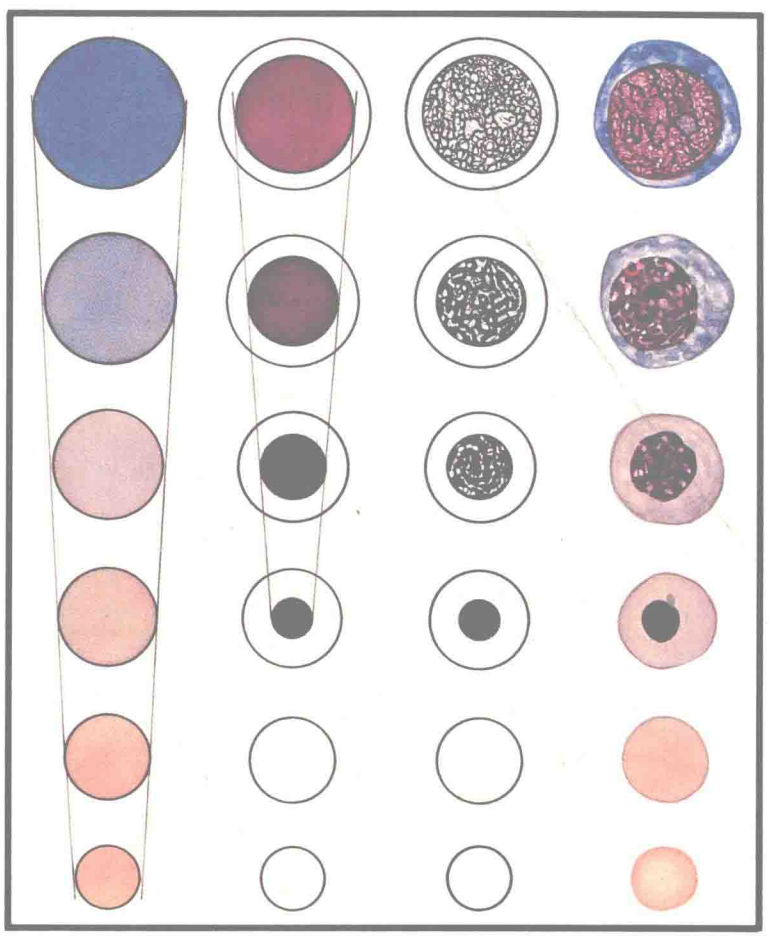
ABBOTT

The Morphology of Human Blood Cells

DIGGS
STURM
BELL



FIFTH EDITION

The Morphology of Human Blood Cells

L. W. Diggs, MD; Dorothy Sturm; and Ann Bell, MS
University of Tennessee Center for Health Sciences
Department of Medicine, Division of Hematology
and Regional Medical Center
Memphis, Tennessee

FIFTH EDITION

ABBOTT LABORATORIES
Abbott Park, IL 60064

PREFACE

This atlas, which portrays the morphologic characteristics of normal and pathologic cells in smears of blood and bone marrow, is published mainly for the use of medical students, student medical technologists, veterinary students, and other health-science students who are learning to identify the various types of hemic cells. This monograph also is an aid for teachers of morphologic hematology and for technologists and technicians who are responsible for the examination of smears by manual or automated methods and for the reporting of differential counts. A knowledge of morphology is also useful for residents in clinical and anatomic pathology, pediatrics, and medicine.

Major emphasis is placed on the anatomical characteristics of individual cells in the various stages of their maturation as revealed by light microscopy, employing an oil-immersion objective. Unless otherwise stated, the cells that are described and visually depicted by the artist, Dorothy Sturm, are those present in thin, air-exposed, dried smears or imprints that have been stained by a combination (Wright or equivalent stain) of the blue dye (methylene blue) and the red dye (eosin).

The procedure was to select an ideally prepared stained smear of fresh blood or aspirate of bone marrow without admixture with an anticoagulant and without fixation except by air drying and by methyl alcohol in the stain—and then to locate a typical cell in the thin portion of a smear. Dorothy Sturm, a skilled artist as well as a biologically trained scientist, painted the various colors and structures of the cells as she viewed them. Water colors were employed for the color plates.

It is impossible to portray by means of a relatively few cells all the infinite variations of nuclear and cytoplasmic structures of normal and pathologic cells. The authors have attempted to portray cells that are representative. Once selected, the cell was painted as that individual cell appeared. Unless otherwise specified, all cells are reproduced at a magnification of approximately × 1,800.

The microscopic examination of the cells of the blood and blood-forming organs is an extension of the history and physical examination. The identification and enumeration of cells in stained smears is of value as a screening procedure to detect normality as well as an aid in the diagnosis and differential diagnosis of various diseases, the establishment of prognosis, the indications for treatment, and the response to therapy—and as a safeguard against drug toxicity.

The development of new instruments and new technologies such as electron, stereoscan, and fluorescent microscopy and the employment of cytochemical, enzymatic, radioisotopic, centrifugal, serologic, electrophoretic, and other procedures have expanded our knowledge and understanding of normal and pathophysiologic mechanisms and the interpretation of morphologic characteristics as revealed by optical microscopy. These new, ingenious, important, and welcomed techniques are supplements to—not replacements of—the simple, time-honored, and relatively inexpensive microscopic procedures.

The reader is referred to textbooks of hematology for a discussion of etiologic factors, the clinical and anatomical manifestations, and the treatment of various diseases of blood and blood-forming organs. The reader is also referred to other sources for information about techniques, cell kinetics, pathophysiology, and lists of diseases characterized by an increase or decrease in the total leukocyte count and in the relative and absolute numbers of nucleated cells of different types.

Appreciation is expressed to numerous individuals who assisted in various ways in the preparation of this atlas. These include Mrs. Jane Tyler and other technologists in the special hematology laboratory at Baptist Memorial Hospital; Mrs. Lew Bailey and Mrs. Linda Mason, medical technologists at the Regional Medical Center; Mrs. Sara Cobb at St. Jude Children's Research Hospital—and from the University of Tennessee Center for the Health Sciences, Division of Hematology, Miss Helen Goodman, a hematology technologist; Miss Jeanie Peeples, research technician; and Drs. Luther Burkett, Marion Dugdale, Henry Herrod, Alfred Kraus, Alvin Mauer, Charles Neely, Gerald Plitman, and J.D. Upshaw. The authors wish to thank Mrs. Beatrice Diggs for library work, editing, and typing; Mr. Thomas Craig, from Abbott Laboratories professional relations, for his cooperation and understanding in the technical phases of the editing and printing; and Abbott Laboratories for publishing this atlas as a service to the medical profession.

