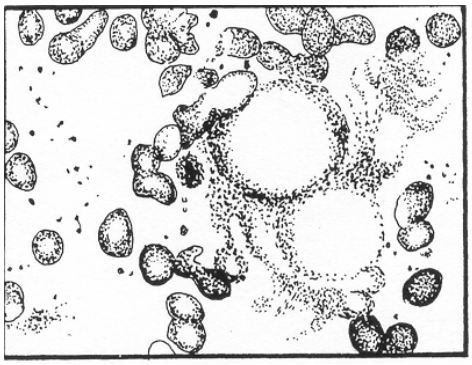


Figure 4-37. Megakaryocytic series: Megakaryoblast: bone marrow.

(1) 20 to 50 microns in diameter.

(2) Nucleus. One to two large oval or kidney-shaped nuclei are present. There is a fine chromatin pattern. Multiple nucleoli may be present which stain blue.

(3) Cytoplasm. The cytoplasm is blue, nongranular, and may have small, blunt pseudopods. It is usually seen as a narrow band around the nucleus.

b. **Promegakaryocyte**.

(1) Size. 20 to 60 microns in diameter.

(2) Nucleus. One-to-two indented round or oval nuclei are present. They may show slight lobulation. The nuclear chromatin is purple, coarse, and granular. Multiple nuclei are present but may be indistinct.

c. **Megakaryocyte**. See figure 4-38.

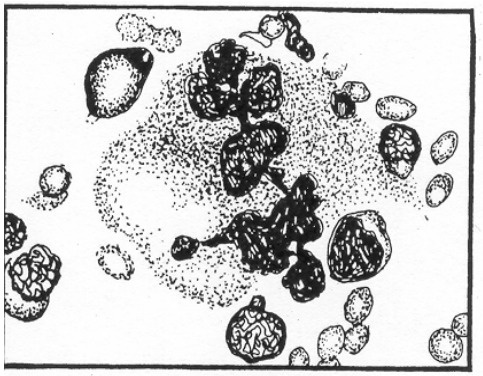


Figure 4-38. Megakaryocytic series: Megakaryocyte

(1) Size. 40 to 120 microns in diameter.

(2) Nucleus. Two-to-sixteen nuclei may be visible or the nucleus may show multilobulation. No nucleoli are visible. The nuclear chromatin is purple, coarse, and granular.

(3) Cytoplasm. The cytoplasm is pinkish-blue in color and very granular. Numerous blue-purple granules begin to aggregate into small bundles that bud off from the cell to become platelets.

d. **Thrombocyte (Platelet)**. See figure 4-39.

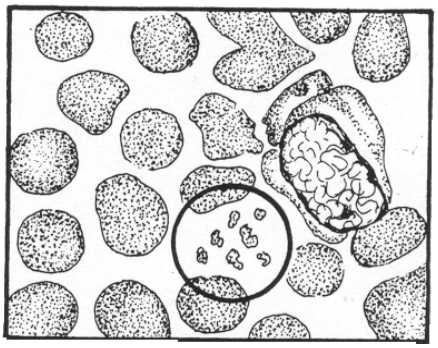


Figure 4-39. Megakaryocytic series: Thrombocytes.

(1) Size. 1 to 4 microns in diameter.

(2) Nucleus. None.

(3) Cytoplasm. The cytoplasm is light blue to purple and very granular. It consists of two parts:

(a) The chromomere, which is granular and located centrally.

(b) The hyalomere, which surrounds the chromomere and is nongranular and clear to light blue.

Continue with Exercises

EXERCISES, LESSON 4

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. The stages of blood cell maturation are:

   a. Irrelevant categories.

   b. Artificial classifications.

   c. Obsolete pigeonholes.

   d. Strictly and universally applicable categories.

2. As a general role for cell identification, the cytoplasm in a mature cell is:

   a. Green.

   b. Dark green.

   c. Blue.

   d. Light orange.

3. Generally speaking, what are the texture and consistency of the nuclear chromatin in an immature cell?

   a. Fine and lacy.

   b. Course and clumpy.

   c. Rough and lacelike.

   d. Fine and crumbled together.

4. Generally speaking, what is the size of the cell and the texture and consistency of the nuclear chromatin in a mature cell?

   a. Larger than an immature cell; fine and lacy.

   b. Smaller than an immature cell; course and clumpy.

   c. Smaller than an immature cell; rough and lace-like.

   d. Larger than an immature cell; fine and crumbled together.

5. Which does **NOT** occur during the development of blood cells?

   a. Nucleus disappears.

   b. Nucleus reduces in size.

   c. Cytoplasm lightens in color.

   d. Nucleus becomes reddish in color.

6. The most immature cell in the erythrocytic series is the:

   a. Rubricyte.

   b. Rubriblast.

   c. Prorubricyte.

   d. Metarubricyte.

   e. Erythrocyte.

7. The rubricyte cell has:

   a. Cytoplasm staining a bluish-buff and a purple nucleus.

   b. A large oval, homogeneous blue-black mass for a nucleus.

   c. Dense, irregular clumpy chromatin and a small nucleus.

   d. Light blue reticulum strands in the cytoplasm, but no nucleus.

8. Which cell has a nucleus and usually a few nucleoli?

    a. Rubricyte.

    b. Rubriblast.

    c. Megakaryocyte.

    d. Metarubricyte.

9. In which stage of erythrocyte development does hemoglobin first become visible?

    a. Rubricyte.

    b. Rubriblast.

    c. Prorubricyte.

    d. Metarubricyte.

10. A diffusely basophilic erythrocyte is a(n):

    a. Erythrocyte.

    b. Rubricyte.

    c. Reticulocyte.

    d. Metarubricyte.

11. The metarubricyte has a(n) _____ nucleus.

    a. Pyknotic.

    b. Absent.

    c. Round and small.

    d. Purple.

12. The immediate precursor of the polychromatophilic normoblast erythrocyte is the:

    a. Rubricyte.

    b. Rubriblast.

    c. Prorubricyte.

    d. Metarubricyte.

13. Which cell does not have a nucleus?

    a. Rubricyte.

    b. Reticulocyte.

    c. Prorubricyte.

    d. Metarubricyte.

14. The term normocyte is synonymous with what blood cell?

    a. Rubricyte.

    b. Metarubricyte.

    c. Prorubricyte.

    d. Erythrocyte.

15. The average diameter of a normal prorubricyte is:

    a. 3.8 microns.

    b. 5.3 microns.

    c. 12.0 microns.

    d. 18.8 microns.

16. Abnormal variation in the size of erythrocytes is called:

    a. Hypochromia.

    b. Ovalocytosis.

    c. Anisocytosis.

    d. Poikilocytosis.

17. Which cells are triangular in shape and are spiny looking?

    a. Ovalocytes.

    b. Sickle.

    c. Acanthocytes.

    d. Burr.

18. In microcytosis, the microcytes are erythrocyte variations that are:

    a. Larger than normal.

    b. Smaller than normal.

    c. Abnormally varied in size.

    d. Abnormally varied in shape.

19. RBC fragments that are helmet shaped erythrocytes are called:

    a. Crenated erythrocytes.

    b. Schistocytes.

    c. Drepancytes.

    d. Poikilocytosis.

20. Which cell is particularly characteristic of congenital hemolytic anemia (called hemolytic jaundice in the text)?

    a. Target.

    b. Crenated erythrocyte.

    c. Spherocyte.

    d. Siderocyte.

21. Which cell has an irregular outline?

    a. Acanthrocytes.

    b. Burr cells.

    c. Target.

    d. Crenated erythrocytes.

22. Which cell does NOT indicate a possible hereditary disorder?

    a. Ovalocyte.

    b. Spherocyte.

    c. Sickle cell.

    d. Crenated erythrocyte.

23. An increase of globulin and fibrinogen presents a stack-of-coins appearance for erythrocytes. This is called a(n):

    a. Irregularly-shaped erythrocyte.

    b. Pale ring.

    c. “Sausage" shape.

    d. Rouleaux formation.

24. In hypochromic erythrocytes, the normal central pallor is increased as a result of _____ like many _____.

a. Cellular immaturity; nucleated stages.

b. An increased hemoglobin content; sickle cell abnormalities.

c. A decreased hemoglobin content; anemias.

d. Basophilic cytoplasm; mature cells.

25. A megaloblastic cell is caused by what deficiency?

a. Vitamin $B_{6.}$

b. Vitamin $B_{12.}$

c. Vitamin B_1.

d. Vitamin $B_{3.}$

26. An immediate precursor of the neutrophilic band cell is the:

a. Myeloblast.

b. Promyelocyte.

c. Neutrophilic myelocyte.

d. Neutrophilic metamyelocyte.

e. Neutrophilic segmented cell.

27. In the granulocytic series, the immediate precursor of the promyelocyte is the:

a. Monoblast.

b. Myeloblast.

c. Neutrophilic myelocyte.

d. Neutrophilic metamyelocyte.

28. The neutrophilic segmented cell belongs to which series?

    a. Monocytic series.

    b. Plasmocytic series.

    c. Erythrocytic series.

    d. Granulocytic series.

29. Which cell is the least mature and stains unevenly?

    a. Neutrophilic myelocyte.

    b. Neutrophilic band cell.

    c. Neutrophilic metamyelocyte.

    d. Neutrophilic segmented cell.

30. Which cell has two or more blue nucleoli, no cytoplasmic granules, and the nucleus occupying a ratio of 4:1 nucleus-cytoplasm?

    a. Myeloblast.

    b. Promyelocyte.

    c. Neutrophilic myelocyte.

    d. Neutrophilic metamyelocyte.

31. Which cell has only dark nonspecific granules within the cytoplasm, with the granules overlying the nucleus?

    a. Myeloblast.

    b. Promyelocyte.

    c. Neutrophilic myelocyte.

    d. Neutrophilic metamyelocyte.

32. A myeloblast cell has a:

    a. Small, relatively light area of pink granules among dark azurophilic granules and has a nucleus that is round, oval, or flattened on one side?

    b. Nucleus that is indented and a nucleus-cytoplasm ratio of about 1:5:1.

    c. Narrow, deep blue, nongranular rim around the nucleus.

    d. Nucleus with narrow filament that separates the nucleus lobes.

33. Which cell has a kidney-shaped nucleus and many small, light pink granules within the cytoplasm?

    a. Myeloblast.

    b. Promyelocyte.

    c. Neutrophilic myelocyte.

    d. Metamyelocyte.

34. The normal stages of granulocytes are:

    a. Myeloblast, myelocyte, and neutrophilic.

    b. Myeloblast, promyelocyte, and myelocyte.

    c. Promyelocyte, myelocyte, and esoinophilic.

    d. Neutrophilic, esoinophilic, and basophilic.

35. Numerous blue to black granules obscure the nucleus of the:

    a. Erythroblast.

    b. Mature basophile.

    c. Mature eosinophil.

    d. Neutrophilic segmented cell.

36. The promonocyte is a part of what leukocyte series?

    a. Monocytic.

    b. Lymphocytic.

    c. Plasmocytic.

    d. Granulocytic.

37. The lymphocyte has:

    a. No nucleus.

    b. A segmented nucleus.

    c. An indented, round or oval nucleus.

    d. A spongy, sprawling nucleus.

38. What are the stages of the lymphocytic series?

    a. Myeloblast, lymphoblast, and basophilic.

    b. Lymphocyte, myeloblast, and lymphoblast.

    c. Lymphoblast, lymphocyte, and monocyte.

    d. Lymphoblast, prolymphocyte, and lymphocyte.

39. Azurophilic (reddish-purple) granules may be found in the cytoplasm of:

    a. Lymphocytes.

    b. Erythrocytes.

    c. Mature eosinophils.

.

    d. Neutrophilic segmented cells.

40. Auer rods are frequently found in:

    a. Anemia.

    b. Leukemia.

    c. Multiple myeloma.

    d. Infectious mononucleosis.

41. Toxic granulation of neutrophilic cells occurs in:

    a. All of the below.

    b. Severe infections.

    c. Chemical poisoning.

    d. Burns.

42. Which leukocyte variation is often produced in blood that has been oxalated too long?

    a. Vacuoles.

    b. Auer rods.

    c. Hyposegmentation.

    d. Toxic granulation.

43. A hypersegmented neutrophilic cell has how many segments?

    a. One to three.

    b. Three or four.

    c. One to five.

    d. Six to ten.

44. Vacuolated cytoplasm is common in the atypical _____ characteristic of infectious mononucleosis.

    a. Monocyte.

    b. Lymphocyte.

    c. Plasmocyte.

    d. Neutrophilic segmented cell.

45. A segmented neutrophil that has phagocytized a homogeneous mass of nuclear material is called:

    a. A rosette.

    b. An L.E. cell.

    c. A tart cell.

    d. A Dohle body.

46. Platelets (thrombocytes) have a diameter of:

    a. 1 to 4 microns.

    b. 4 to 6 microns.

    c. 6 to 8 microns.

    d. 8 to 10 microns.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 4.