L. W. Diggs, MD

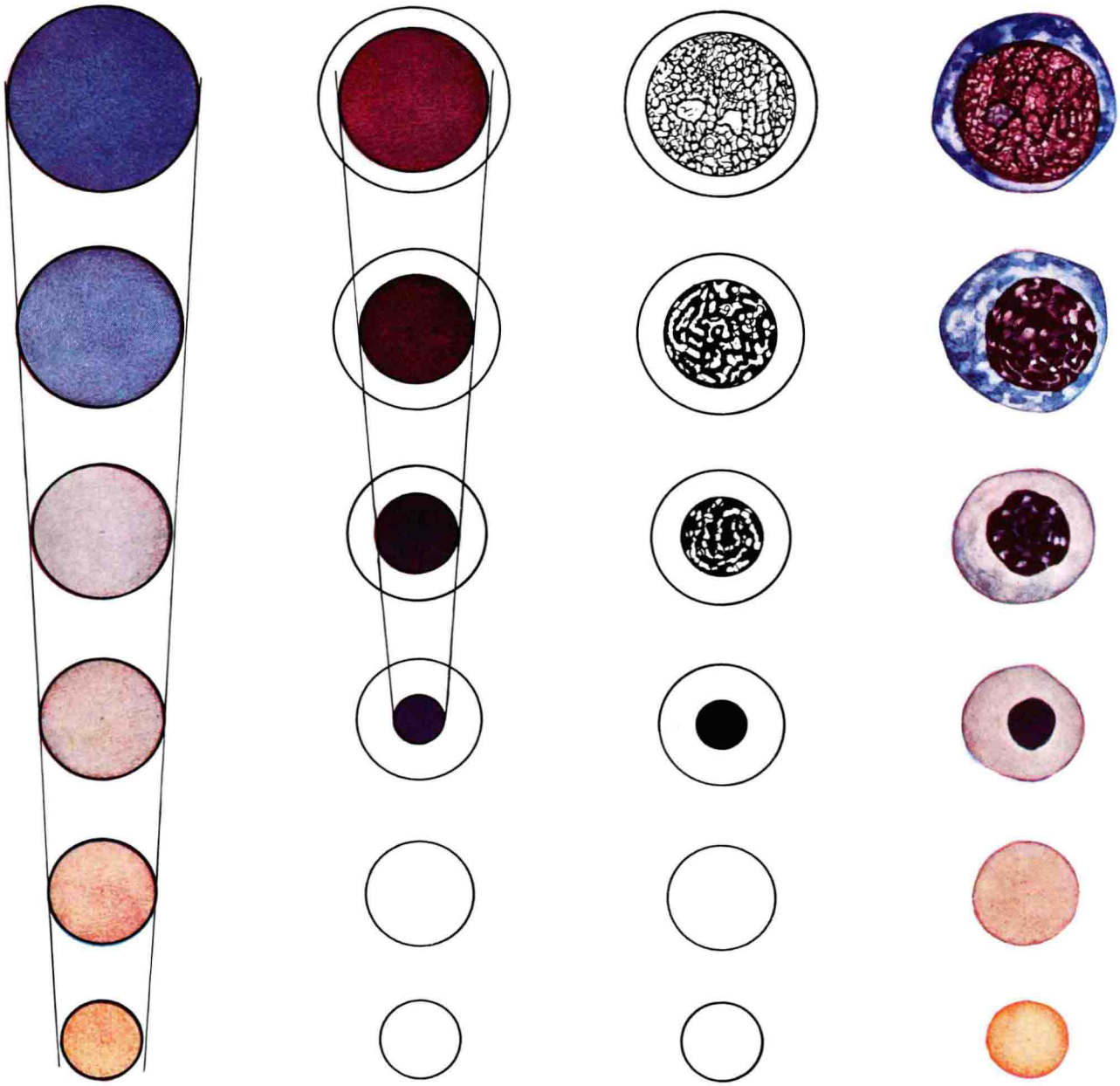


PLATE 1—MATURATION SEQUENCE

A Cell size and cytoplasm color
B Nuclear size and color
C Nuclear chromatin structure
D Composite (Top to bottom: Rubriblast, prorubricyte, rubricyte, metarubricyte, diffusely basophilic erythrocyte, erythrocyte)

CONTENTS

CELL MORPHOLOGY — GENERAL

ALL BLOOD CELLS originate from undifferentiated mesenchymal cells (Table 1, Fig 20). From these stem cells, clones of cells differentiate and ultimately appear in the circulating blood as red cells, platelets, and various types of white cells. The earliest cells of each cell line have similar morphologic characteristics and cannot be differentiated by appearance alone. They are given specific names such as "myeloblast," "lymphoblast," or "rubriblast" depending on the tissue in which they are found, the cells with which they are associated, and the definitive cell that they are destined to produce. As the embryonic cells change from their primitive forms to mature cell types, they undergo changes in nuclear and cytoplasmic characteristics common to all cells. These changes are detailed in Plate 1.

Maturation sequence. Immature cells as a class are large and become progressively smaller as they mature (Plate 1A). The nuclei of young cells of the maturation sequence are large and relatively large in relation to the cytoplasm. As the cells age, the absolute and relative size of the nucleus decreases (Plate 1B). In cells of the erythrocytic series, the small and degenerated nuclei in the older cells are extruded.

The cytoplasm of a primitive cell is predominantly blue and contains large amounts of ribonucleic acid (RNA), which has an affinity for the basic or blue dye (methylene blue). As the cytoplasmic structures and secretory products are manufactured, the color of the cytoplasm becomes more red and less blue (Plate 1A). The nuclear chromatin strands of immature cells contain deoxyribonucleic acid (DNA), which has an affinity for the acidophilic (eosinophilic) red dye. As the nucleus ages, it stains more intensely, and the color changes from purplish-red to dark blue (Plate 1B).

The most reliable criterion of the age of a given cell and its position in the maturation sequence is the structure of the nuclear chromatin. In the most-primitive cells, the nuclear chromatin strands are distinctly visible. No part of the chromatin is darker or more compact than other portions. In some cells, the pattern is linear, whereas in others, the superimposed, tortuous and twisted chromatin threads appear as red granules or short rods. If injured in the process of aspiration or spreading on a slide, the delicate and lacelike chromatin strands become thick and ropy but remain intact and distinct. When the nucleus degenerates, the bonds of the helical structure of the elongated DNA molecules are broken, and the chromatin strands widen and become more coarse and clumped. In the terminal degenerative and senile stages, the nucleus is small, round, dark, and structureless (Plate 1C).

One of the signs of immaturity in blood cells is the presence of nucleoli in the nucleus. These small islands of cytoplasmic material, manufactured within the nucleus, are signs of metabolic activity and growth. Nucleoli are best seen in very-thin smears and may be indistinct and obscured in thick or darkly stained smears. Nucleoli vary in number, shape, and size, but usually there are one to four nucleoli, round or oval in shape, with diameters in the 2 to 4 μm range. They have a fairly homogeneous structure and a color similar to that of the cytoplasm. In methylene blue and eosin stains, the color is predominantly blue. Nucleoli are not bounded by membranes, but the expanding intranuclear masses tend to compress the surrounding chromatin strands to give the appearance of a dark boundary (Plate 1D).

The combined changes in size and color of the cytoplasm and the size, color, and structure of the nucleus are visualized in Plate 1D.

The cytoplasmic shape of cells is influenced by the mechanical trauma to which the cells are subjected, the pressure of surrounding cells, and their ameboid activity. Primitive cells tend to be fixed by their cytoplasmic extensions in the ground substance. When torn away from their attachments, as in the process of bone marrow aspiration, the margins are disrupted, and the edges have a frayed and jagged appearance. Mature and free cells in the circulating blood usually have smooth margins. Cells of the monocytic-macrophage system are slowly motile cells that tend to adhere to glass and plastic surfaces. They continue to spread out during the drying process. These cells often reveal blunt cytoplasmic extensions (pseudopods) seldom seen in other cells. Granulocytes and lymphocytes are actively motile cells that do not stick readily to the surfaces of slides. These cells tend to round up and to become spherical when exposed to the air and as they slowly dry in the thicker portions of smears.

Table 1—Blood Cells

Stem Cell	HEMATOPOIETIC TISSUES					CIRCULATING BLOOD
			E. Myelocyte			Eosinophil
	Myeloblast	Promyelocyte	N. Myelocyte	N. Metamyelocyte	Neutrophil Band	Neutrophil Segmented
			B. Myelocyte			Basophil
	Monoblast	Promonocyte				Monocyte
	Megakaryoblast	Promegakaryocyte	Megakaryocyte	Metamegakaryocyte		Thrombocyte
	Rubriblast	Prorubricyte	Rubricyte	Metarubricyte	Diffusely Basophilic Erythrocyte	Erythrocyte
	Lymphoblast	Prolymphocyte				Lymphocyte
	Plasmoblast	Proplasmocyte			Plasmocyte	

Tissue cells and early cells which move slowly have round, oval, or slightly indented nuclei. Actively motile and mature cells, such as monocytes and granulocytes, have indented, kidney-shaped, lobulated, or segmented nuclei. The nuclei of mature lymphocytes are usually round, but they may be slightly indented. The nuclei of red cells and plasma cells in all stages of maturation are round.

Immature cells that are metabolically active reveal in their cytoplasm a relatively light zone adjacent to the nucleus. This area, known as the Golgi area, contains a smooth endoplasmic reticulum and centrioles. In this juxtanuclear area, colorless (achromatic) mitochondria tend to aggregate. The light zone near the nucleus is best exemplified in plasmocytes but is visible in immature blood cells of all types.

Granules are not present in stem cells. In the series of cells that characteristically develop granules, the primary granules are dark and predominantly blue. The specific and secondary granules that later develop in the more-mature cells stain less intensely and are more red and less blue, as exemplified by eosinophils and neutrophils (Plate 3). Manufactured products within cells, such as globulin in plasmocytes or hemoglobin in red cells, are indicative of maturation.

Phagocytosis of particulate matter is a manifestation of functional activity characteristic of differentiated cells, but the absence of phagocytosis in a given cell at a given time has no value in determining the maturity of a cell.

The cytoplasmic, granular, and nuclear characteristics are usually well-synchronized in normal cells, but in pathological conditions, the maturation sequences of the various cell structures may be out of step with each other. This is the case, for example, in the nucleated red cells of pernicious anemia which may have advanced hemoglobin synthesis and immature nuclear characteristics, or in the cells of iron-deficiency anemia, in which the hemoglobin is inadequately formed in cells with pyknotic nuclei.

Reproductive sequence. In addition to the changes in cell morphology that are manifestations of differentiation and maturation, there are morphologic changes that are manifestations of reproduction and multiplication. A few primitive cells in hematopoietic tissues remain undifferentiated and undergo mitotic division that likewise produces undifferentiated cells. Other cells, after the first mitosis, differentiate to a degree before they divide again. When these slightly more-mature cells undergo mitosis, the daughter cells maintain the cytoplasmic characteristics of the parent cells from which they derive. The majority of cells enter the mitotic cycle in the intermediate stages of maturation, as for example, in the promyelocyte or myelocyte stages of the granulocytes or the prorubricyte or rubricyte stages of the nucleated red cells. After one or several mitotic cycles, the nucleus degenerates and loses its ability to divide.

After the mitotic division of the nucleus, the cleavage of the cytoplasm, and the formation of two cells from the parent cell, the nuclear membrane re-forms around the chromosomes. The cell and its nucleus progressively enlarge. Nucleoli appear in the nucleus. During the last few hours of the division cycle, the nuclear membrane and the nucleoli disappear, and the chromatin condenses into dark, compact masses. Each chromatin thread divides, spindles extend outward from the

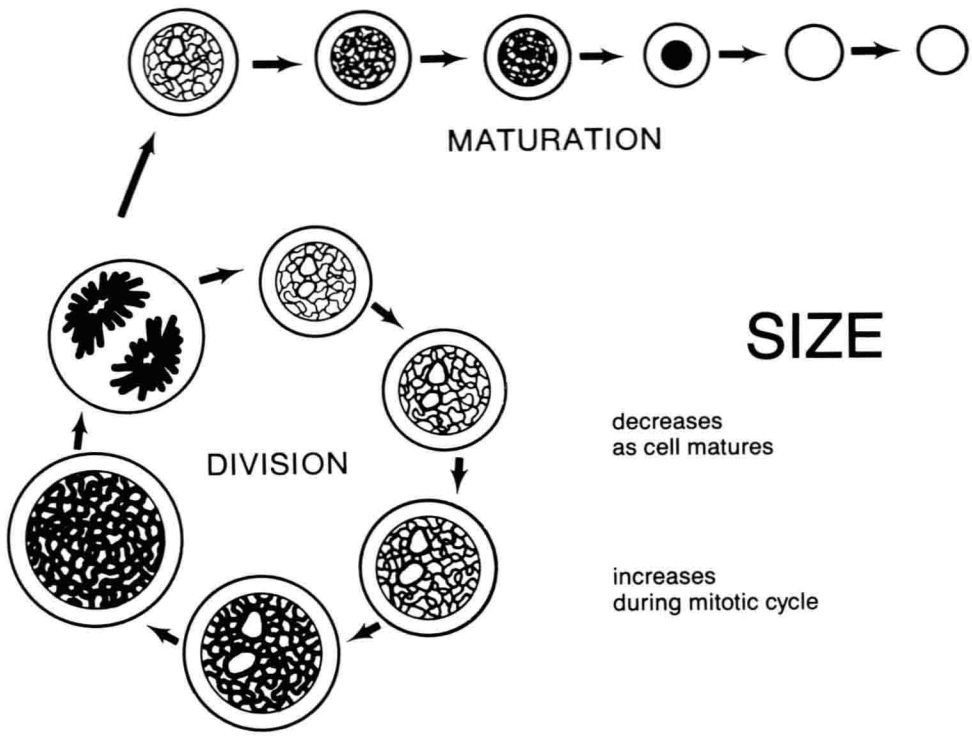


Fig 1—Cell size.

centrosomes, and the chromosomes migrate to the opposite poles. Lines of cleavage appear in the cytoplasm, and two new cells are formed. At the time of mitosis, the cytoplasm, like the nucleus, is in a state of unrest and assumes a granular or bubbly appearance.

The volume of any given nucleated cell that is capable of replication at any given time is dependent on the quantity of DNA in the nucleus and the quantity of RNA and constituents in the cytoplasm. Each cell, in preparation for mitosis, approximately doubles its component of genetic material and cytoplasmic organelles before that cell divides. During the early growth (G_1) phase, the cell is appreciably smaller than that same cell will be a few hours later. During DNA synthesis (S_1) and the later growth (G_2) interval, there is a progressive increase in volume. Frequency-distribution curves of cell diameters in smears of normal, as well as pathologic, cells reveal bell-shaped curves. Between the largest and smallest cells, there are intermediate diameters. Cells that have adequate nutrition and long periods of growth between divisions are larger than are cells with inadequate nutrition, inability to utilize nutrient factors, or shortened mitotic cycles.

Mitotic figures are not demonstrable in smears of peripheral blood of individuals who are in good health. Mitotic figures in bone marrow smears of normal individuals are difficult to find. The presence of more than one cell in mitosis per 1,000 nucleated marrow cells is an indication of abnormal proliferative activity.

The cytoplasmic shape of cells throughout interphase is variable. Cells observed during the time of mitosis often have very-irregular shapes and blunt and frayed cytoplasmic protrusions caused by the violence of cytoplasmic movement during the act of tearing apart and separating.

The nuclei of cells during interphase are round. During prophase and in the later stages of mitosis (metaphase, anaphase, and telophase), the nuclear shape is irregular.

The color of the cytoplasm of undifferentiated and blast cells participating in the division cycle is blue, but in the more differentiated cells, such as hemoglobin-containing nucleated red cells or eosinophils undergoing mitosis, the color of the cytoplasm and the structures within the cytoplasm are dependent on the stage of development of the individual cell at the time it entered the reproductive cycle. Whether cells become more differentiated while they are in the premitotic phase is a moot point.

The nuclei of cells may undergo one or more mitotic divisions without the corresponding division of the cytoplasm, producing giant cells with multiple nuclei as exemplified by megakaryocytes and by double nuclei in plasmocytes and in nucleated red cells. In other cells, the chromosomes may replicate without disruption of the nuclear membrane and without division of the nucleus (endomitosis). Other abnormalities of nuclear division include: nuclei with too few or too many chromosomes; multiple nuclei within a single cell which differ in size, color, and structure; uneven number of nuclei; and clefts or cleavage lines.

Nomenclature. Nucleated cells which are morphologically undifferentiated (anaplastic) and that cannot, by associ-

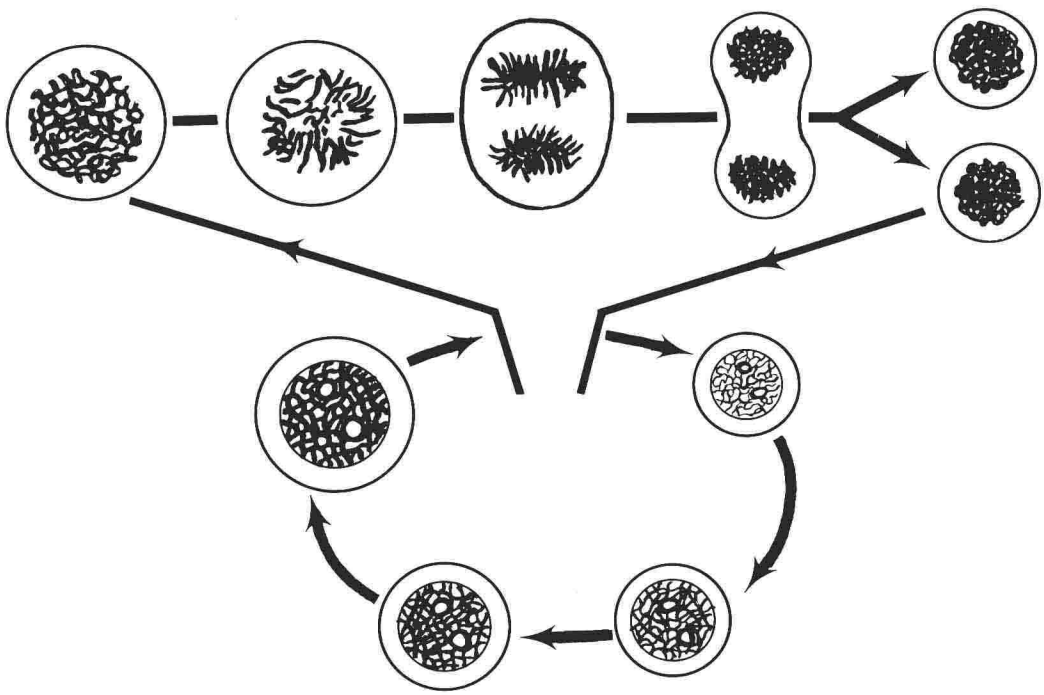


Fig 2—Mitotic cycle.

ation with other cells, be identified as blast cells of any given cell line are tallied and reported as "stem cells" (Table 1).