1. b (para 4-1b)
2. c. (para 4-2c)
3. a (para 4-2e)
4. b (para 4-2a, e)
5. d (para 4-2b-d)
6. b (para 4-3a; figure. 4-1)
7. c (para 4-4c)
8. b (para 4-4a(2))
9. a (para 4-4c(3))
10. c (para 4-4e)
11. a (para 4-4d(2))
12. c (para 4-3a)
13. b (para 4-4e(2))
14. d (para 4-3a)
15. c (para 4-4b(1)
16. c (para 4-5a(l))
17. d (para 4-5b(6))
18. b (para 4-5a(3))
19. b (para 4-5b(9))
20. c (para 4-5b(3))
21. c (para 4-5b(5))
22. d (para 4-5b(8))
23. d (para 4-5b(10))

24. c (para 4-5c(1))

25. b (para 4-5e)

26. c (para 4-6)

27. b (para 4-6)

28. d (para 4-6)

29. a (para 4-6)

30. a (para 4-6a(2), (3))

31. b (para 4-6b(3))

32. c (para 4-6a)

33. d (para 4-6e(2), (3))

34. b (para 4-6)

35. b (para 4-6k(2), (3))

36. a (para 4-9b)

37. c (para 4-8c(2))

38. d (para 4-8)

39. a (para 4-8c)(3))

40. b (para 4-11b)

41. a (para 4-11c))

42. a (para 4-11e)

43. d (para 4-11f)

44. b (para 4-11g)

45. b (para 4-11h(1))

46. a (para 4-13d(1))

End of Lesson 4

LESSON ASSIGMMENT

LESSON 5 Manual Cell Counts.

TEXT ASSIGNMENT Paragraphs 5-1 through 5-6.

LESSON OBJECTIVES After completing this lesson, you should be able to:

5-1. Select the statement that best describes what specimen and methods used for cell counts.

5-2. Select the statement that best describes the materials, procedures, and calculations needed to perform manual counts for blood specimens (WBC, total eosinophil, and reticulocyte cell counts).

5-3. Select the statement which best describes the materials and procedures to perform a total eosinophil count.

5-4 Select the statement which best describes the materials, procedures, and calculations needed to perform a reticulocyte count.

5-5 Select the statement which best describes the materials, procedures, and calculations needed to perform manual counts for other body fluids (cerebrospinal fluid).

5-6 Select the statement which best describes the materials and procedures to perform a semen analysis.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 5

MANUAL CELL COUNTS

Section I. MANUAL COUNTS, BLOOD SPECIMENS

5-1. INTRODUCTION

a. Blood cells are subject to quantitative variations as well as the qualitative variations described in Lesson 4. Some diseases stimulate the production of blood cells while others prevent or diminish the production of blood cells. For this reason a cell count gives valuable information to the physician concerning his patient's condition. Furthermore, in the case of the leukocyte count, the total count is necessary to calculate absolute counts for each type of leukocyte. This is done by multiplying the total count by the percentage of the particular cell type.

b. Cell counts can be performed by a variety of methods. Erythrocytes and leukocytes are counted by manual methods or automated methods. Other cell counts are performed only by manual methods. It is important when performing a cell count to maintain good quality control. Great care should be taken when performing any cell count.

c. The following paragraphs outline procedures for white blood cell (WBC) count, total eosinophil count, and reticulocyte count. The WBC counts are routinely done; they are performed either by the hemacytometer method (manually) or by automated methods. Total eosinophil counts are performed by a hemacytometer method (manually) using special diluting fluids to accentuate these cells. Reticulocytes are demonstrated by using a supravital stain. Semen analysis and cerebrospinal fluid (CSF) counts use a hemacytometer to perform the procedure as well. They are included in the next section.

5-2. WHITE BLOOD CELL COUNT

Unopette 1: 20 or 1:100 dilution.

a. **Principle** - Whole blood is mixed with a weak acid solution to dilute the blood and hemolyze the red blood cells. Then loaded into a Hemacytometer and counted.

b**. Specimen** - Whole blood may be obtained from a venous EDTA sample or a free flowing capillary puncture and diluted 1:100 with a Unopette.

c. **Reagents**: A prepared kit is available that uses the Unopette system for white blood cell counts. The reservoir contains 3% glacial acetic acid. A 25 ul capillary pipette is used to aspirate the blood sample and make a 1:20 dilution in the reservoir. (Alternatively, the Unopette containing ammonium oxalate and result in a 1:100 dilution may be used). Functions of reagents are:

(1) Diluent.

(2) Lyse RBCs.

(3) Preserve WBCs and platelets.

d. **Procedure**.

(1) Dilute the specimen - let stand for 10 minutes to allow red cells to hemolyzed.

(2) Expel first 3 to 4 drops of diluted specimen to clean capillary bore.

(3) Charge the Hemacytometer (both sides) with the diluted specimen.

(4) Cells must settle for a minimum 3 to 5 minutes after placing Hemacytometer in moist chamber.

(5) Count white blood cells. See figure 5-1.

(a) Use low power objective and low light.

(b) Viewed under low power, leukocyte nuclei appear slightly iridescent but not retractile; cells should have a visible cell wall and nucleus; use fine focus to differentiate them from artifacts.

(c) Count all WBCs within the 9 large squares (1:100) and those WBCs touching upper and right-hand perimeter lines.

(d) Count second side of Hemacytometer in the same manner.

(e) Validation that each side of the chamber was charged equally - Total number of cells counted on each side of the counting chamber should agree within 10 percent of each other - calculate acceptable range using lower count.

NOTE: Count both sides of the Hemacytometer to calculate the average and to provide a more accurate WBC count.

NOTE: Count cells that fall on top and left line of the squares but not the bottom and the right.

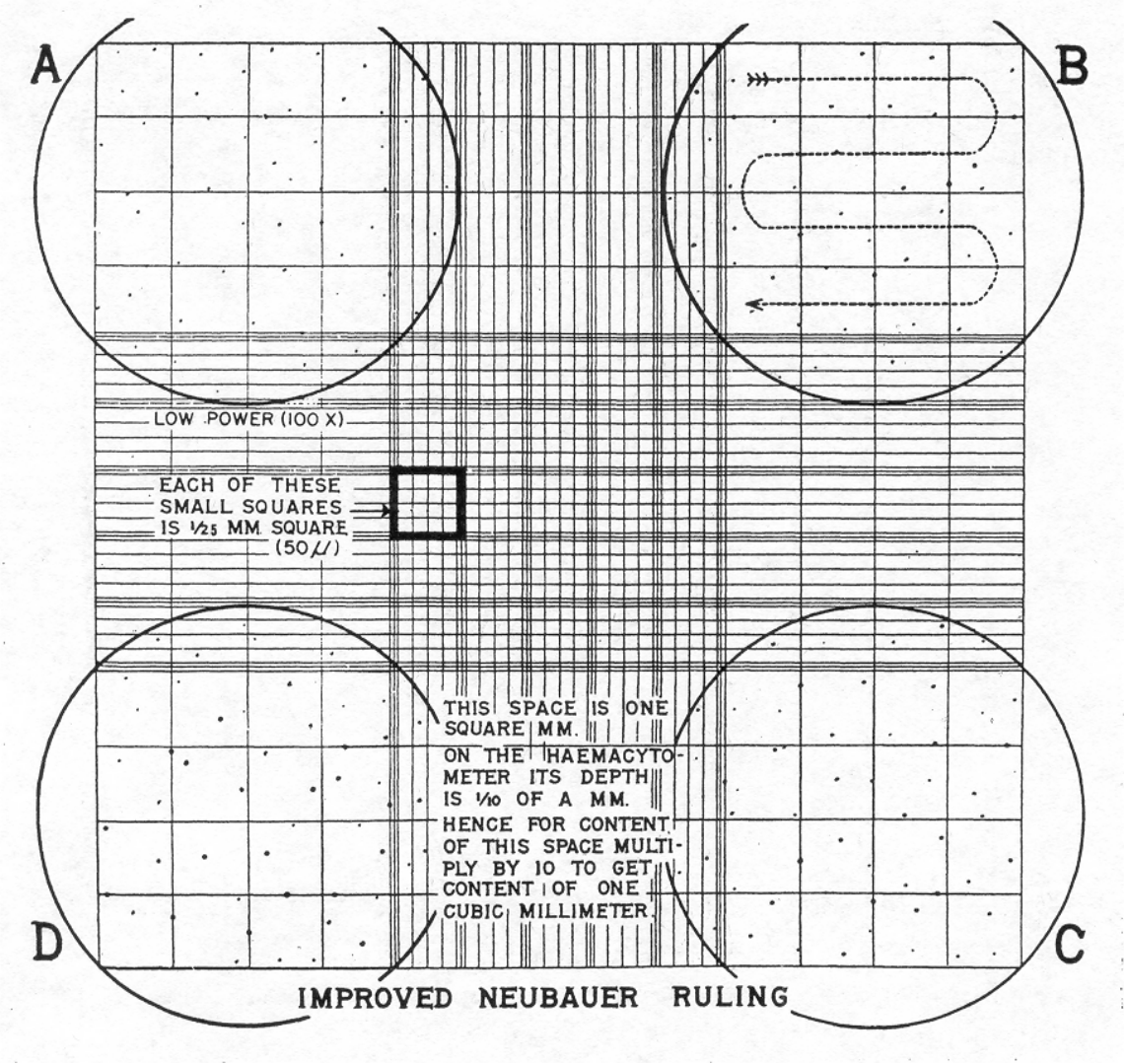


Figure 5-1. Hemacytometer counting chamber (WBCs). Areas marked A, B, C, and D are used to count white blood cells.

e. **Calculation**

(1) The Unopette system delivers a dilution of 1:20.

(2) The depth of the counting chamber is 0.1 mm and the area counted is 4 sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore, 4 X 1.0 sq mm = a total of 4 sq mm). The volume counted is: area X depth = volume. 4 mm^2 X 0.1 mm = 0.4 mm^3 (cubic millimeters).

(3) The formula is as follows:

$$\text{WBCs per cu mm} = \frac{\text{Average number of chambers (2) WBCs counted X dilution (20)}}{\text{Volume (0.4)}}$$

(4) For example:

First Chamber Cells counted in each square	Second Chamber Cells counted in each square
35	45
40	37
44	36
39	44
158 WBCs counted	162 WBCs counted

Average of the 2 chamber counts. Total the amount and divide by 2:

$$\begin{array}{r} 158 \\ +162 \\ \hline 320 \end{array} \qquad \frac{320}{2} = 160 \text{ WBCs}$$

Then: $\frac{60 \times 20}{0.4} = 8{,}000/mm^3$ or 8.0×10^9 WBCs/ L

f. **Sources of Error**.

(1) Improper collection of blood specimens causes variable results.

(2) Wet or dirty pipets.

(3) Not allowing cells to settle for an adequate amount of time.

(4) Poor pipetting technique causes high or low counts. Poor pipetting technique includes:

(a) Undershooting Unopette with blood.

(b) Overfilling Unopette with blood.

(c) Air bubbles in the shaft.

(d) Not mixing the blood specimen thoroughly.

(5) Failure to expel 3 or 4 drops in the pipet tips before charging the Hemacytometer.

(6) Overfilling the chamber of the hemacytometer, which causes erroneously high counts.

(7) Not mixing the diluted specimen prior to filling the Hemacytometer.

(8) Uneven distribution of cells in the counting chamber causes erroneous results.

(9) Counting artifacts.

(10) Dirty or scratched Hemacytometer.

(11) Failure to mix anticoagulated blood thoroughly before use.

g. **Discussion**.

(1) The counting chamber must be scrupulously clean and free of debris that might be mistaken for cells.

(2) The minimum blood sample recommended for performing routine white blood cell counts is that obtained using one pipet and counting two chambers as previously outlined.

(3) If nucleated erythrocytes are present, the count is corrected by the following formula:

$$\text{corrected count} = \frac{\text{observed count X 100}}{\text{100 + percent nucleated erythrocytes}}$$

(4) The percent nucleated erythrocyte is obtained from the differential count, which is discussed in another subcourse.

h. **Normal Values**

(1) Adults (both sexes): 4,500 to 11,500 WBCs per cu mm or 4.5-11.5 X 10^9 WBCs/L.

(2) Childhood: 6,000 to 14,000 WBCs per cu mm or 6.0-14.0 X 10^9 WBCs/L.

(3) Birth: 9,000 to 30,000 WBCs per cu mm or 9.0-34.0 X 10^9 WBCs / L.

5-3. TOTAL EOSINOPHIL COUNT

a. **Principle**. A sample of blood is diluted with a solution that selectively stains the eosinophils and eliminates all other leukocytes and erythrocytes from view. Following mixing, the specimen is introduced into the counting chamber and the number of eosinophils in a known volume of blood is counted.

b. **Reagent.** A prepared kit is available that uses the Unopette system for absolute eosinophil counts. The reservoir contains phloxine B solution in propylene glycol and distilled water. A 25 ul capillary pipette is used to aspirate the blood sample and make a 1:32 dilution in the reservoir. Alternatively, stains including Pilot's solution or Randolph's stain, may be prepared by the laboratory as described elsewhere.

c. **Procedure**.

(1) Add the sample for the Unopette pipette to the reservoir.

(2) Mix by gently shaking the pipets for 30 seconds. Prolonged and harsh shaking will tend to cause rupturing of the eosinophils.

(3) Let stand for 10 minutes to allow red cells to hemolyze.

(4) Expel first 3 to 4 drops of diluted specimen to clean capillary bore.