Each clone of definitive cells is given a family name. The suffix "blast" is reserved for the least-differentiated cells of each specific cell line. The suffix "cyte" is employed for all of the more-mature cells. The prefix "pro" is used for the second cell in the maturation sequence. Cells characterized by four maturation stages—such as myelocytic, rubricytic, and megakaryocytic series—are given the prefix "meta" for the fourth cell (Table 4).

The most-frequently employed synonyms for nucleated red cells in their various maturation stages are given in Table 5.

The terms "reticulum cell," "reticuloendothelial cell," and "histiocyte" are purposely avoided because there is lack of agreement concerning the morphologic characteristics of blood cells designated by these names.

Table 2—Bone Marrow Cells: Normal Adult Values

Stem cell	0-0.01%
Myeloblast	0-1
Promyelocyte	1-5
N. Myelocyte	2-10
N. Metamyelocyte	5-15
N. Band	10-40
N. Segmented	10-30
Eosinophil	0-3
Basophil	0-1
Lymphocyte	5-15
Plasmocyte	0-1
Monocyte	0-2
Other cells	0-1
Megakaryocyte	0.1-0.5
Rubriblast	0-1
Prorubricyte	1-4
Rubricyte	10-20
Metarubricyte	5-10
WBC:Nucleated RBC Ratio 4:1	

Table 3—Peripheral Blood Cells: Normal Adult Values

	Percent	Per mm³
N. Band	1-5	50-500
N. Segmented	50-70	2,500-7,000
Eosinophil	1-3	50-300
Basophil	0-1	0-100
Lymphocyte	20-40	1,000-4,000
Monocyte	1-6	50-600

Table 4—Nomenclature

PREFIX	SUFFIX	MYELOCYTIC SERIES	MONOCYTIC SERIES	MEGAKARYOCYTIC SERIES	ERYTHROCYTIC (RUBRICYTIC) SERIES	LYMPHOCYTIC SERIES	PLASMOCYTIC SERIES
	___blast	myeloblast	monoblast	megakaryoblast	rubriblast	lymphoblast	plasmoblast
pro___	___cyte	promyelocyte	promonocyte	promegakaryocyte	prorubricyte	prolymphocyte	proplasmocyte
	___cyte	myelocyte	monocyte	megakaryocyte	rubricyte	lymphocyte	plasmocyte
meta___	___cyte	metamyelocyte	_____	metamegakaryocyte	metarubricyte	_____	_____

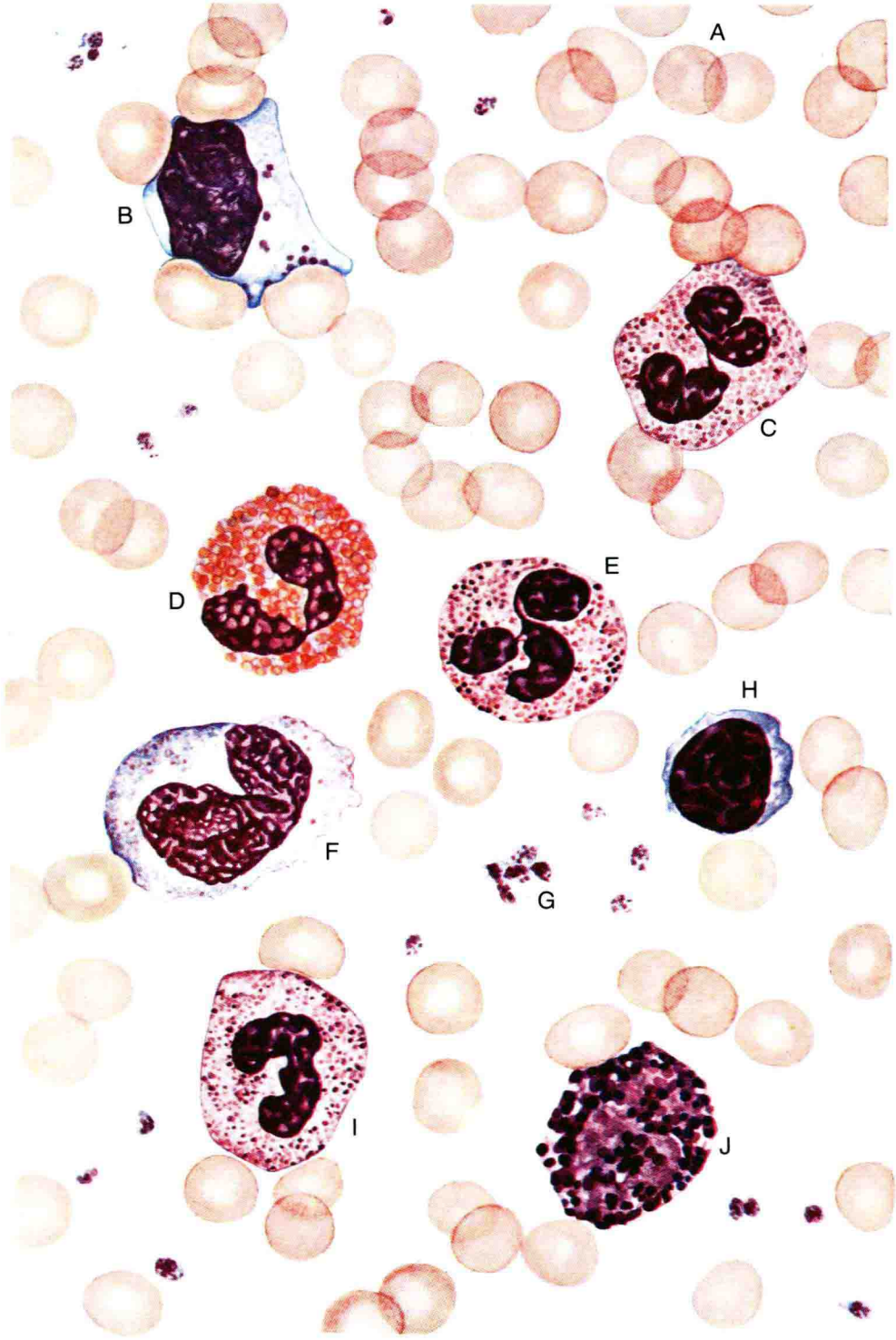


PLATE 2—CELL TYPES FOUND IN SMEARS OF PERIPHERAL BLOOD FROM NORMAL INDIVIDUALS

A Erythrocytes
B Large lymphocyte with purplish-red (azurophilic) granules and deeply indented by adjacent erythrocytes
C Neutrophilic segmented
D Eosinophil
E Neutrophilic segmented
F Monocyte with gray-blue cytoplasm, coarse linear chromatin, and blunt pseudopods
G Thrombocytes
H Lymphocyte
I Neutrophilic band
J Basophil

The arrangement is arbitrary, and the number of leukocytes in relation to erythrocytes and thrombocytes is greater than would occur in an actual microscopic field.

II LEUKOCYTES, ERYTHROCYTES, THROMBOCYTES

Neutrophilic Leukocytes

GRANULAR leukocytes (granulocytes) develop in the bone marrow from undifferentiated stem cells (Fig 20) and from precursor cells called myeloblasts. Maturation of the myelocytic series of cells is characterized by the development of dark and blue-staining primary granules which are later replaced by secondary granules that differ in their affinity for various dyes. Cells that have granules with an affinity for blue or basic dye are called basophils; those that are stained reddish-orange with the acid dye, eosin, are called eosinophils; and the cells with granules that do not stain intensely with either dye are called neutrophils. As the cells acquire mobility, the nuclei of the neutrophilic, eosinophilic, and basophilic systems of granular cells undergo progressive changes from round to multilobular forms designated respectively as myelocytes, metamyelocytes, band, and segmented forms (Plate 3).

Myeloblast. This cell varies in diameter from 15 to 20 μm. There is a moderate amount of bluish nongranular cytoplasm, which stains unevenly and is lighter next to the nucleus than at the periphery. Cytoplasmic tags are often demonstrable. The nucleus is round and stains predominantly red. The interlaced chromatin strands are delicate, well defined, and evenly stained. Two or more nucleoli are usually demonstrable (Plate 3).

Promyelocyte (Progranulocyte). A cell ceases to be a myeloblast and becomes a promyelocyte when it develops distinct granules (Plate 3). The earliest granules are dark-blue or reddish-blue. Most of the granules are round, but some may be elongated, curved, and irregular in shape. Granules may be visible above and below the relatively lightly stained and purple-red nucleus.

The nucleus is round or oval and relatively large in relation to the cytoplasm (Plate 3). The chromatin structure is slightly coarser, and the strands of chromatin are bluer and not as red as in the myeloblast. Nucleoli may be visible but are usually indistinct. The cytoplasm is blue with a relatively light zone adjacent to the nucleus. The cytoplasmic margins are smooth, and the cell does not indent nor is it compressed by neighboring cells. Since some early granulocytes are still capable of reproduction, the size may be quite variable, depending on the stage of a given cell in the mitotic cycle.

A promyelocyte becomes a myelocyte when the granules differentiate to such a degree that one can identify the granules as basophilic, eosinophilic, or neutrophilic.

Neutrophilic myelocyte. The first sign of neutrophilic differentiation or "dawn of neutrophilia" is the development of a small, relatively light island of ill-defined reddish granules adjacent to the nucleus (Plate 3, Plate 6). In older myelocytes, the dark granules become less prominent, and the neutrophilic granules predominate. Neutrophilic myelocytes are usually smaller than progranulocytes and have relatively larger amounts of cytoplasm. The nuclei are round, oval, or flattened on one side. The chromatin strands are unevenly stained and thickened. Nucleoli are indistinct.

Neutrophilic metamyelocyte (Juvenile). A neutrophilic metamyelocyte has a slightly indented nucleus and small, pinkish-blue granules. As a class, these cells are slightly smaller than myelocytes and have relatively smaller nuclei and less-well-defined chromatin structures (Plate 3, Plate 6). Neutrophilic metamyelocytes are rarely seen in normal peripheral blood but are often found in conditions in which there is myelocytic hyperplasia.

Neutrophilic band (N. nonsegmented, N. nonfilamented, N. staff or stab). As the neutrophilic metamyelocyte matures, the nuclear indentation becomes more marked until a stage is reached in which the indentation is greater than half the width of the hypothetical round nucleus. The opposite edges of the nucleus become approximately parallel for an appreciable distance giving a horseshoe appearance (Fig. 3). Neutrophilic bands are slightly smaller than metamyelocytes. The nucleus shows degenerative changes, and there is usually a dark pyknotic mass at each pole where the lobe is destined to be. The granules of band neutrophils are small and evenly distributed and stain various shades of pink and blue (Plate 3).

Neutrophilic band forms constitute from 1% to 5% of the leukocytes in the peripheral blood of healthy individuals. An increase in nonfilamented forms and other immature neutrophils is known as a "shift to the left" and is an indication of an abnormal response.

Neutrophilic segmented (N. filamented, N. polymorphonuclear, PMN, polymorphonuclear neutrophilic granulocyte). This cell differs from the neutrophilic band in that the nucleus is now separated into definite lobes with a very-narrow filament or strand connecting the lobes. The mature neutrophil is approximately twice the size of an erythrocyte. The cytoplasm in an ideal stain is light pink and the small, numerous, and evenly distributed granules have a light-pink to bluish-purple color (Plate 2, Plate 3).

Segmented neutrophils in the peripheral blood of older children and adults range from 50% to 70% with an average of 60%. On the average, 5% of the neutrophils have one lobe, 35% two lobes, 41% three lobes, 17% four lobes, and 2% five or more lobes. In pernicious anemia and related B_{12} and folic-acid deficiencies, there is an increase in hyperlobulated (six or more lobes) neutrophils (Plate 27, Plate 31).

The transition between the various stages of granulocytes is gradual. Many cells are borderline and difficult to distinguish from each other. The major difficulty is that of differentiating between band and segmenting forms and deciding whether the margins of the isthmus between two lobes are parallel and whether the connecting link is wide enough to be interpreted as a "band" or narrow enough to be identified as a "filament." A "band" is defined as a connecting strip or isthmus with parallel sides and wide enough to reveal two distinct margins with nuclear chromatin material visible between the margins. A "filament" is defined as a threadlike connection between two lobes so narrow that there is no visible chromatin between the two sides. In its most characteristic form, the shape of a "band" (nonsegmented, nonfilamented) nucleus is that of a bent stick, a horseshoe, or a curved link of sausage. Lobes of nuclei often touch or are superimposed so it is impossible to see connecting links. If the margin of a given lobe can be traced as a definite and continuing line from one side across the isthmus to the other side, it is assumed that a filament is present even though it is not visible (Fig 4). In differentiating between segmented (filamented) and band (nonsegmented, nonfilamented) nuclei, do not restrict evaluation to any single morphological characteristic but combine features including parallel sides and width of the connecting link, visibility of chromatin at the

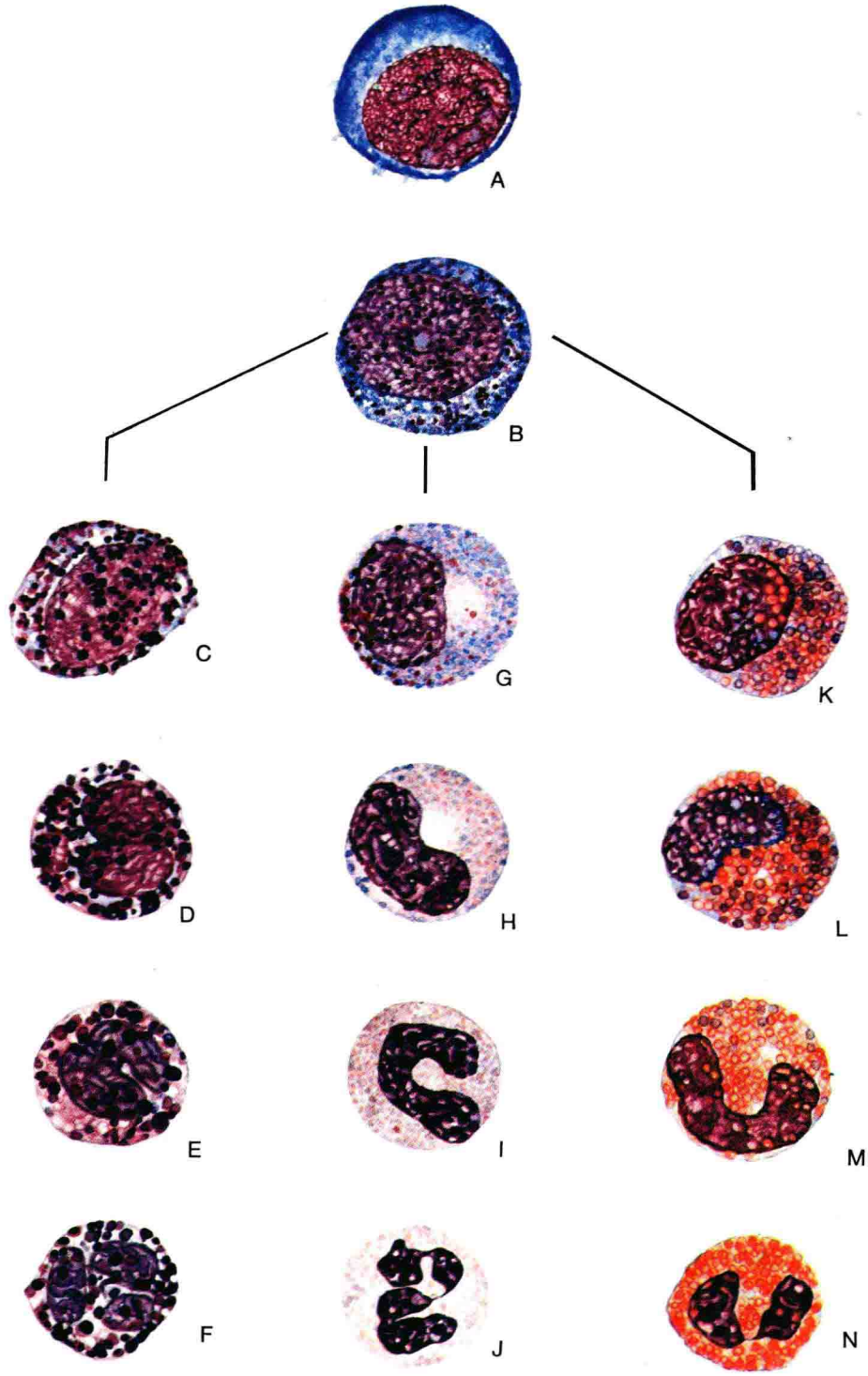


PLATE 3—MYELOCYTIC (GRANULOCYTIC) SYSTEM

A Myeloblast
B Promyelocyte (progranulocyte)