(5) Using one pipet, charge both chambers of a hemacytometer and with the other pipet charge both chambers of the second Hemacytometer.

(6) Allow both hemacytometers to stand for 15 minutes to permit staining of the eosinophils. To prevent evaporation, the hemacytometers are placed on a damp towel and covered with Petri dish covers.

(7) Under low-power magnification, count the red-stained eosinophils in the entire ruled area (9 sq mm) each of the four chambers (a total area of 36 sq mm). The chamber has a depth of 0.1 mm so the total vo1ume is 3.6 cu mm.

d. **Calculations**.

$$\text{Number of eosinophils Per cu mm} = \frac{\text{Number of eosinophils counted X dilution (10)}}{\text{volume (3.6)}}$$

e. **Source of Error**. See paragraph 5-2e.

f. **Discussion**.

(1) Fuchs-Rosenthal or Levy Hemacytometer is preferable to a standard counting chamber since its greater volume (4.0 X 4.0 X 0.2 mm) allows for counting of more cells, thereby reducing the statistical error. Counting two of these chambers is equivalent in accuracy to seven standard chamber counts.

(2) In eosinopenia, it is necessary to set up more chambers to provide an optional number of cells to be counted.

(3) The eosinacetone diluting fluids are unsatisfactory and should not be used.

(4) Estimation of eosinophils on a stained blood smear is too inaccurate for use because of poor cellular distribution.

(5) The propylene glycol in Pilot's solution renders the erythrocytes invisible, and the sodium carbonate causes lysis of all the leukocytes except the eosinophils. The phloxine stains the eosinophils.

(6) In the Thorn test an eosinophil count must be made prior to the initiation of the test proper. This establishes the patient's total eosinophil count, to which the response of the adrenal cortex to adrenocorticotropic hormone (ACTH) can be judged. The ACTH is then injected and, at an interval of 4 hours, another eosinophil count is made. The interpretation of this test is as follows:

(a) Normal--approximately a 50 percent drop in eosinophils.

(b) Cushing's disease (hyperadrenalism)--0-30 eosinophils per cu mm.

(c) Addison's disease (hypoadrenalism) no change in eosinophil count.

(7) Nasal smears are also submitted for eosinophil evaluation. These smears are stained with Wright's stain and examined for the presence of eosinophils.

g. **Normal Value**. Normal value: 150-300 eosinophils per cu mm.

5-4. RETICULOCYTE COUNT

a. **Principle**. Nonnucleated immature erythrocytes contain nuclear remnants of RNA and the cell is known as a reticulocyte. To detect the presence of this RNA, the red cells must be stained while they are still living. This process is called supravital staining. With supravital staining, the RNA appears as a reticulum within the red cell.

b. **Reagent**.

(1) New Methylene Blue Solution. Dissolve 0.5 grams of new methylene blue, 1.4 grams of potassium oxalate, and 0.8 grams of sodium chloride in distilled water. Dilute to 100 ml. Filter before use.

(2) Brilliant Cresyl Blue Solution. Dissolve 1.0 grams of brilliant cresyl blue in 99 ml of .85 per cent sodium chloride. Filter before use
.

c. **Procedure**.

(1) Mix equal amounts of blood and new methylene blue stain (2-3 drops or 50 uL each) and allow to incubate at room temperature for 3-6 min.

(2) This allows the reticulocytes adequate time to take up the stain.

(3) At the end of 15 minutes, mix the contents of the tube well.

(4) Place a small drop of the mixture on two clean glass slides and prepare a thin smear (prepare two smears).

(5) Counter stain with Wright's stain, if desired.

(6) Allow smear to air-dry.

(7) Place the slide on the microscope stage and, using the low power objective, locate the thin portion of the smear in which the red cells are evenly distributed and are not touching each other.

(8) Switch to oil immersion magnification and count the number of reticulocytes in five fields of 200 RBCs.

d. **Calculation**.

$$\frac{\text{Number of reticulocytes counted}}{10} = \%\ \text{reticulocytes}$$

e. **Sources of Error**.

(1) Equal volumes of blood and stain give optimum staining conditions. An excess of blood causes the reticulum to understain. An excess of stain usually obscures the reticulum.

(2) Crenated erythrocytes and rouleaux formation make an accurate count difficult to perform.

(3) Stain precipitated on erythrocytes causes them to appear as reticulocytes.

(4) Dirty slides cause uneven spreading.

(5) The dye solution should have adequate time to penetrate the cell and stain the reticulum.

f. **Discussion**.

(1) Reticulocytes are nonnucleated erythrocytes that exhibit blue reticulum strands within their cytoplasm when stained supravitally. When stained only with Wright's stain, they are buff-pink in color and larger and darker than erythrocytes.

(2) Reticulocytes serve as an index of the activity of the bone marrow in blood regeneration. As such, these counts are of value in following anti-anemia therapy. Satisfactory response to therapy is evidenced by an increase of reticulocytes in the peripheral blood. Increased reticulocyte counts also occur whenever there is rapid bone marrow activity as in leukemia or blood regeneration associated with hemorrhage or hemolysis. Decreased reticulocyte counts occur in conditions in which the bone marrow is not producing adequate red blood cells, such as aplastic anemia.

(3) Several methods for staining and counting reticulocytes are in common use. Compared to the use of alcoholic solutions of dye, methods employing saline solutions of new methylene blue can give slightly higher values for reticulocytes. For comparative studies, the same method should be used throughout the work.

(4) Precipitated stain is often confused with reticulum but can be recognized by its presence throughout the smear and apart from the red cells. Precipitation can be eliminated as a source of error by frequently filtering the stain.

(5) An alternate method of counting reticulocytes utilizes the Miller disk that is placed inside the microscope eyepiece. This disc consists of 2 squares as shown below in figure 5-2. The area of the smaller square (B) is a tenth that of square A. Therefore, if there are 40 red cells in square A, there should be four red cells present in square B. When employing this method to count reticulocytes, the red cells in square B are counted in successive fields on the slide, until a total of 500 red cells have been counted. At the same time, the reticulocytes in square A are enumerated. At the completion of the count, theoretically, the reticulocytes obtained in this way are divided by 50, in order to obtain the percent reticulocytes present in the blood.

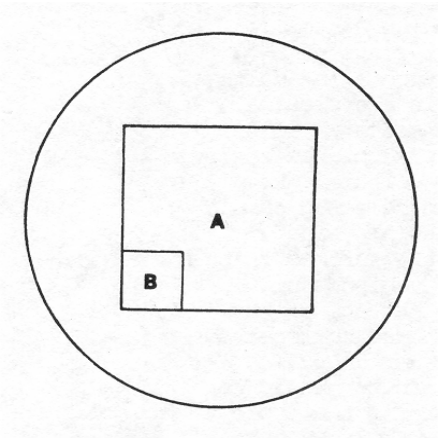


Figure 5-2. Miller disc.

g. **Normal Values**.

(1) Birth to 1 day. Two and one-half to 6.0 percent.

(2) 1 day to 2 weeks. 0.30 to 1.5 percent.

(3) 2 weeks to adult. 0.50 to 2.20 percent.

Section II. MANUAL COUNTS, OTHER BODY FLUIDS

5-5. CEREBROSPINAL FLUID CELL COUNTS

a. **Principle**. Cerebrospinal fluid is delivered to a counting chamber and examined microscopically for blood cells. Normally, spinal fluid is clear. If it is cloudy, dilute before charging the counting chamber.

b. **Reagent**. Normal saline.

c. **Procedure**.

NOTE: Set up cell counts on spinal fluids within 30 minutes after withdrawal of the specimen.

(1) Clear spinal fluid is set up as follows:

(a) With a transfer pipet introduce a drop of well-mixed spinal fluid into both counting chamber of a Hemacytometer.

CAUTION: Avoid contamination by careful handling of spinal fluid.

(b) Examine the entire ruled area for the presence of cellular elements. If both leukocytes and erythrocytes are observed, note the condition of the red cells (fresh or crenated).

(c) Count all cells in the entire ruled area (0.9 cu mm).

(2) Turbid sample – Perform dilution using normal saline.

(a) Slightly hazy – 1:10 dilution.

(b) Hazy – 1:20 dilution.

(c) Mix the specimen well.

(d) Discard the fluid in the capillary portion of the pipet.

(e) Charge the counting chamber and allow the cells to settle for five minutes.

(f) Under low-power magnification count all cells in the entire ruled area (0.9 cu mm).

(g) Switch to high-power and perform a rough differential count on prepared smear.

d. **Calculations**.

(1) Clear spinal fluid:

Number of cells counted x dilution factor x area factor x depth factor = total cells / *u*l

(2) Turbid spinal fluid:

Number of cells counted X dilution (10) x area factor x depth factor = total cells / *u*l

(3) Very clouded spinal fluid:

Number of cells counted X dilution (20) x area factor x depth factor = total cells / *u*l

e. **Source of Error**. See paragraph 5-2e.

f. **Discussion**.

(1) If more than 100 leukocytes per cu mm are present, centrifuge the undiluted specimen, make a smear, and stain with modified Wright's stain. Perform a routine differential count and also estimate the ratio of erythrocytes to leukocytes.

NOTE: It may be necessary to use egg albumin or cell-free serum to make the sediment adhere to the slide.

(2) Normally the spinal fluid is water clear. It can be turbid if cell count is 500 or more cells per cu mm. If there is fresh blood with spontaneous clotting, the indications are those of a bloody tap. Xanthochromia develops after subarachnoid hemorrhage has been present for a few hours and is due to disintegration of blood pigments. Xanthochromia may also develop from tumors, abscesses, and inflammation.

(3) Cell counts above ten are considered to be evidence of intracranial disease. The predominant cell in most viral infections, syphilis, and tuberculous meningitis is the lymphocyte. Bacterial infections due to meningococcus, pneumococcus, and so forth, usually result in a predominance of the neutrophil. Cerebral and extradural abscesses as well as subdural hemorrhages produce a neutrophilic response although bacteria are not demonstrated.

(4) Biochemical, bacteriological, virological, serological, and hematological ands are all necessary to reflect the true condition of the cerebrospinal fluid. The current laboratory standing operating procedures should give guidance for the most efficient method to accomplish all the necessary ands.

g. **Normal Value**: Zero to five cells per cu mm (chiefly lymphocytes).

5-6. SEMEN ANALYSIS

a. **Principle**. Semen analysis involves gross examination (volume, color, turbidity, viscosity, and pH) and microscopic examination (motility and spermatozoa count).

b. **Reagent**. Tap water.

c. **Collection Instructions**. A physician will usually give the instructions; however, the patient should be reminded of several critical points.

(1) The patient may be required to abstain from intercourse for 48 to 72 hours.

(2) The specimen is collected in a clean container that has been pre-warmed to body temperature.

(3) The specimen should be delivered to the laboratory within 1 hour.

(4) The specimen must be kept at body temperature (37^{o}C) and not subjected to extremes of heat or cold.

d. **Gross Examination**.

(1) Record. the time of collection and receipt of the specimen.

(2) Measure and record the volume.

(3) Observe and record the color (white, gray, yellow, and so forth), turbidity (clear, opalescent, opaque, and so forth), and viscosity (viscid, gelatin, liquid).

(4) Determine the pH with a pH reagent strip and record this.

e. **Motility Examination**.

(1) When the specimen becomes fluid (within 15 to 30 minutes after collection, the semen liquifies by the action of fibrinolysin), place one drop on a slide (pre-warmed to 37ºC) and place a cover slip on it.

(2) Under high dry power, count motile and nonmotile spermatozoa in two or more areas until a total of at least 200 spermatozoa have been observed. It is necessary to focus through the entire depth of a given field so as to include nonmotile spermatozoa that may have settled to the bottom of the slide. Only those that move forward actively are considered motile. Record the percent of motile spermatozoa seen.

(3) Repeat this procedure in three hours and six hours, using a new drop from the original specimen each time.

f. **Spermatozoa Count**.

(1) Make a 1:20 dilution of seminal fluid with diluent (tap water).

(2) Mix sample thoroughly and charge a Hemacytometer.

(3) Count the spermatozoa in the same manner as you would count white blood cells.

(4) After counting the sperm, examine the morphology and report the percent of abnormal forms. Morphologically normal sperm are quite uniform in appearance. Any sperm with rounded, enlarged, small, or bilobed heads are abnormal. Abnormal tails are enlarged, small, irregular in length, absent, or multiple. See figure 5-3 for morphology of spermatozoa.