C Basophilic myelocyte
D Basophilic metamyelocyte
E Basophilic band
F Basophilic segmented

G Neutrophilic myelocyte
H Neutrophilic metamyelocyte
I Neutrophilic band
J Neutrophilic segmented

K Eosinophilic myelocyte
L Eosinophilic metamyelocyte
M Eosinophilic band
N Eosinophilic segmented

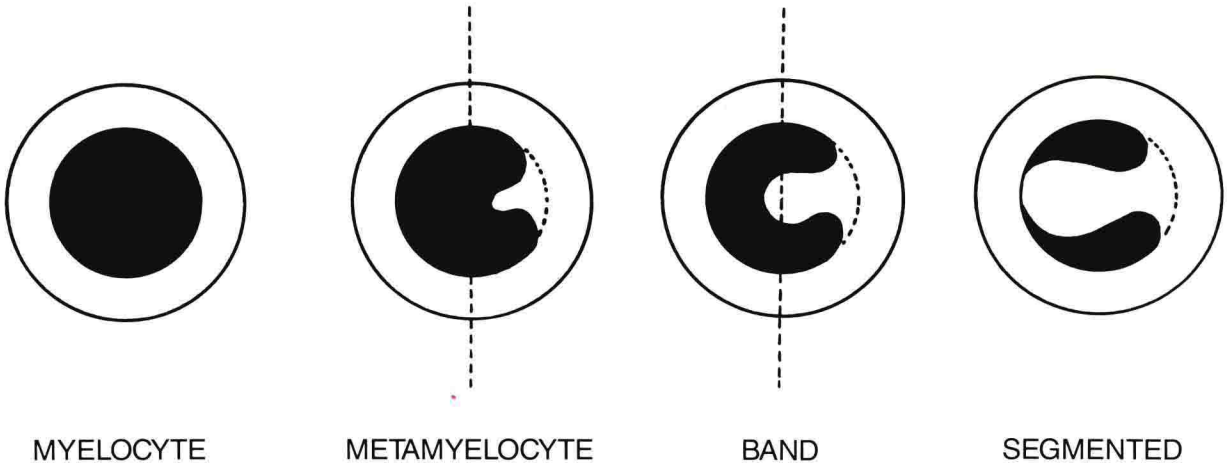


Fig 3—Terminology based on indentation of nuclei.

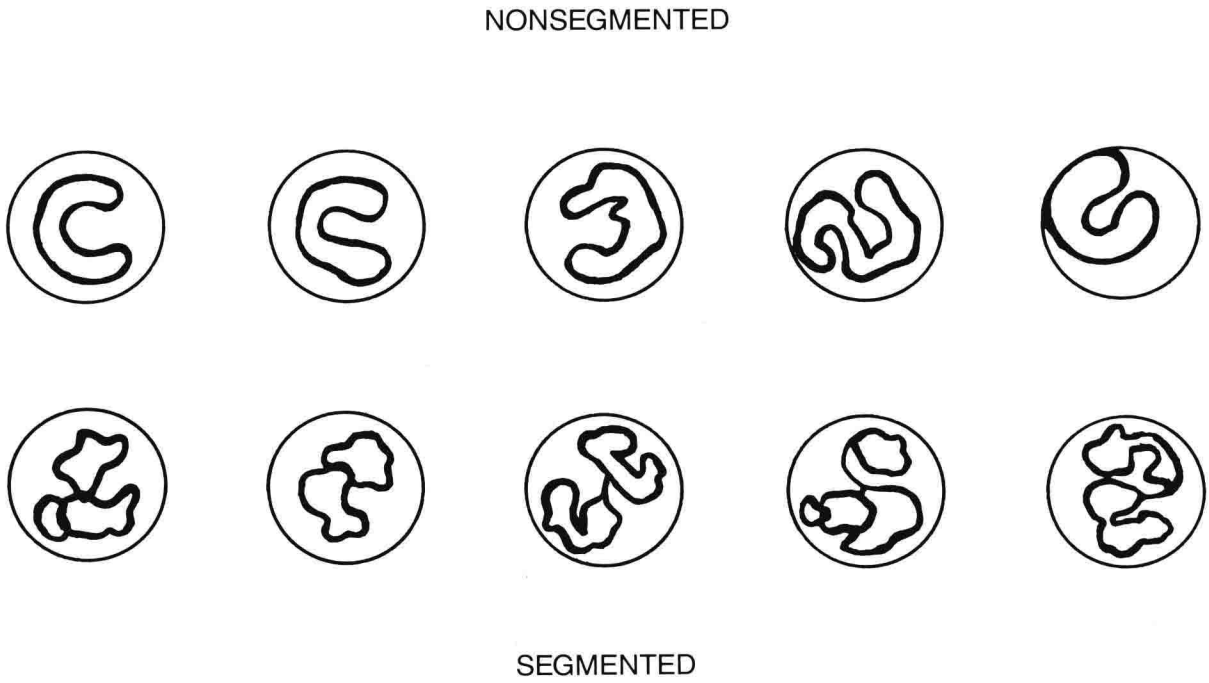


Fig 4—Neutrophil nonsegmented and segmented cells.

narrowest portion, and superimposition of lobes. In case of doubt, the rule is to place the questionable cell in the more-mature or segmented category most likely to be correct.

Morphological Abnormalities. Numerous structural abnormalities that are visible by light microscopy in the cytoplasm and nuclei of neutrophils are useful in the diagnosis and differential diagnosis of various diseases and conditions.

Hyperlobulated metamyelocytes and nonsegmented (band) neutrophils and segmented cells with five or more lobes are characteristic findings in smears of blood of patients with pernicious anemia and related B_{12}-folic acid deficiencies (Fig 5, Plate 31, Plate 27). As a rule, hyperlobulated and hypersegmented neutrophils are larger than normal cells. Hypersegmentation of giant neutrophils, as well as hypersegmentation of cells of normal size, may be inherited characteristics.

Hypolobulation of the nuclei of neutrophils and eosinophils is a characteristic feature of the inherited and benign condition known as "Pelger-Huët anomaly." In the homozygous form, the nuclei of most of the neutrophils are round. In the heterozygous condition, the nuclei of the majority of neutrophilic leukocytes have two round lobes which are closely approximated and connected by short filaments (pince-nez form). Other nuclei are round or have dumbbell or kidney shapes (Plate 33). The nuclear chromatin and granules of hypolobulated neutrophils have normal features. Occasionally, asynchronous Pelger-like (pseudo-Pelger) hypolobulated cells may be demonstrable in small numbers in smears of blood and bone marrow of patients with myelocytic leukemia, severe infections, or other diseases.

Prominent purplish and blue-black granules, designated as "toxic granules," often are demonstrable in the cytoplasm of neutrophils in association with severe infections and other toxic states. Toxic granules vary in size, shape, and distribution as well as in color (Plate 31). Morphologically, toxic granules resemble the primary granules of promyelocytes and early neutrophilic myelocytes. Toxic granules are manifestations of asynchronism between the maturation of nuclei and lysosomes.

Döhle bodies (Amato bodies) are inconspicuous pale sky-blue cytoplasmic inclusions. They most frequently are demonstrable in band and segmented neutrophils. Based on electron microscopic evidence, the cerulean-blue areas are residual aggregates of rough endoplasmic reticulum. Döhle bodies vary in number, distribution, and size. Some have round or oval shapes, while others appear as blue splotches with nebulous extensions. Focal areas of basophilia often are demonstrable in leukocytes that also contain "toxic granules." Döhle bodies may be demonstrable in numerous infectious diseases, as well as burns, toxemia of pregnancy, diseases due to exposure to cytotoxic chemicals, plant and animal poisons, and the hereditary May-Hegglin anomaly (Plate 36).