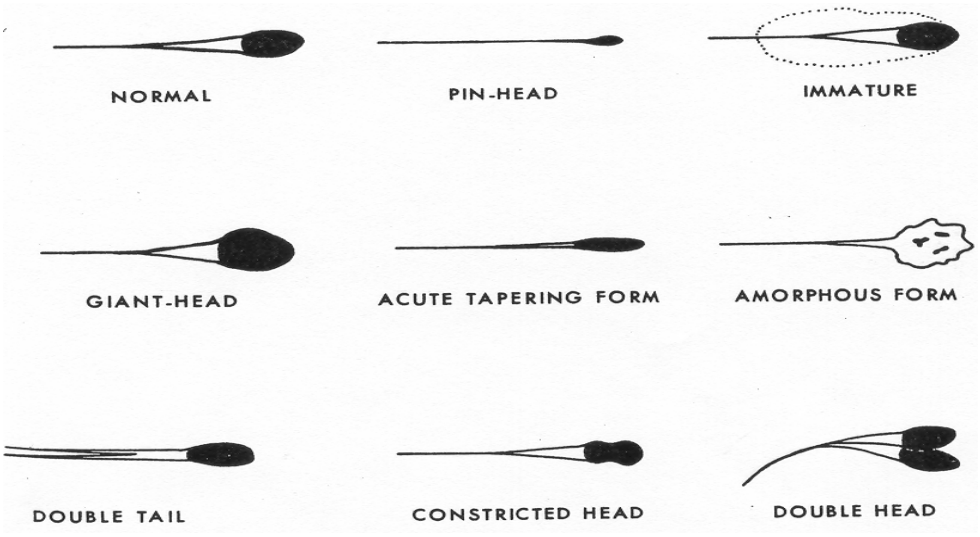


Figure 5-3. Morphology of spermatozoa.

g. **Calculations**.

$$\frac{\text{Number of sperm counted X dilution (20) x } 10^3}{\text{Number of squares counted X volume (0.4)}} = \text{Number of sperm x } 10^6 \text{ sperm/ml}$$

h. **Sources of Error**.

(1) Delay in analysis results in a lower percentage of motile forms and a lower count.

(2) Temperature extremes cause spermatozoa to die.

(3) See paragraph 5-2e for sources of error when counting.

i. **Discussion**.

(1) Semen analyses are usually performed as part of infertility studies or following a vasectomy.

(2) Semen analysis can be performed for medico-legal cases involving rape or to support or disprove a denial of paternity on the grounds of sterility.

(3) Semen is derived from the following: testes, seminal vesicles, prostate, epididymides, vasa deferentia, bulourethral glands, and urethral glands.

j. **Normal Values**.

(1) Volume: 2.0-5.0 ml.

(2) pH: 7.3-7.8.

(3) Motility: > 50-60 percent.

(4) Spermatozoa Count: 20-160 x 10^6 sperm/ ml.

Continue with Exercises

EXERCISES, LESSON 5

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answer the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. For a WBC count, after drawing blood into the diluting pipet, it is necessary to expel first 3 to 4 drops of diluted specimen in order to:

   a. Clean capillary bore.

   b. Dirtying the pipet.

   c. Dirtying the hemacytometer cover glass.

   d. Waste cells.

2. When doing a WBC count, to what mark should the diluting fluid be drawn?

   a. 3.

   b. 7.

   c. 11.

   d. 13.

3. When performing a WBC count, which reagents may be used as diluants?

   a. Acetic acid or Ammonium Oxalate.

   b. Acetic acid or citric acid.

   c. Acetic acid or hydrochloric acid.

   d. Acetic acid or trisphosphoric acid.

4. For the WBC count, immediately after the contents of the pipet have been mixed for about three minutes, it is necessary to:

   a. Use a mechanical mixer.

   b. Expel three to four drops.

   c. Observe for even distribution of cells.

   d. Refill both chambers of the Hemacytometer.

5. After the WBCs have settled for about three minutes during a manual WBC count, which powered magnification and lighting arrangements are used to focus on the ruled area to observe for even distribution of WBC?

   a. Low-power (10X); reduced light.

   b. High-power (43X); bright light.

   c. Oil immersion (97X); bright light.

   d. High-power (43X); reduced light.

6. When counting WBCs, a variation of more than _____ cells between any of the four areas counted or a variation of more than _____cells between sides of the Hemacytometer indicate uneven distribution and require that the procedure be repeated.

   a. 6; 12.

   b. 7; 9.

   c. 10; 20.

   d. 10; 18.

7. How long do you let the WBC diluent sit to allow RBCs to lyse?

    a. 10 min.

    b. 20 min.

    c. 15 min.

    d. 5 min.

8. Which WBCs are counted?

    a. Those touching the inner left-hand bottom lines.

    b. All WBCs outside the squares.

    c. All WBCs within the square and those touching the upper and right hand center lines.

    d. All WBCs within the square and those touching the upper and left- hand center lines.

9. How many 1-sq-mm comer areas and chambers are used to count WBCs?

    a. 3; 4.

    b. 4; 2.

    c. 2; 6.

10. Which chemical is mixed with whole blood when obtaining a WBC count?

    a. Sodium chloride.

    b. Weak acid.

    c. Weak base.

    d. Calcium carbonate.

11. Let diluted blood stand for _____ minutes and allow cells to settle for _____ minutes after placing in the hemacytometer.

    a. 45, 5-10.

    b. 15, 1-2.

    c. 10, 3-5.

    d. 1, 6-8.

12. The usual blood dilution for the manual WBC count is:

    a. 1:10.

    b. 1:20.

    c. 1:100.

    d. 1:200.

13. The volume is the:

    a. Area X width.

    b. Width X length.

    c. Width X depth.

    d. Area X depth.

14. Using the Hemacytometer counting chamber, the formula for calculating the WBC count is:

    a. $\frac{\text{Average number of WBCs counted X Dilution}}{\text{Volume}}$ = WBCs per cu mm

    b. $\frac{\text{Average number of WBCs counted X Dilution}}{\text{Volume}}$ = WBCs per sq in

    c. $\frac{\text{Average number of WBCs counted X Volume}}{\text{Dilution}}$ = WBCs per cu mm

    d. $\frac{\text{Average number of WBCs per cu mm X WBCs counted}}{\text{Volume}}$ = Dilution

15. If blood is drawn in Unopette with a dilution of 1:20, what is the patient if the average of two chamber counts is 163?

    a. 3,260 WBCs per cu mm.

    b. 8,530 WBCs per cu mm.

    c. 8,150 WBCs per cu mm.

    d. 10,320 WBCs per cu mm.

16. If blood is drawn in Unopette with a dilution of 1:20, what is the patient if the average of two chamber counts is 214?

    a. 4,280 WBCs per cu mm.

    b. 8,350 WBCs per cu mm.

    b. 9,984 WBCs per cu mm.

    c. 10,700 WBCs per cu mm.

17. If blood is drawn in Unopette with a dilution of 1:20, what is the patient if the average of two chamber counts is 198?

    a. 3,960 WBCs per cu mm.

    b. 9,900 WBCs per cu mm.

    c. 9,984 WBCs per cu mm.

    d. 10,540 WBCs per cu mm.

18. What are common sources of error when performing a manual Hemacytometer WBC count?

    a. Wet or dirty pipets.

    b. Poor pipetting techniques.

    c. Improper collection of blood specimen.

    d. All of the above.

19. Overfilling the chamber of the Hemacytometer can cause:

    a. Erroneous low counts.

    b. Erroneous high counts.

    c. Erroneous equal counts.

    d. No errors to the counts.

20. If the WBC count is 10,210 and the differential indicates there are 19 nucleated RBCs per 100 WBCs, What is the corrected WBC count?

    a. 8,650.

    b. 8,580.

    c. 9,580.

    d. 1,021,000.

21. If the WBC count is 9,640 and the differential indicates there are 14 nucleated RBCs per 100 WBCs, What is the corrected WBC count?

    a. 7,390.

    b. 8,256.

    c. 8,456.

    d. 946,000.

22. What is the normal range of the WBC count in adults?

    a. 4,500-11,500 WBCs per cu mm.

    b. 6,000-14,000 WBCs per cu mm.

    c. 9,000-30,000 WBCs per cu mm.

    d. 4.2-5.4 million WBCs per cu mm.

23. If 88 eosinophils are counted in a 36-sq mm area of a Hemacytometer using a 1:10 dilution, what is the eosinophil count?

    a. 20 eosinophil per cu mm.

    b. 150 eosinophil per cu mm.

    c. 244 eosinophil per cu mm.

    d. 300 eosinophil per cu mm.

24. An unchanged eosinophil count 4 hours after the injection of ACTH is indicative of:

    a. Addison’s disease.

    b. Hyperadrenalism.

    c. Cushing’s disease.

    d. Normal adrenocortical function.

25. Which stain is used to evaluate eosinophil nasal smears?

    a. Pink.

    b. Orange.

    c. Supravital.

    d. Wright's.

26. The reticulocyte is an immature:

    a. Rubriblast.

    b. Erythroyte.

    c. Prorubricyte.

    d. Metarubricyte

27. If 15 reticulocytes are counted in a total of 1,000 erythrocytes, what percentage of reticulocytes should be reported?

    a. 0.015 percent.

    b. 1.15 percent.

    c. 2.5 percent.

    d. 1.5 percent.

28. If 86 reticulocytes are counted in a total of 1,000 erythrocytes, what percentage of reticulocytes should be reported?

    a. 8.6 percent.

    b. 4.6 percent.

    c. 66 percent.

    d. 86 percent.

29. In tuberculosis meningitis, the predominant WBC type usually found in the spinal fluid is the:

    a. Monocyte.

    b. Eosinophil.

    c. Lymphocyte.

    d. All of the above.

30. If 198 cells are counted in an undiluted spinal fluid, what is the cell count?

    a. 2.2 per cu mm.

    b. 22 per cu mm.

    c. 220 per cu mm.

    d. 2,200 per cu mm.

31. If 47 cells are counted in a spinal fluid diluted 1:10, what is the cell count?

    a. 4.7 per cu mm.

    b. 52.2 per cu mm.

    c. 470 per cu mm.

    d. 522 per cu mm.

32. White blood cell counts on spinal fluid that are above _____ are usually considered indicative of some type of intracranial disease.

    a. 10 per cu mm.

    b. 15 per cu mm.

    c. 20 per cu mm.

    d. 25 per cu Im1.

33. In most viral infections, the predominant cell usually found in the spinal fluid is the:

    a. Neutrophil.

    b. Basophil.

    c. Eosinophil.

    d. Lymphocyte.

34. In subdural hemorrhages, the predominant cell type found in spinal fluid is usually the:

    a. Lymphocyte.

    b. Neutrophil.

    c. Monocyte.

    d. Segmented lymphocyte.

35. The neutrophil cell is predominant in which disease or infection?

    a. Tuberculous meningitis.

    b. Syphilis.

    c. Bacterial infections.

    d. Hodgkin’s disease.

36. Except for the diluting fluid used, the spermatozoa count is almost identical in procedure to the:

    a. RBC count.

    b. WBC count.

    c. Reticulocyte count.

    d. Total eosinophil count.

37. What is the patient required to abstain from prior to having a Semen analysis collected?

    a. Water intake.

    b. Using a pre-warmed container.

    c. Intercourse.

    d. Using the Miller disk.

38. What three factors should be observed and recorded during gross and of the semen specimen?

    a. Color, viscosity, and temperature.

    b. Color, amount of blood, and viscosity.

    c. Mucus dissolved, temperature, and color.

    d. Viscosity, color, and turbidity.

39. During a motility and of spermatozoa, which cells are considered to be motile?

    a. The entire mixture.

    b. The entire depth of the field.

    c. All active ones moving forward.

    d. Only those that are floating.

40. When should the motility procedure be repeated when examining spermatoza specimens?

    a. Every 15 minutes.

    b. In 3 hours and 6 hours.

    c. Within 30 minutes of collection.

41. Fibrinolysin causes what type of change to the semen?

    a. It solidifies.

    b. It is modified with Wright's stain.

    c. It causes it to liquefy.

    d. Nothing.

42. Semen analysis can be performed for _____ cases involving rape or in support or denial of paternity on the grounds of _____.

    a. Medico-legal; sterility.

    b. Medico-vasa deferentia; sterility.

    c. Abnormal; epididymides.

43. From which of the following male body parts is semen derived?

    a. Testes, seminal vesicles, prostate, epididymides, vasa deferentia, reticulocytes, and urethral glands.

    b. Testes, seminal vesicles, prostate, erythrocytes, vasa deferentia, bulbourethral glands, and urethral glands.

    c. Testes, motility spermatozoa, seminal vesicles, prostate, epididymides, vasa deferentia, bulbourethral glands, and urethral glands.

    d. Testes, seminal vesicles, prostate, epididymides, deferentia, bulbourethral glands, and urethral glands.

44. Which of the following is a correct normal value of spermatoza?

    a. Volume: 0.5-5.0 ml.

    b. pH: 7.4-7.6.

    c. Motility: greater than 50 percent.

    d. Spermatozoa Count: 25 to 50 million per ml.

45. Which of the following is a correct normal value of spermatoza?

a. Volume: 1.5-5.2 ml.

b. pH: 7.3-7.8.

c. Motility: 60 to 94 percent.

d. Spermatozoa Count: 60-170 million per ml.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 5

1. a (para 5-2d(2))
2. c (para 5-2c(3))
3. c (para 5-2b)
4. b (para 5-2d(2))
5. a (para 5-2d(5a))
6. c (para 5-2d(5e))
7. d (para 5-2c(1))
8. c (para 5-2d(5c))
9. b (paras 5-2d(5c, d))
10. b (para 5-2a)
11. c (paras 5-2d(1), (4))
12. b (para 5-2c)
13. d (para 5-2e(2))
14. a (paras 5-2e(3), (4))
15. c (paras 5-2e(3), (4))
16. d (paras 5-2e(3), (4))
17. b (paras 5-2e(3), (4))
18. c (paras 5-2f(1), (2), (4))
19. b (para 5-2f(6))
20. b (para 5-2g(3))
21. c (para 5-2g(3))
22. a (para 5-2h(1))

23. c (para 5-3d)

24. a (para 5-3f(6))

25. d (para 5-3f(7))

26. b (para 5-4a)

27. d (para 5-4d)

28. a (para 5-4d)

29. d (para 5-5f(3))

30. c (para 5-5d(1))

31. d (para 5-5d(2))

32. a (para 5-5f(3))

33. d (para 5-5f(3))

34. b (para 5-5f(3))

35. c (para 5-5f(3))

36. b (para 5-6f(5))

37. c (para 5-6c(1))

38. d (para 5-6d(3))

39. c (para 5-6e(2))

40. b (para 5-6e(3))

41. c (para 5-6e(1))

42. a (para 5-6i(2))

43. d (para 5-6i(3))

44. c (para 5-6j(3))

45. b (para 5-6j(2))

End of Lesson 5

LESSON ASSIGMMENT

LESSON 6	Hematocrit, Erythrocyte Sedimentation Rate, and Hemoglobin.
TEXT ASSIGMMENT	Paragraphs 6-1 through 6-12.
TASK OBJECTIVES	After completing this lesson you should be able to:

6-1. Select the statement that best describes the procedures used to perform the microhematocrit test.

6-2. Select the statement that best describes the procedures used to perform the erythrocyte sedimentation using the Wintrobe-Landsberg and modified Westergren techniques.

6-3. Select the statement that best describes hemoglobin, the compounds *of* hemoglobin, and the variations *of* hemoglobin.

6-4. State the four basic ways to measure hemoglobin concentrations and correctly describe the cyanmethemoglobin method.

6-5. Select the procedures required to perform the detection of hemoglobin S and to demonstrate the sickle cell phenomenon.

6-6. Select the statement that correctly describes the purpose of hemoglobin electrophoresis.

6-7. Select the statement that correctly describes the procedures to perform a fetal hemoglobin test.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 6

HEMATOCRIT, ERYTHROCYTE SEDIMENTATION RATE, AND HEMOGLOBIN

Section I. HEMATOCRIT

6-1. INTRODUCTION

a. The hematocrit or the packed-cell volume is the percentage of the total volume of red blood cells in relation to the total volume of whole blood. The term "hematocrit" is derived from two Greek words: Hemato, meaning blood; and Krites, meaning to judge (then reduced to crit, meaning to separate). The procedure is performed by filling a capillary tube with blood and centrifuging at a constant speed for a constant period of time. The packet cell volume is then measured. The hematocrit can also be determined by automated sequential analyzers but is usually a calculated value.

b. The hematocrit is the most useful single index for determining the degree of anemia or polycythemia. It can be the most accurate (2-4 percent error) of all hematological determinations. In contrast, the direct red blood cell chamber count has a percent error of 8-10 percent. The hematocrit is, therefore, preferable to the red blood cell count as a screening test for anemia. The values for the hematocrit closely parallel the values for the hemoglobin and red blood cell count.

6-2. MICROHEMATOCRIT

a. **Principle**. A capillary tube is filled with whole blood by capillary action to within one to two cm of the end. The unfilled end is sealed and the tube is centrifuged. After centrifugation, the capillary tube is placed in a reading device and the hematocrit value determined.

b. **Procedure**.

(1) If anticoagulated venous blood is the specimen, fill a plain capillary tube with blood. If blood without anticoagulant is used, fill a heparinized capillary tube with the blood specimen. A heparinized capillary tube is identified by red line on the tube specimen. A heparinized capillary tube is identified by a red line on the tube.

(2) Allow blood to enter two capillary tubes until they are approximately 2/3 filled with blood. Air bubbles denote poor technique, but do not affect the results of the test.

(3) Seal the unfilled ends of the tubes with clay.

(4) Place the two hematocrit tubes in the radial grooves of the centrifuge head exactly opposite each other, with the sealed end away from the center of the centrifuge.

(5) Screw the flat centrifuge head cover in place.

(6) Centrifuge at 10,000 rpm for 5 minutes.

(7) Remove the hematocrit tubes as soon as the centrifuge has stopped spinning. Determine the hematocrit values with the aid of a microhematocrit reader. Results should agree within ±1 percent. If they do not, repeat the procedure.

NOTE: Since there are a variety of readers available, it is necessary that the technician carefully follow the directions of the manufacturer for the particular device utilized.

c. **Sources of Error**.

(1) Inadequate mixing of the blood prior to sampling.

(2) Improper sealing of the capillary tube causes the blood to blow out of the capillary tube during centrifugation.

(3) Capillary tubes must be properly identified. Numbered holders for capillary tubes are available. Place the tubes in slots on the holder and record the numbers on the laboratory request slip.

(4) Improper centrifugation leads to varied results. For good quality control, maintain prescribed centrifuge speed and time.

(5) Misreading the red cell level by including the buffy coat causes elevated values.

d. **Discussion**.

(1) The microhematocrit technique is advantageous because of speed, and because only a small quantity of blood is necessary for the determination. An additional advantage is the ease with which this procedure is adapted to infants and small children. The microhematocrit technique requires only a simple capillary puncture whereas in the Wintrobe method venous blood must be used. Another advantage is the use of disposable capillary tubes.

(2) If the microhematocrits cannot be read promptly, the capillary tubes must be properly identified and placed in a vertical position. Slanting of the cell layer will occur if tubes are left in a horizontal position for more than 30 minutes.

(3) Hematocrit results may also be obtained or computed through use of an automated cell counter. Hematocrit calculated on the automated hematology analyzer is calculated from the determination of MCV and RBCs. The computation makes no allowance for the trapped plasma that always occurs to some degree in manual packing procedures.

e. **Normal Values**.

(1) Birth: 45 to 60 percent.

(2) Male: 40 to 52 percent.

(3) Female: 36 to 48 percent.

Section II. ERYTHROCYTE SEDIMENTATION RATE

6-3. ERYTHROCYTE SEDIMENTATION RATE

a. **Introduction**. When anticoagulated whole blood is allowed to stand for a period of time, the red blood cells settle out from the plasma. The rate at which the red cells fall is known as the erythrocyte sedimentation rate (ESR). The ESR is affected mainly by three factors: erythrocytes, plasma, and mechanical and technical factors.

b. **Erythrocytes**. Size and shape of erythrocytes cause the ESR to fluctuate. Microcytes tend to settle slower than normal cells, while macrocytes fall more rapidly. An increase in spherocytes and/or bizarrely-shaped red cells retards the sedimentation rate. With a decreased hematocrit there is less retardation of sedimentation by the erythrocytes themselves and they tend to settle faster. Corrections for anemic blood are available; however, most experts consider this correction useless and invalid. An increased hematocrit (above 55 percent) retards the sedimentation rate.

c. **Plasma Composition**. In certain diseases plasma proteins, namely fibrinogen and globulin, may be altered, causing rouleaux formation. The speed of the sedimentation corresponds to the length of the rouleaux formation. Increases in fibrinogen or globulin will produce long rouleaux that are difficult to disperse. This leads to a larger mass and an increased sedimentation velocity.

d. **Mechanical Technical Factors**. It is important that the ESR tube be exactly perpendicular. A tilt of 30 can cause errors up to 30 percent. Vibration or movement of the ESR tube or holding rack can affect the ESR as can extreme changes in temperature.

e. **Erythrocyte Sediment Rate**. Determination of the ESR is performed by many methods of the more common methods; the Wintrobe-Landsberg and modified Westergen methods are stated in this lesson.

6-4. DETERMINATION OF SEDIMENTATION RATE (WINTROBE-LANDSBERG)

a. **Principle**. Anticoagulated blood is placed in a narrow tube. The blood cells settle out of the suspension, leaving clear plasma above them. The distance that the erythrocytes fall within a given interval of time is measured.

b. **Procedure**.

(1) Draw 5 ml of blood by venipuncture and place in a test tube containing ethylenediaminetetraacetic acid (EDTA) (lavender top vacuum tube).

(2) Thoroughly mix the blood and anticoagulant by gently inverting the tube several times, being careful not to cause bubbles.

(3) Draw the blood into the capillary pipet and fill the Wintrobe tube to the “0” mark. This is done by inserting a capillary pipet to the bottom of the Wintrobe tube, while holding it at an angle of 45°. As the tube is filled, slowly withdraw the pipet so that the tip is always just below the level of the blood. The blood count must be free of bubbles.

(4) Place the filled Wintrobe tube in a rack in an exactly vertical position and note the time and room temperature.

(5) At the end of exactly 1 hour, read the level to which the red cells have settled on the descending scale etched on the tube. Each mark equals 1.0 mm while each numbered mark equals 10 mm (1 cm). The figure obtained is reported in mm per hour as the "uncorrected" erythrocyte sedimentation rate.

(6) If a "corrected" sedimentation is requested, perform a hematocrit. Calculators to correct for sedimentation rate are available in the Federal Supply Catalog.

c. **Sources of Error**.

(1) The blood specimen must be properly mixed with the proper anticoagulant to obtain an undiluted representative sample.

(2) The test should be set up within two hours after the blood sample is collected to avoid a false low sedimentation rate.

(3) Increase in temperature accelerates the rate. Desirable temperature range is 22º C to 27º C.

(4) The tube must be vertical. A three-degree variation from the vertical rate accelerates the rate by 30 percent.

(5) Dirty Wintrobe tubes or capillary pipets can decrease the rate.

(6) Tubes should be placed free from vibration or disturbance.

d. **Normal View**.

(1) Males: 0 to10 mm/hr.

(2) Females: 0 to 15 mm/hr.

(3) Children: 0 to 10 mm/hr.

6-5. DETERMINATION OF SEDIMENTATION RATE

a. **Principle**. Well-mixed, whole blood is diluted with 0.85 percent sodium chloride placed in a Westergren pipet, and allowed to stand for exactly one hour. The number of millimeters the red cells fall during this tie period constitutes the ESR result.

b. **Procedure**.

(1) Mix the whole blood and the anticoagulant by gently inverting the tube several times or by placing on a rotator for two minutes.

(2) Place 0.5 ml of 0.85 saline in a plain 13 X 100 mm test tube (usually commercially prepared and pre-measured).

(3) Add 2.0 ml of well-mixed whole blood to the test tube.

(4) Mix the tube for two minutes.

(5) Make certain that the Westergren ESR rack is exactly level.

(6) Fill the Westergren pipet to exactly the “0” mark, making certain there are no air bubbles in the blood.

(7) Place the pipet in the rack. Be certain the pipet fits snugly and evenly into the grooves provided for it.

(8) Allow the pipet to stand for exactly 60 minutes.

(9) At the end of 60 minutes, record the number of millimeters that the red cells have fallen. This result is the erythrocyte sedimentation rate in millimeters per hour.

c. **Sources of Error**. See para 6-4c.

d. **Normal Values**.

(1) Males: 0 to 10 mm/hr.

(2) Females: 0 to 15mm/hr.

(3) Children: 0 to10 mm/hr.

e. **Discussion**.

(1) The erythrocyte sedimentation rate is a nonspecific test that suggests the possibility of a disease process and tissue damage in the body. It is not diagnostic but is extremely useful in following the course of some diseases.

(2) The rate is usually increased in inflammatory infections, toxemia, cell or tissue destruction, severe anemia, active tuberculosis, syphilis, acute coronary thrombosis, rheumatoid arthritis, and malignant processes.

(3) Sickle cell anemia, polycythemia, hypofibrinogenemia, and certain drugs usually decrease the rate.

Section III. HEMOGLOBIN

6-6. GENERAL INFORMATION

Hemoglobin is a conjugated protein composed of the basic protein globin linked to 4 heme molecules. Ninety-eight percent of all the iron found in the blood is contained in hemoglobin. Hemoglobin transports oxygen and carbon dioxide. This important substance reacts with oxygen to form oxyhemoglobin. In the tissues, oxygen is released and reduced hemoglobin is formed. Hemoglobin can react with acids, bases, and oxidizing and reducing agents. It also can exist in a variety of forms. These hemoglobin compounds and variants are discussed briefly in the following paragraphs. For more detailed information, refer to the standard hematological texts.

6-7. COMPOUNDS OF HEMOGLOBIN

a. **Oxyhemoglobin**. Oxygen combines loosely with iron (ferrous state) in hemoglobin. The loosely attached oxygen diffuses into the tissues for oxidative processes. The hemoglobin then binds carbon dioxide and exists as reduced hemoglobin.

b. **Carboxyhemoglobin**. Hemoglobin combines with carbon monoxide to form carboxyhemoglobin. Carbon monoxide has an affinity 200 times greater for hemoglobin than oxygen does. Hemoglobin in this combination is incapable of oxygen transport.

c. **Methamoglobin**. This compound is formed when the ferrous state of the heme is oxidized to the ferric state. This compound is incapable of oxygen transport.

d. **Sulfhemoglobin**. This compound results from the combination of inorganic sulfides and hemoglobin. This compound is incapable of oxygen transport. This is an irreversible reaction.

e. **Cyanmethemoglobin**. This compound results when methemoglobin combines with the cyanide radical. This compound is used in hemoglobinometry.