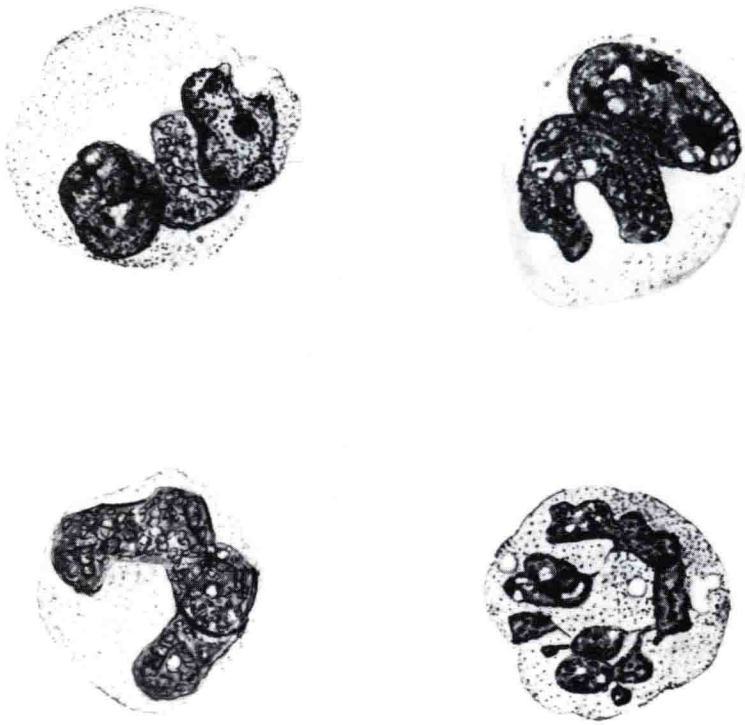


Fig 5—Hyperlobulated macroneutrophils, pernicious anemia.

The cytoplasm of neutrophils as well as other cells of patients with Alder's (Alder-Reilly) anomaly contains multiple dark-blue round or oval granules (Plate 34).

The lysosomes in the cytoplasm of granulocytes in patients with Chédiak-Higashi syndrome are grossly enlarged and vary in color from blue-black to light red and pink (Plate 35).

Auer bodies (Auer rods) are elongated narrow purplish-red structures of uncertain nature that are demonstrable in the cytoplasm of myeloblasts and monoblasts and, in rare instances, in more-mature granulocytic cells. The number in a given cell varies, but, as a rule, there is only one (Plate 40). They reveal positive reactions when peroxidase, Sudan black, and acid phosphatase stains are used. Auer rods are not demonstrable in cells of the lymphocytic, plasmocytic, erythrocytic, and megakaryocytic systems.

Spherical chromophobic areas (vacuoles) in the cytoplasm or in nuclei are manifestations of degenerative changes (Plate 31).

Lupus erythematosus is an auto-immune disease characterized by the presence of antinuclear antibodies. When aspirates of bone marrow or blood are allowed to stand outside of the body before the preparation and staining of smears, some of the nuclei in some of the leukocytes undergo lytic changes (pre-LE cells) (Plate 32). Motile neutrophils are attracted to the cells that have undergone degenerative changes. These neutrophils form clusters or rosettes around the lysed cells (Plate 32). Viable neutrophils phagocytize nuclear material, producing within the digestive vacuoles of their cytoplasm round or oval inclusions which have a fairly homogeneous structure and stain purplish-red (Plate 32). Neutrophils that contain inclusions that are reddish-purple and structureless are called LE cells. Neutrophils that have engulfed lysed nuclear masses may themselves undergo degenerative changes producing post-LE cells (Plate 32). Morphological changes of the types described above are not specific for lupus erythematosus. The final diagnosis should be based on combined clinical manifestations and other laboratory tests.

Structureless red-staining cytoplasmic inclusions of the type demonstrable in stained smears of bone marrow and body fluids in patients with lupus erythematosus are to be differentiated from linear and/or lumpy nuclei that stain dark blue and that are contained in the cytoplasm of monocytes (Plate 31). Monocytes that have ingested nonlysed nuclei are designated as "Tart cells," so named because they resemble the cells that were observed and reported in smears of bone marrow of Mr. Tart who was a patient at the Mayo Clinic.

Eosinophils

EOSINOPHILS are characterized by relatively large, spherical granules that have a particular affinity for the acid eosin stain. The earliest recognizable eosinophils have a few dark and bluish primary granules intermingled with the secondary and specific red granules. As the eosinophils pass through their various developmental stages, the bluish granules, characteristic of the progranulocyte and the early myelocyte stages, disappear (Plate 3). Because the percentage of eosinophils is usually low in bone marrow and 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 in which the eosinophils are greatly increased, an analysis of the incidence of the various stages is indicated.

The eosinophils as seen in normal peripheral blood smears are about the size of neutrophils, and usually have band or two-lobed nuclei. The granules are spherical and uniform in size. They usually are evenly distributed in the cell and fill it. Rarely do they overlie the nucleus (Plate 2, Plate 3). In good stains, the granules take a bright reddish-orange stain with brownish tints. On focusing up and down, one can bring out highlights on individual granules or reveal them as little circles. Often this spherical shape permits identification of the cell when the stain is unsatisfactory. (Eosinophils can be identified readily in moist preparations without the use of stains, because the granules are distinct, round, relatively large, and of a brownish color.) Often it is difficult to distinguish eosinophils from neutrophils when the granules of the neutrophil are prominent and stain darkly. In case of doubt, the questionable cell should be called a neutrophil. By moving to the thin part of the field (with brighter illumination)—and paying attention to the size, uniformity, and shape of the granules rather than to the color alone—one can often make distinctions that otherwise would be guesswork.

Eosinophils, like neutrophils and basophils, are derived from progenitor myeloblasts and myelocytes (Plate 3, Plate 54).

Eosinophils constitute from 1% to 3% of leukocytes in smears of peripheral blood of normal individuals. There is a relative and absolute eosinophilia in association with the invasion, migration, and encystment of animal (metazoa) parasites. Other diseases and conditions characterized by an increase in eosinophils include bronchial asthma, hay fever, Löffler's syndrome, extensive skin lesions, recovery from bacterial and other infections, chronic myelocytic and eosinophilic leukemia, and some cases of neoplastic malignancy.

Eosinopenia occurs in conditions of stress, such as shock, severe burns, and severe infections. The absence of eosinophils in blood in surgical conditions is an unfavorable omen and the presence of—or an increase in—eosinophils is a favorable sign. Eosinophils are decreased by the administration of adrenal corticosteroids.

Eosinophilic leukocytes are motile cells that are infrequently phagocytic. They leave the blood and enter tissue spaces at the sites of antigen-antibody contacts. It is thought that the histamine liberated from basophils during the initial stage of an allergic reaction serves as a chemotactic factor. Eosinophils aid in the restraint and control of inflammatory reactions. They also have cytotoxic properties against invading animal parasites.

Basophilic Leukocytes (Basophils, Basophilic Granulocytes)

BLOOD BASOPHILS which are derived from myeloblasts and progranulocytes have round, indented, band, or segmented nuclei (Plate 2, Plate 3, Plate 54, Fig 6). Based on the shape of their nuclei, they may be classified as basophilic myelocytes, metamyelocytes, and band and segmented forms, but the cells are so few in peripheral blood and bone marrow smears that there is no clinical advantage in placing the cells in separate categories.

Blood basophils in all stages of their maturation are smaller than promyelocytes, and their size is approximately the same as that of neutrophils in the same or closely adjacent microscopic fields (Fig 6). The margins are smooth, and the shape is round or oval. Nuclei, in contrast to the darkly stained granules, are inconspicuous. The color of the nuclei is purple-red.

Basophilic granules vary in size from 0.2 to 1.0 μm. Most of the granules are round. They are numerous and unevenly distributed. The color of the granules varies from deep purplish-blue to dark purple-red. Due to intense staining, the granules stand out in sharp contrast to the light purplish color of the cytoplasm. The darkly stained granules are visible above and below the lightly stained nuclei.

The basophilic granules are water soluble. In smears, imprints, or sections of bone marrow that are exposed to moisture before being fixed or that are poorly fixed during the staining process, the granules disappear, leaving small, round, and colorless cytoplasmic areas.

Granules of the type present in basophilic leukocytes have a love or an affinity for basic dyes. When stained by a single blue dye such as toluidine blue; azure A, B, or C; or other blue and basic dyes, the granules, instead of revealing the blue color of the dye that was used, are red (metachromasia). The Wright stain contains two dyes: the red dye, eosin, and the blue dye, methylene blue. The granules of basophilic leukocytes, when stained by the Wright method, reveal varying shades of red and blue. The predominant color is blue.

The granules of basophilic leukocytes yield negative or very weak peroxidase, Sudan black, and alkaline phosphatase reactions. Some basophils react positively when periodic acid Schiff (PAS) stain is used.

Basophils differ from neutrophils and monocytes in that they are not phagocytic. The granules in basophils are membrane-bound sacks containing secretory products including heparin, histamine, and other chemicals. They are not lysosomes that contain digestive enzymes. When properly stimulated, the granules present in basophilic leukocytes eject the chemicals contained in their storage areas (exocytosis).

The number of leukocytes with basophilic granules in smears of peripheral blood or bone marrow of normal individuals is less than one per 100 nucleated cells.