6-8. VARIATIONS OF HEMOGLOBIN

The variations of hemoglobin occur due to structural differences in the globin protein. These differences are genetically controlled. The normal hemoglobin components are hemoglobin A (HbA), hemoglobin A_2 (HbA_2), and fetal hemoglobin (HbF). HbA constitutes most of the hemoglobin of a normal adult while HbA_2 constitutes a much smaller amount. HbF is present during the first 4 to 6 months of life and not normally present in adults. Hemoglobin S and hemoglobin C are the most commonly occurring abnormal hemoglobins. Others (D, E, H, etc.) are found in rare occurrences associated with several types of anemia. The various types of hemoglobin are separated by electrophoresis.

6-9. HEMOBLOGINOMETRY

The hemoglobin concentration is directly proportional to the oxygen-combining capacity of blood. Therefore, the measurement of the hemoglobin concentration in the blood is important as a screening test for diseases associated with anemia and for following the response of these diseases to treatment. There are four basic ways to measure the hemoglobin concentration: (1) measurement of the oxygen-combining capacity of blood (gasometric), (2) measurement of the iron content (chemical method), (3) colorimetric measurement of specific gravity (gravimetric method), and (4) the cyanmethemoglobin method is the method of choice and is most widely used. The cyanmethemoglobin is recommended by the Technical Subcommittee on Hemoglobinometry of the International Committee for Standardization in Hematology.

6-10. CHYANMETHEMOGLOBIN METHOD

a. **Principle**. Blood is diluted with a dilute solution of potassium ferricyanide and potassium cyanide at a slightly alkaline pH. The ferricyanide converts the hemoglobin to methemoglobin. The cyanide then reacts with the methemoglobin to form the stable cyanmethemoglobin. The color intensity is measured in a spectrophotometer at a wavelength of 540 mm. The optical density is proportional to the concentration of hemoglobin.

b. **Discussion**.

(1) Cyanmethemoglobin is the most stable of the various hemoglobin pigments showing no evidence of deterioration after 6 years of storage in a refrigerator. The availability of prepared standards is a distinct advantage of this technique. All hemoglobin derivatives are converted to cyanmethemoglobin with the exception of sulfhemoglobin.

(2) This method is highly accurate and is the most direct analysis available for total hemin or hemoglobin iron. Its disadvantage is the use of cyanide compounds, which, if handled carefully, should present little hazard.

(3) For accuracy in hemoglobin determinations, it is absolutely necessary that the spectrophotometer and Sahli pipets be accurately calibrated.

(4) Venous samples give more constant values than capillary samples.

(5) If the procedure is performed properly, the degree of accuracy is $\pm$2 to 3 percent.

c. **Normal Values**.

(1) Infants at birth: 15 to 20 g/dL.

(2) Males: 13 to 18 g/dL.

(3) Females: 12 to 16 g/dL.

6-11. DETECTION OF HEMOGLOBIN S AND NON S SICKLING HEMOGLOBINS

a. **Principle**. Erythrocytes are introduced into a phosphate buffer solution containing a reducing agent and hytic agent. The red cells are lysed and the hemoglobin is reduced. Reduced sickling types of hemoglobin are insoluble in phosphate buffer and turbidity results. On addition of urea, hemoglobin S dissolves.

b. **Reagents**.

(1) Add the entire content of one vial of Sickledex reagent powder to one bottle of Sickledex test solution. (Reagents are available commercially.)

(2) Dissolve the Sickledex reagent powder completely in the Sickledex test solution by shaking the bottle vigorously for a few seconds. The Sickledex test solution is then ready for use. Date the reconstituted test solution. This reagent remains stable under refrigeration at 4ºC for approximately 60 days. See figure 6-1.

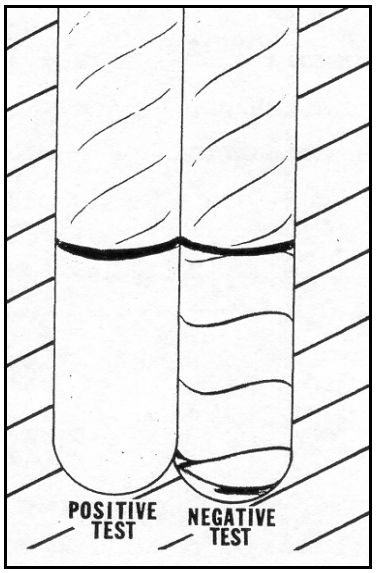


Figure 6-1. Sickledex tube test interpretation.

c. **Procedure**.

(1) Pipet 2 ml of Sickledex reagent in 12 x 75 mm test tube.

(2) Add 0.02 ml of well-mixed anticoagulated blood (collected in EDTA).

(3) Mix the contents and al1a.v to stand at room temperature for a minimum of 6 minutes (see figure 6-1).

(4) After 6 minutes examine the tube for turbidity against a lined reader. Hemoglobin five, if present, produces turbidity in the tube.

d. **Sources of Error**.

(1) Other unstable hemoglobins.

(2) Out dated reagents. The freshness of this reagent must be checked with positive and negative controls.

(3) Hemoglobin concentration less than 7 g/dL can cause a false negative

(4) False negative results could occur if the blood sample for testing is drawn within 4 months of transfusion.

e. **Discussion**.

(1) Hemoglobin S is an inherited type of hemoglobin found primarily in blacks and people from Mediterranean areas.

(2) The degree of erythrocyte sickling is dependent on the concentration of hemoglobin. SS, SC, and SD cells sickle more rapidly than AS cells. Newborns with sickle cell anemia have erythrocytes more resistant to sickling due to the presence of hemoglobin F.

(3) The dithionite test also detects other sickling types of hemoglobin. Urea causes hemoglobin S (and structural variants of hemoglobin S) to dissolve. Other hemoglobins remain turbid in the presence of urea.

(4) This test is a rapid screening test for hemoglobin S. All positive dithionite tests should be electrophoresed for confirmation.

f. **Interpretation**. Hemoglobin S causes turbidity in the tube. Hemoglobin A is soluble in the phosphate buffer.

6-12. HEMOGLOBIN ELECTROHORESIS (CELLULOSE ACETATE)

a. **Principle**. Hemoglobin fractions are separated by the rate of their protein migration in an electrical medium. The fractions are stained with ponceau S and quantitated on a densitometer. The order of mobility from the cathode toward the anode is A_3> A_I> F> S-D>C-A_2.

b. **Discussion**.

(1) A_2 hemoglobin migrates identically to hemoglobin C. They are distinguished by the quantity present. If this band is 40 percent or more of the total hemoglobin, it is C. A_2 hemoglobin should always be less than 20 percent.

(2) Two slow-moving, nonhemoglobin components are seen using this technique. These fractions are carbonic anhydrases I and II (CA_I and CA_{II})

(3) Hemoglobin A_2 is elevated in thalassemia minor.

(4) Genotype SS is found in patients with sickle cell anemia.

(5) Genotype AS is found in patients with sickle cell trait.

(6) This method separates hemoglobin A_2 in the presence of hemoglobin S in patients manifesting sickle-thalassemia disease.

(7) Hemoglobin F is quantitated by the alkali denaturation test because it migrates close to the hemoglobin A_1 fraction on the electrophoretic pattern.

(8) Include known A, S, and C controls in each analysis.

c. **Normal Values**

(1) A_3 Hemoglobin: One and three tenths to 3 percent.

(2) Genotype: AA.

(3) F Hemoglobin: 0 to 2 percent (except in infants).

Continue with Exercises

EXERCISES, LESSON 6

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answer the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. When blood is centrifuged, the volume percentage occupied by the packed red cells is known as the:

   a. Erythrocyte sedimentation rate (ESR).

   b. Hematocrit.

   c. Hemoglobin concentration.

   d. Mean corpuscular volume.

2. The procedure for determining hematocrit is performed by:

   a. Filling a capillary tube capillary with blood.

   b. Centrifuging at constant speed for a constant period of time.

   c. Measuring the packed-cell volume.

   d. Automated sequential analyzers (the results are usually a calculated value).

   e. All of the above.

3. The hematocrit is the most useful single index in determining the degree of:

   a. Anemia.

   b. Hypochromia or anemia.

   c. Leukopenia.

   d. Thrombocytopenia or thrombocytosis.

4. The hematocrit error rate for determining the degree of polycythemia is:

    a. 1 to 3 percent.

    b. 6 to 15 percent.

    c. 2 to 4 percent.

    d. 3 to 6 percent.

5. In contrast to hematological determinations, what is the percent error rate for the direct red blood cell chamber count?

    a. 2 to 4 percent.

.

    b. 5 to 9 percent.

    c. 6 to 8 percent.

    d. 8 to 10 percent.

6. The hematocrit values closely parallel the values for the:

    a. Packed WBCs and hemoglobin.

    b. Packed-cell blood count and reagent.

    c. WBC count and hemoglobin.

    d. Hemoglobin and RBC count.

7. Which microhematocrit principle is correct?

    a. A capillary tube is filled with plasma by capillary action to within 1 to 2 cm of the end. The unfilled end is sealed and the tube is centrifuged. After centrifugation, the capillary tube is placed in a reading device and the hematocrit value determined.

    b. A capillary tube is filled with whole blood by capillary action to within 1 to 2 cm of the end. The unfilled end is sealed and the tube is centrifuged. After centrifugation, the capillary tube is placed in a reading device and the hematocrit value determined.

    c. A capillary tube is filled with whole blood by capillary action to within 2 to 4 cm of the end. The unfilled end is sealed and the tube is centrifuged. After centrifugation, the capillary tube is placed in a reading device and the hematocrit value determined.

    d. A capillary tube is filled with whole blood by capillary action to within 1 to 2 cm of the end. The filled end is sealed and the tube is centrifuged. After centrifugation, the capillary tube is read and recorded.

8. Centrifugation for the microhematocrit lasts:

    a. 30 seconds.

    b. 30 minutes.

    c. 1 minute.

    d. 5 minute.

9. During the microhematocrit test, blood without anticoagulant is identified by a heparinized capillary tube with a _____ line.

    a. Green.

    b. Red.

    c. Yellow.

    d. Pink.

10. When performing the microhematocrit test, if blood contains anticoagulant, how far up should the capillary tube be filled with blood?

    a. One forth.

    b. Halfway.

    c. Three fourths.

    d. Completely.

11. Measuring the microhematocrit test, when blood is allowed to enter the two capillary tubes to approximately 2/3 full and air bubbles appear, what does this signify?

    a. A poor technique was used but it does not affect the results of the test.

    b. The heparinized capillary tube line was passed.

    c. The tubes were dirty.

    d. The seal was broken.

12. At what rpm and for how long are the two hematocrit tubes centrifuged for the microhematocrit test?

    a. 4,000 rpm; 2 minutes.

    b. 5,000 rpm; 4 minutes.

    c. 10,000 rpm; 5 minutes.

    d. 15,000 rpm; 7 minutes.

13. When using the microhematocrit reader, the results should agree within _____. If they do not, then what should occur?

    a. ±1 ;nothing.

    b. ±15; nothing.

    c. ±1; repeat the procedure.

    d. ±5; repeat the procedure.

14. Slanting of the cell layer in a microhematrocrit tube will occur if tubes are left in a _____position for more than _____ minutes.

    a. upright; 10.

    b. vertical; 60.

    c. horizontal; 45.

    d. vertical; 30.

15. The rate at which red blood cells fall when anticoagulated whole blood is allowed to stand is known as:

    a. Plasma composition.

    b. Erythrocyte sedimentation.

    c. Coulter models.

    d. Spherocytosis.

16. Erythrocyte sedimentation is retarded when the hematocrit exceeds:

    a. 35 percent.

    b. 40 percent.

    c. 45 percent.

    d. 55 percent.

17. The normal hematocrit readings for adult males and adult females are respectively:

    a. 38-47 percent and 34-41 percent.

    b. 44-64 percent and 34-41 percent.

    c. 44-64 percent and 40-54 percent.

    d. 40-52 percent and 36-48 percent.

18. Size and shape of the erythrocytes cause the erythrocyte sedimentation rate (ESR) to:

    a. Increase.

    b. Remain the same.

    c. Decrease.

    d. Fluctuate.

19. During erythrocyte sedimentation and with certain diseases, what kind of formation may form in the plasma protein if the plasma protein fibrinogen and globulin are altered?

    a. Round.

    b. Spiral.

    c. Rouleaux.

    d. Short.

20. What happens to the mass of plasma and to the sedimentation rate when the plasma protein of fibrinogen and globulin are altered?

    a. Mass enlarges; rate increases.

    b. Mass decreases; rate decreases.

    c. Mass shrivels; rate increases.

    d. Mass enlarges; rate decreases.

21. Keeping in mind the mechanical and technical factors, why is it important that the ESR tube be exactly perpendicular?

    a. A tilt of 50 can cause errors up to 55 percent.

    b. A tilt of 30 can cause errors up to 30 percent.

    c. A tilt of 10 can cause errors up to o. 05 percent.

    d. There are no other factors that affect the ESR tube.

22. What other mechanical and technical factors are important and why when working with the ESR tube or holding rack?

    a. Spilling the ESR tube or tilting holding rack can affect the ESR, as can extreme changes in temperature.

    b. Static electricity or movement of the ESR tube or holding rack can affect the ESR, as can extreme changes in temperature.

    c. Vibration or movement of the ESR tube or holding rack can affect the ESR as can extreme changes in temperature.

    d. There are no other factors.

23. When determining the ESR, using the Wintrobe-Landsberg method, what happens to the anticoagulated blood and how is this procedure measured?

    a. The anticoagulated blood is placed in a narrow tube.

    b. The blood cells settle out of the suspension, leaving clear plasma above them.

    c. The distance that the erythrocytes fall within a given interval of time is measured.

    d. a, b, and c all happen.

    e. a and d happen.

    f. None of the above.

24. With the Wintrobe-Landsberg method, which tube is used to draw 5 ml of blood by venipuncture?

    a. Green top vacuum tube.

    b. Lavender top vacuum tube.

    c. Red lined tube.

    d. Blue lined tube.

25. After the Wintrobe tube is placed in a rack in an exactly vertical position and the time and room temperature are noted, when is a reading taken and what is observed?

    a. At the end of exactly 1 hour, read the level to which the red cells have settled on the descending scale etched on the tube.

    b. At the end of exactly 2 hour, read the level to which the red cells have settled on the descending scale etched on the tube.

    c. Within 15 minute, read the level to which the red cells have settled on the ascending scale etched on the tube.

    d. In 5 minutes, read the level to which the red cells have settled on the descending scale etched on the tube.

26. If measurement of the ESR is delayed more than 2 hours after blood collection, the reading may be inaccurate because of a:

    a. False varied sedimentation rate.

    b. False low sedimentation on rate.

    c. False high sedimentation rate.

    d. Varied sedimentation rate.

27. For the Wintrobe-Landsberg method, to determine the ESR fill the Wintrobe tube to the _____ mark while holding it at _____ angle.

    a. 0, 30 degrees.

    b. 0, 45 degrees.

    c. 5, 10 degrees.

    d. 10, 50 degrees.

28. If the tube is at a 3^0 variation from vertical this is a source of error and will accelerate the ESR by _____ percent.

    a. 30.

    b. 70.

    c. 40.

    d. 50.

29. When using the modified Westergren method, whole blood is diluted with _____ percent saline and mixed for _____ minutes.

    a. 0.85, 2.

    b. 0.90, 3.

    c. 0.95, 4.

    d. 0.80, 2.

30. Using the modified Westergren method, what is the normal value ESR for children?

    a. 0-15 mm/hr.

    b. 0-20 mm/hr.

    c. 0-10 mm/hr.

    d. 0-25 mm/hr.

31. Once hemoglobin gives up its oxygen to the tissues, it is known as:

    a. Methemoglobin.

    b. Carboxyhemoglobin.

    c. Cyanmethemoglobin.

    d. Reduced hemoglobin.

32. Hemoglobin reacts with oxygen to form:

    a. Oxyhemoglobin.

    b. Methemoglobin.

    c. Cyanmethemoglobin.

    d. Carboxyhemoglobin.

33. Which compound results when methemoglobin combines with the cyanide radical?

    a. Oxyhemoglobin.

    b. Sulfhemogobin.

    c. Cyanmethemoglobin.

    d. Carboxyhemoglobin.

34. As ferrous iron in hemoglobin is oxidized to the ferric state, which of the following is produced?

    a. Methemoglobin.

    b. Carboxyhemoglobin.

    c. Carbaminohemglobin.

    d. Reduced hemoglobin.

35. Which constitutes most of the hemoglobin of a normal adult?

    a. Hemoglobin F.

    b. Hemoglobin $A_{2.}$

    c. Hemoglobin A.

    d. Hemoglobin S.

36. Which is normally present in infants of less than 6 months but not normally present in adults?

    a. Hemoglobin A.

    b. Hemoglobin $A_{2.}$

    c. Hemoglobin F.

    d. Hemoglobin S.

37. When hemoglobin combines with oxygen, its iron must be in what state?

    a. Ferrous.

    b. Globulin.

    c. Anemic.

    d. Active.

38. How many basic ways are there to measure the hemoglobin concentration?

    a. 2.

    b. 3.

    c. 4.

    d. 5.

39. Which method is the most widely used to measure the hemoglobin concentration of blood?

    a. Gasometric.

    b. Cyanmethemoglobin.

    c. Chemical.

    d. Specific gravity.

40. What does the spectrophotometer's 540 mm wavelength measure during the hemoglobin reaction using the cyanmethemoglobin method?

    a. Specific gravity.

    b. Proportionalism.

    c. Color intensity.

    d. Concentration.

41. Although the cyanmethemoglobin method is accurate, what is a disadvantage of using it?

    a. It is not the most direct method.

    b. If the cyanide compounds are handled incorrectly, they can be hazardous.

    c. Venous samples give erratic values.

    d. Its hemoglobin pigments are not stable.

42. The normal concentration of hemoglobin in blood of the adult male is:

    a. 10-15 g/dL.

    b. 12-16 g/dL.

    c. 13-18 g/dL.

    d. 18-27 g/dL.

43. Which cells sickle more rapidly than AS cells?

    a. SS cells.

    b. SC cells.

    c. SD cells.

    d. a, b, and c.

    e. a and c.

44. Erythrocytes of persons with sickle cell anemia or trait will assume a sickle shape when:

    a. The oxygen tension is lowered.

    b. The oxygen tension is raised.

    c. An electrophoretic pattern is run.

    d. Highly oxygenated blood is observed.

45. Sickle cell anemia is caused by:

    a. Endocrine disorders.

    b. Massive hemorrhage.

    c. Chronic hemorrhage.

    d. An inherited protein abnormality of hemoglobin.

46. Sickledex reagent is:

    a. Totally stable.

    b. Very stable.

    c. Not stable after 20 days.

    d. Stable for 60 days.

Check Your Answers on Next Page

SOLUTIONS TO EXCERCISES, LESSON 6

1. b (para 6-1a)
2. e (para 6-1a)
3. a (para 6-lb)
4. c (para 6-lb)
5. d (para 6-lb)
6. d (para 6-lb)
7. b (para 6-2a)
8. d (para 6-2b(6))
9. b (para 6-2b(1))
10. d (para 6-2b(1))
11. a (para 6-2b(2))
12. c (para 6-2b(6))
13. c (para 6-2b(7))
14. d (para 6-2d(2))
15. b (para 6-3a)
16. d (para 6-3b)
17. d (para 6-2e)
18. d (para 6-3b)
19. c (para 6-3c)
20. a (para 6-3c)

21. b (para 6-3d)

22. c (para 6-3d)

23. d (para 6-4a)

24. b (para 6-4b(1))

25. a (para 6-4b(5))

26. b (para 6-4c(2))

27. b (para 6-4b(3))

28. a (para 6-4c(4))

29. a (para 6-5a,b(4))

30. c (para 6-Sd(3))

31. d (~as 6-6,6-7a)

32. a (para 6-7a)

33. c (para 6-7e)

34. a (para 6-7c)

35. c (para 6-8)

36. c (para 6-8)

37. a (para 6-7a)

38. c (para 6-9)

39. b (para 6-9)

40. c (para 6-10a)

41. b (para 6-10b(2))

42. c (para 6-10c(3))

43. d (para 6-11e(2))

44. a (para 6-12a)

45. d (paras 6-8,12d(1))

46. d (para 6-1lb(2))

End of Lesson 6

APPENDIX

GLOSSARY OF TERMS

A

Agranulocyte: A leukocyte without definite cytoplasmic granules.

Agranulocytosis: Complete or nearly complete absence of the granular leukocytes from the blood and bone marrow.

Aleukemic Leukemia: A fatal condition of the blood-forming tissues, characterized by marked proliferation of immature cells in the bone marrow, without their presence, in any great numbers, in the blood steam.

Anemia: A condition in which the blood is deficient in quantity or quality of erythrocytes.

Anisocytosis: Variation in size of the erythrocytes.

Anomaly: Abnormality.

Anoxemia: Lack of normal proportion of oxygen in the blood.

Antecubital Space: The area on the forearm frontal to the elbow.

Anticoagulant: A substance that prevents the coagulation of blood. Commonly used ones are potassium oxalate, sodium oxalate, sodium citrate, EDTA and heparin.

Aplasia: Incomplete or defective blood development; cessation of blood cell formation.

Aplastic Anemia: Anemia characterized b incomplete or effective blood development.

Asynchronous: Uncoordinated development as in abnormal cell development.

Azurophilic Granule: Rounded, discrete, reddish-purple granule, smaller than the granules of neutrophils; 1-10 are common in lymphocytes, and they are very numerous, and smaller, in the cytoplasm of monocytes

B

Band Form: In the Schilling classification, a neutrophil with the nucleus unsegmented and ribbonlike; also stab, staff, nonfilamented.

Basket Cell: A degenerated primitive cell which has ruptured and in which the cell nucleus appears as a pale staining smear without prescribed form or shape.

Basopenia: An abnormal decrease in the number of basophils.

Basophil: A granular leukocyte, the granules of which have affinities for the basic dye of Wright stain (methylene blue). The granules are large, irregular and blue-black in color.
Basophilia: An abnormal increase in the number of basophils.

Basophilic: Staining readily with basic dyes, for example, blue with Ramanovsky type stains.

Binary Fission: Simple cell division.

Bleeding Time: The time required for a small standardized wound, made in the capillary bed of the finger or ear lobe, to stop bleeding.

Blood Dyscrasia: A disease of the blood or blood-forming organs.

Buffy Coat: The layer of leukocytes that collects immediately above the erythrocytes in sedimented or centrifuged whole blood.

C

Cabot's Rings: Lines in the form of loops or figures-of-eight seen in erythrocytes in severe anemias.

Centriole: A minute cell organoid within the centrosome.

Centrosome: An area of condensed cytoplasm active in mitosis.

Chemotaxis: the phenomenon of movement of leukocytes caused by a chemical influence.

Chromatin: The more stainable portion of the cell nucleus contains genetic materials.

Clot Retraction: The rate and degree of contraction of the blood clot.

Coagulation Time: The time required for venous blood, in the absence of all tissue factors, to clot in glass tubes under controlled conditions.

Cocatalyst: A substance that works in tandem with another group of chemicals to accelerate a reaction velocity without being used up in the reaction.

Color Index: The ratio between the amount of hemoglobin and the number of red blood cells.

Complete Blood Count: A hematology study which consists of a red cell count, white cell count, hematocrit, hemoglobin, and blood smear study including differential white cell count.

Congenital: Born with a person; existing at or before birth.

Cooley's Anemia (Mediterranean Disease or Thalassemia): A chronic progressive anemia commencing early in life and characterized by many normoblasts in the blood, unusual facies, splenomegaly and familial and racial incidence. Target type red blood cells are often present in the peripheral blood.

Crenation: The scalloped or notched appearance of the periphery of erythrocytes found when the cells are suspended in a hypertonic solution. Also found in smears, caused by dirty glassware, slow drying, and poor smearing technique.

Cytoplasm: Protoplasm of a cell excluding the nucleus.

D

DNA: Deoxyribonucletic acid.

Differential Count: An en1.meration of the types of white blood cells seen on a stained blood smear.

Discrete: Separate.

Dyscrasia: Abnormality.

E

Ecchymosis: Subcutaneous extravastion *of* blood covering a large area.

Endothelial Leukocyte: Monocyte.

Eosinopenia: An abnormal decrease in eosinophils.

Eosinophil: A granular leukocyte, the granules of which have an affinity for the acid dye of Wright's stain (eosin). The granules are large, round, uniform in size, red-orange in color and are shiny and refractile.

Eosinophilia: A relative or absolute leukocytosis in which the main increase is in eosinophils.

Eosinophilic: Readily stained with eaosin, red-orange stain.

Epigastric: Pertaining to the upper middle portion of the abdomen.

Erythremia: A disease marked by persistent polycythemia and increased blood volume also polychythemia vera.

Erythrocyte: Red blood cell.

Erythrocytosis: An increase in the total number of erythrocytes.

Erythrogenic: Producing erythrocytes.

Erythroleukemia: An abnormal condition characterized by proliferation of erythroblastic and myeloblastic cells.

Erythropenia: A decrease in the number of red cells in the blood.

Erythropoiesis: The production of erythrocytes.

Etiology: The theory of the causation of a disease.

Extravascular: Occurring outside the blood vessels.

Extrinsic: Originating outside of the particular area.

F

Fibril: A microscopic filament often composed of fibrin.

Fibrin: The end product of the clotting mechanism that forms a network of fibers that enmesh the formed elements of blood.

Fibrinogen: The precursor of fibrin that is present normally in the plasma and produced by the liver.

Fragility Test (Osmotic): A test devised to measure the resistance of the erythrocytes to break down (hemolyze) when subjected to varying concentrations of hypotonic salt solutions.

Fulminating: Sudden and severe.

G

Golgi Apparatus: A meshwork of lipid containing fibrils within the cytoplasmic portion of a cell.

Granulocyte: A white blood cell that contains specific cytoplasmic granules (neutrophils, eosinophils, and basophils); these granules are peroxidase positive.

Granulocytosis: The presence of increased numbers of granulocytes in the blood.

Granulocytopenia (Granulopenia): A decrease in the number of granulocytes in the blood.