The most striking relative and absolute increase in basophilic leukocytes in the peripheral blood as well as in the bone marrow occurs as a manifestation of basophilic (mast cell) leukemia. There is a relative and absolute, but less-marked, increase in the number of basophils in chronic myelocytic leukemia and in myeloblastic crisis.

Since basophils in blood and bone marrow smears are present in such small numbers, the failure to find these cells during a differential count of several hundred cells is without clinical significance.

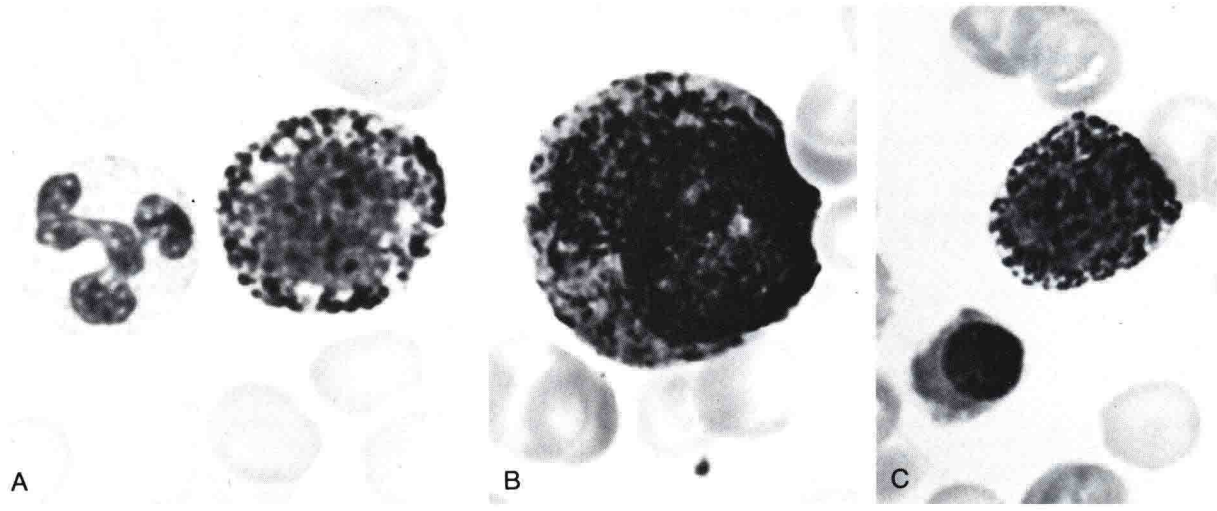


Fig 6—A Segmented neutrophil, basophil
B Promyelocyte
C Nucleated red cell, basophil

Monocytes (Large Mononuclears)

MONOCYTES are phagocytic leukocytes of the blood which, together with tissue macrophages and neutrophilic leukocytes, play a major role as a first line of defense against pathogenic organisms and nonself or foreign cells.

In smears of peripheral blood from healthy individuals, monocytes usually range from 1% to 6%. In smears of bone marrow from persons in good health, monocytes usually constitute less than 2%.

Monocytes as a class are slightly larger than neutrophils, and their diameters are three to four times those of erythrocytes in the same microscopic fields (Plate 2). Generally, there is a large amount of cytoplasm in relation to the nucleus.

The shape of monocytes is variable (Plate 4). Many are round or oval. Others reveal blunt pseudopods which are manifestations of their slow motility. These ameboid and aggressive cells continue to move while the blood film is drying and become fixed before there is time to retract their cytoplasmic extensions. The pseudopods vary in size and in number. The outer portion of the outstretched cytoplasm (ectoplasm) often has a transparent or hyaline appearance as contrasted with the granular inner cytoplasm (endoplasm).

The cytoplasm in the Wright-stained smear is dull gray-blue as contrasted with the color of the cytoplasm of neutrophils in adjacent fields which is less-intensely stained and is pink.

The granules of monocytes are usually fine, lightly stained, numerous, and evenly distributed, giving to the cells a ground-glass appearance. In other cells, there may be, in addition to the small granules, varying numbers of prominent granules. Vacuoles are often prominent in the cytoplasm. Phagocytized erythrocytes, leukocytes, nuclei, cell fragments, pigment, bacteria, and fungi may be demonstrable in digestive vacuoles.

The nucleus of the monocyte is usually round or kidney-shaped, but may be deeply indented or have two or more lobes separated by narrow filaments. One of the most distinctive and diagnostic features of the monocyte is the presence of brainlike convolutions. Another feature of the nucleus of value in identification is the tendency of the nuclear chromatin to be loose with light spaces in between the chromatin strands, giving a coarse, linear pattern in contrast to the lymphocyte with its clumped chromatin.

Monocytes are derived from stem cells in the bone marrow. As these cells grow, they are transformed into macrophages too large to pass readily through capillaries. Extremely large mononuclear phagocytes are seldom seen in blood smears but are demonstrable in body fluids other than blood. Some of the macrophages become anchored in connective tissues where they are entrapped by reticular and collagen fibers. The smaller monocytes, the larger wandering macrophages, and the semifixed or fixed phagocytes are thought to be capable of reversible transformation from one to the other.

Promonocytes and Monoblasts are not identifiable as such in bone marrow or peripheral blood smears except in conditions in which there is marked proliferation of cells of the monocytic type as in monocytic leukemia. The identification of early mononuclear cells is based on the indented and folded nuclei and the association with more mature monocytes having blunt pseudopods, vacuoles, and phagocytic particles in the cytoplasm. The cells in monocytic leukemia are called monoblasts when the nuclear chromatin is fine and distinct, nucleoli are demonstrable, and there are no granules in the cytoplasm (Plate 41).

Monocytes vs Neutrophils vs Large Lymphocytes. Monocytes are the cells of the peripheral blood most difficult to identify and to differentiate from other cells (Plate 6). They are frequently mistaken for neutrophils, for they may have prominent granules and lobulated nuclei. The monocyte often is mistaken for a large lymphocyte, because its cytoplasm is blue, or because the granules may be indistinct, the nucleus round or only slightly indented, the linear chromatin pattern ill defined, and the distinctive blunt pseudopods and digestive vacuoles missing.

The three most characteristic features of the monocyte and the most helpful in diagnosis are the dull gray-blue color of the cytoplasm, the blunt pseudopods, and the brainlike convolutions of the nucleus.

The color of the cytoplasm must not be compared with a textbook picture or hypothetical ideal cells, but with known neutrophils in the same or adjacent fields. The cytoplasm of a neutrophil is relatively light and reddish in comparison with the cytoplasm of the monocyte which is relatively dark, more bluish, and more opaque. Neutrophils practically never have blunt pseudopods.

To distinguish monocytes from large lymphocytes, the nuclear structure, the character of the cytoplasm, and the shape of the cells are most useful. The nucleus of a lymphocyte tends to be clumped, rather than linear (Plate 6). There is a greater tendency for the nuclear chromatin to be condensed at the periphery in the lymphocyte. Brainlike convolutions are not present in the lymphocyte. Monocytes and large lymphocytes may have distinct bluish-red granules (lysosomes). In addition, the cytoplasm of the monocyte has a finely granular or ground-glass appearance, whereas the cytoplasm of the lymphocyte has a background that is nongranular.

Monocytes tend to indent and to compress adjacent cells rather than to be indented by them. Large lymphocytes, on the other hand, are often deeply indented by neighboring cells (Plate 2, Plate 5).

Morphological Abnormalities. Extremely large monocytes (macrophages) in smears of peripheral blood may be demonstrable in patients with subacute bacterial endocarditis and other chronic infectious states. These large cells, some of which may contain phagocytized particulate matter, are most likely to be found at the feather edge of smears made from blood of the ear rather than from other sites.

In its cytoplasm, a monocyte may contain intact and hemoglobin-containing red cells, red-cell fragments, and hemosiderin granules, as well as phagocytized leukocytes and leukocyte fragments.

Alveolar monophagocytes ingest and degrade pathogenic organisms. They also phagocytize particles containing inspired air and serve as a means of transportation of pollutants by ciliated epithelial cells lining the respiratory tract.

Phagocytic cells of varying size and mobility remove from the circulating blood injured and dead cells, cell fragments, microorganisms, and insoluble particles. Motile monophagocytes escaping between epithelial lining cells of the upper and lower respiratory tracts and the gastrointestinal and genitourinary organs perform a scavenger function, clearing the body of insoluble and unneeded debris.

An important function of micromonocytes as well as macromonocytes (macrophages) is to ingest and kill pathogenic organisms and to ingest and degrade noxious external agents. Monophagocytes also play a role in phagocytizing old and degenerated cells and cell fragments, malignant cells, and cells that spontaneously undergo mutations in the body.