Granulopoiesis: The production of granulocytes.

H

Hemacytometer: A calibrated chamber in which blood cells are counted.

Hematin: A brown or blue-black amorphous iron substance that unites with globin and forms hemoglobin.
Hematocrit: The packed cell volume (PVC) of red blood cells obtained by globin and forms hemoglobin.
Hematology: The branch of medicine that deals with the study of blood cells, blood producing organs and the manner in which these cells and organs are affected in disease.

Hematoma: Subcutaneous effusion of blood with resulting swelling, pain, and discoloration, forming a tumorlike mass.

Hematopoietic (Hemopoietic): Blood forming.

Hemoglobin: The coloring matter of the red blood cells. A complex iron-bearing pigment that carries oxygen and carbon dioxide.

Hemoglobinuria: The presence of free hemoglobin in the urine.

Hemogram: The blood picture.

Hemolysis: The dissolution or dissolving of the erythrocytes.

Hemolytic Anemia: That type of anemia characterized by excessive intra-vascular destruction of red cells.

Hemophilia: A hereditary disease characterized by a prolonged coagulation time and repeated hemorrhages, occurring only in males and transmitted only by females and affected males. The cause is a deficiency in a plasma factor (antihemophilic globulin or thromboplastinogen) resulting in a defect in thromboplastic activity.

Hemoptysis: The spitting of blood; coughing up blood.

Hemostasis: The checking of the flow of blood, especially from a vessel.

Hepatic: Originating from the liver.

Heterozygous: Derived from germ cells unlike in respect to one or more factors.

Hemeostasis: Stability in normal body states.

Homozygous: Derived from germ cells that are alike.

Howell-Jolly Bodies: Small basophilic particles sometimes found in erythrocytes, remnants of nuclear material.

Hygroscopic: Readily taking up and retaining water.

Hyperplasia: An increase in cell formation.

Hypertonic: Greater than isotonic concentration.

Hypertrophy: Enlargement of an organ or part due to increase in the size of the constituent cells.

Hypochromia: A decrease in color of the erythrocytes, hence a decrease in their hemoglobin content.

Hypoplasia: A decrease in cell formation.

Hypotonic: Less than isotonic concentration.

I

Idiopathic: Disease of unknown cause.

Inclusion: Usually lifeless, an accumulation of fats, proteins, crystals pigments or secretory granules within a cell cytoplasm.

Inhibitor: A substance, directed against a coagulation factor or factors, which interferes with the coagulation process.

Intravascular: Occurring within the blood vessels.

Intrinsic: Situated within the particular part.

In Vitro: Within a test tube (glass, etc.).

In Vivo: Within the living organism, as in life.

Isotonic: Solutions with the same osmotic pressure.

J

Jaundice: Yellow mass of the skin and eyes due to the presence of blood pigments in the blood; follows excessive destruction of the blood, obstruction of the bile passage, diffuse liver disease, certain infections, toxic chemical agents and drugs.

Juvenile Cell: In the Schilling classification, the cell between the myelocyte and band forms; also metamyelocyte.

K

Karyolysis: Apparent destruction of the nucleus of a cell.

Karyorrhexis: Fragmentation of the nucleus; a degenerative process usually followed by karyolysis.

L

L.E. Cell: A large segmented neutrophil or eosinophil that contains ingested autolyzed nuclear fragments in its cytoplasm.

Leukemia: An ultimately fatal disease of the blood-forming organs characterized by increased numbers of leukocytes and associated anemia.

Leukemoid Crisis or Reaction: A temporary appearance of immature leukocytes in the blood stream, with a marked increase in the total white count. In the laboratory sometimes temporarily indistinguishable from leukemia.

Leukocyte: White blood cell.

Leukocytosis: An increase in leukocytes in the blood.

Leukopenia: A reduction in the number of leukocytes in the blood.

Leukopoiesis: Leukocyte formation.

Lymphoblast: The parent cell of the lymphocytic series.

Lymphocyte: A white blood cell having a round or oval nucleus and sky blue cytoplasm. The nuclear chromatin is densely clumped but separated by many clear areas giving a "hill and valley" effect. A few red-purple (azurophilic) granules may be present in the cytoplasm.

Lymphocytosis: A relative or absolute increase in the number of circulating lymphocytes.

Lymphopenia: An abnormal decrease in the number of lymphocytes.

Lysis: Destruction by a specific agent.

M

Macrocyte: An erythrocyte larger than normal.

Macrocytosis: An increase in the number of macrocytes.

Mast Cell: A basophil or a true tissue cell.

Maturation Factor: A substance that will cause cells to ripen and care to maturity.

Mean Corpuscular Hemoglobin (MCH): The average amount of hemoglobin in the red blood cell.

Mean Corpuscular Hemoglobin Concentration (MHC): The average percent hemoglobin saturation in the red blood cell.

Mean Corpuscular Volume (MCV): The volume of the average red blood cell.

Megakaryoblast: The parent cell of the megakaryocytic series.

Megakaryocyte: An extremely large cell with an irregular lobed, ring or doughnut-shaped nucleus that stains blue-purple. The cytoplasm is abundant, light blue and is packed with fine azurophilic granules. This cell gives rise to thrombocytes.

Megaloblast: The type of red cell precursor found in pernicious anemia. This differs from the normal erythrocyte precursor (normoblast) in that the megaloblast is larger and the nuclear chromatin has a fine meshwork or scroll design.

M.E. Ratio: The ratio of myeloid to erythroid cells in the bone marrow.

Mesentery: The fold of peritoneum that attaches the intestine to the posterior abdominal wall.

Metamyelocyte: Juvenile cell of Schilling.

Metarubricyte: An erythrocyte with a pyknotic, contracted nucleus. Also called orthochromatophilic normoblast.

Methemoglobin: A spectroscopically detected compound of hemoglobin found in nitrobenzol, and other poisonings. The blood is a chocolate brC1.oln color to the eye.

Microcyte: An erythrocyte smaller than normal.

Microcytosis: An increase in the number of microcytes

Micron: One-thousandth of a millimeter, the common unit of microscopic measure.

Mitochondria: Granular components of a cell cytoplasm active in oxidative processes.

Mitosis: A series of changes through which the nucleus passes in indirect cell division. A tissue showing many cells in mitosis indicates rapid gro.&1th of that tissue.

Monoblast: The parent cell of the monocytic series.

Monocyte: A large white blood cell with a pale blue-gray cytoplasm containing fine azurophilic granules. The nucleus is spongy and lobulated.

Monocytosis: A relative or absolute increase in the number of circulating monocytes.

Mucosa: Mucous membrane.

Myeloblast: The parent cell of the granulocytic or myelocytic series.

Myelocyte: The stage in development of the granulocytic series that is characterized by the first appearance of specific granules (eosinophilic, neutrophilic or basophilic) and a round nucleus.
Myeloid Cells: The granular leukocytes and their stem cells.

Myelopoiesis: Formation of bone marrow and the blood cells that originate in the bone marrow.

Myeloproliferative: Rapid production of bone marrow constituents.

N

Necrosis: The death of a circumscribed portion of tissue. Simple necrosis is degeneration of the cytoplasm and nucleus without change in the gross appearance of the tissue.

Neutropenia: A decrease in the number of neutrophils in the blood.

Neutrophil (Polymorphonuclear Neutrophil or Segmented Neutrophil): A granulocyte having fine neutrophilic (pink-violet) granules in the cytoplasm. The nucleus is divided into two or more lobes; each lobe is usually connected by a filament.

Neutrophilia: An increase in neutrophils.

Normoblast. The nucleated precursor of the normal red blood cell. Also called a rubriblast.

Normocyte (Erythrocyte): A red blood cell of normal size.

NRBC: Nucleated red cell, usually a metarubricyte when seen in the peripheral blood smear.

Nucleolus: An intranuclear pale blue body, surrounded: by a dense condensation of chromatin.

O

Occult Blood: The presence of blood that cannot be detected except by special chemical tests.

Oligochromemia: A decrease in hemoglobin.

Oligocythemia: A decrease in the number of erythrocytes.

Organoid: Structures present in cells resembling organs.

Ovalocyte: An elliptical erythrocyte.

Oxyhemoglobin: The bright red hemoglobin that is loosely combined with oxygen and found in arterial blood.

P

Pancytopenia: A reduction in all three formed elements of the blood, namely, the erythrocytes, leukocytes and thrombocytes.
Pathologic Increase (Or Decrease): Due to abnormal function or disease, as contrasted to physiological (due to normal body function).

Pernicious Anemia: A chronic, macrocytic anemia caused by a defect in production of “intrinsic factor” by the stomach. There is accompanying megaloblastic erythropoiesis, poikilocytosis, granulocytic hypersegmentation, achlorhydia, and neurological disturbances.

Petichiae: Small spots on the skin formed by subcutaneous effusion of blood (also purpura and ecchymoses).

Phagocytosis: The destruction of organisms and extraneous matter by a process of envelopment and absorption.

Plasma: The fluid portion of the blood composed of serum and fibrinogen, obtained when an anticoagulant is used.

Plasma Cell: A lymphocyte-like cell with an eccentrically placed deep-staining nucleus. The nuclear chromatin is distributed in a "wheel-spoke" fashion. The cytoplasm is deep blue with a lighter halo about the nucleus.

Platelet: Thrombocyte.

Poikilocyte: A red blood cell having abnormal shape (pear-shape, sickle-shape, etc.).

Poikilocytosis: Increased number of abnormally shaped erythrocytes.

Polychromasia: Diffuse basophilia of the erythrocytes.

Polychromatophilia: The presence in the stained blood smear of immature, nonnucleated, bluish-staining red blood cells.

Polycythemia: An increase in the total number of erythrocytes. (See erythremia.).

Precursor: A substance from which another substance is formed.

Promyelocyte: The precursor of the myelocyte having nonspecific azurophilic (red-purple) cytoplasmic granules.

Prorubricyte: The second stage of development of the red cell.

Prothrombin: The inactive precursor of thrombin that is formed in the liver and present normally in the plasma. Its formation depends upon adequate vitamin K.

Punctate Basophilia: Small basophilic aggregates in the erythrocytes that stain blue with the basic dye of Wright's stain; also basophilic stippling.
Purpura: Small spots on the skin formed by subcutaneous effusion of blood.

Pyknosis: A condensation and reduction in size of the cell and its nucleus.

Q-R

Reduced Hemoglobin: A combination of hemoglobin and carbon dioxide that is found in venous blood.

Reticulocyte: A red blood cell showing a reticulum or network when stained with vital dyes (for example, brilliant cresyl blue). The stage between the nucleated red cell and the mature erythrocyte.

Reticulocytosis: An increase above normal values of reticulocytes *in* peripheral blood.

RNA: Ribonucleic acid.

Rouleaux Formation: The arrangement of red cells with their flat surfaces facing, in which they appear as figures resembling stacks of coins.

Rubricyte: Polychromatophilic normoblast.

S

Sedimentation Rate, Erythrocyte (ESR): The rate at which red cells will settle out in their own plasma in a given time under controlled conditions.

Serum: The fluid portion of the blood, after clot formation.

Shift to the Left: A term used to designate that condition in which the immature forms *of* the neutrophils are increased above their normal number.

Shift to the Right: Increase in mature, pyknotic, and hypersegmented neutrophils.

Sickle Cell: A sickle- or crescent-shaped erythrocyte.

Sickle Cell Anemia: This is a hereditary and familial form of chronic, hemolytic anemia essentially peculiar to Negroes. It is characterized clinically by symptoms of anemia, joint pains, leg ulcers, acute attacks of abdominal pain and is distinguished hematologically by the presence of distinct hemoglobin, peculiar sickle-shaped and oat-shaped red corpuscles, and signs of excessive blood destruction and active blood formation.

Smudge Cell: A ruptured white cell; also basket cell, or degenerated cell.

Spherocyte. A red blood cell that is more spherical, smaller, darker, and more fragile than normal.

Stasis*:* A stoppage of blood flow.

Supravital Stain: A stain of low toxicity that will not cause death to living cells or tissues.

Synchronous: Occurring at the sane time and in a regular pattern.

T

Target Cell (Leptocyte): An abnormal, thin erythrocyte characteristic of Cooley's or Mediterranean anemia.

Triturate: To grind together.

Thrombin: This is an enzyme formed from prothrombin that converts fibrinogen to fibrin. This is not present in circulating blood.

Thrombocyte: A blood platelet.

Thrombocytopenia: A decrease in blood platelets; also thrombopenia.

Thrombocytosis: An increase in blood platelets.

Thromboplastin: The substance that initiates the process of blood clotting. It is released from injured tissue and/or formed by the disintegration of platelets in combination with several plasma factors.

Thrombopoiesis: The production of thrombocytes.

Thrombosis: Formation of a thrombus, or blood clot.

U-V

Vacuole: A space or cavity formed in the protoplasm of a cell.

Venipuncture: The act of puncturing a vein in order to remove a sample of blood.

Viscous Metamorphosis: Friction between molecules resulting in a structural change.

Vitamin K: A vitamin constituent of the normal diet requiring bile salts for absorption. The liver in the production of prothrombin utilizes this vitamin.

W-X-Y-Z

Xanthochromia: A yellowish discoloration, usually associated with spinal fluid.

End of Appendix