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THE WHITE CELL

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Preface

At the time I initiated work on *The White Cell*, I did not fully appreciate the problems of critically reviewing and describing the physiology of the normal leukocyte and its abnormal variants. The difficulties in writing a comprehensive summary were soon apparent. The leukocyte is not a single cell type, but three major classes of cells—granulocytes, lymphoid cells, and mononuclear phagocytes. In addition, there are several distinct subpopulations of lymphoid cells and mononuclear phagocytes. Each of these classes and subclasses has a variety of forms at different levels of maturation. Not surprisingly, each normal subclass and each maturational subset has its characteristic morphology, metabolism, and functions. For each of these normal cells there is an abnormal counterpart occurring in various disease states. The consequence is a vast body of literature on normal and abnormal leukocytes.

The problems of selectively summarizing this literature are compounded by the number of fields of basic science embraced by studies of leukocytes: general cell biology, microbiology, immunology, viral oncology, and cell and tissue kinetics. Some of these areas are changing rapidly so that each new issue of the relevant scientific journals brings important new information and sometimes new insight. Consequently, any survey of the white cell that aims at an understanding of cell form and function is destined to be outdated on the day of publication.

The many basic science disciplines related to the white cell present still another challenge—how much material does the reviewer offer in order to clarify cell physiology without writing a textbook of immunology or of cell kinetics? The answer, I would hope, is a compromise between a superficial treatment and a set of encyclopedias.

My purpose in writing this text was to review critically the existing body of knowledge of normal and abnormal leukocyte physiology in order to present the interested reader with a comprehensive bibliography and source book of information on the leukocyte. I have aimed at a sophisticated audience: primarily the medical student, but also the pupil of cell biology and the postgraduate physician concerned with hematology. I have tried to present concepts of leukocyte physiology in a manner that reveals their experimental basis. In pursuing this goal, I have given due consideration to experimentalists who first enunciated certain concepts or made critical observations; for the history of development of a body of scientific information is often as informative as its present status. Consequently, many references to the older as well as the more recent literature have been included. Because the objective was a comprehensive source book of information, over 6,000 articles were reviewed and cited. I have included a number of discussions of immunologic phenomena and general cell biology in order to promote an understanding of leukocyte physiology and function. These areas have been organized for the student of hematology as the principal reader.

To accomplish my objectives, I have arranged the text in the following manner. Three main divisions correspond to the three major types of leukocytes. The interrela-

tionships among these cell types are defined near the beginning of each of these divisions. Each type of leukocyte is discussed in the following sequence. First, *the normal cell:* development and structure, production, lifespan and distribution, metabolism, and function; then *the abnormal cell:* abnormalities of morphogenesis, production, metabolism, and function. Finally, disease states illustrating each of these abnormalities are discussed.

Despite the difficulties, writing *The White Cell* has been a rewarding experience. It has offered a unique opportunity to reexamine a body of information and to ascertain that which is firmly based on a solid experimental foundation. For the finished work I am grateful to many: to Dr. T. Hale Ham, who suggested the task and followed its development with frequent encouragement; to Emmaline Stump, who thoughtfully edited the text; to Gwen Dangerfield for her aid in preparing the manuscript; to Dr. David Golde, Dr. Robert Lehrer, and many other colleagues, who offered specific suggestions and general intellectual stimulation; and to Dr. Maxwell M. Wintrobe, who has long provided a model for dedicated scholarship.

I gratefully acknowledge a grant from the Commonwealth Fund that permitted me to initiate the work, and also support from the United States Public Health Service (Grants CA 11067 and CA 12822), the American Cancer Society (Grant CI-60), and various funds of the Cancer Research Institute of the University of California in San Francisco.

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Introduction

The Relation of Leukocytes to Other Hematopoietic Cells

The origin of mammalian blood cells can be traced to the blood islands of the fetal yolk sac mesoderm. In the early embryo this organ does not ordinarily contain cells morphologically identifiable as the leukocytes that ultimately circulate in the blood of the adult animal. Rather, it contains progenitor cells capable of giving rise to leukocytes and other hematopoietic cell lines during later embryonic and postnatal development. From the yolk sac, streams of precursor cells migrate to colonize the hematopoietic organs of fetal life: the liver, the spleen, and ultimately the bone marrow. Lymphoid precursors may also migrate from the yolk sac to the epithelial thymic rudiment and thus establish the earliest lymphoid organ. The particular pattern of colonization from the yolk sac differs, of course, among different species.

The migrating hematopoietic progenitors of the early embryo are often referred to as *stem cells.* A hematopoietic stem cell may be defined as a cell with two capabilities: (*a*) the ability to divide and give rise to daughter cells having the same capabilities as the parent; that is, the cell is self-renewing; (*b*) the ability to differentiate into more mature hematologic cells. A stem cell may be either *pluripotent* and capable of giving rise to cells of several hematopoietic lines, or *unipotent* with maturation capabilities along a single line. Both types of stem cells exist in higher animals.

Evidence supporting the existence of pluripotent and unipotent stem cells will be summarized in Chapter 2; however, at the outset it is important to understand that all blood cells are thought to be related in their origins from a pluripotent precursor. The relation between any two types of blood cells may be close or distant, depending upon their point of origin along the branching pathway of potential hematopoietic cell development. This concept is illustrated in Fig. I.1.

The decision for a pluripotent stem cell to develop along one or another potential line appears to be governed directly by local environmental factors operative within a hematopoietic organ, as well as indirectly by specific "cytopoietic" hormones such as erythropoietin. The local microenvironmental factors are poorly understood at present, but almost certainly involve hematopoietic cell interactions that operate over a very short distance. The systemic cytopoietic hormones may be generated by remote organs, which sense the requirements for a given cell line and elaborate a hormonal signal that is distributed systemically.

Leukocyte Functions and Interactions

As indicated earlier, the leukocyte populations of the body are generally considered to be comprised of the granulocytic, lymphoid, and mononuclear phagocyte cell lines. From evidence to be summarized, one can conclude that the origins of granulocytes and monocytes may be more closely related to those of red cells and megakaryocyte precursors than to the origins of lymphoid precursors. For example, certain chromosomal abnormalities may be shared by erythrocyte, megakaryocyte, granulo-

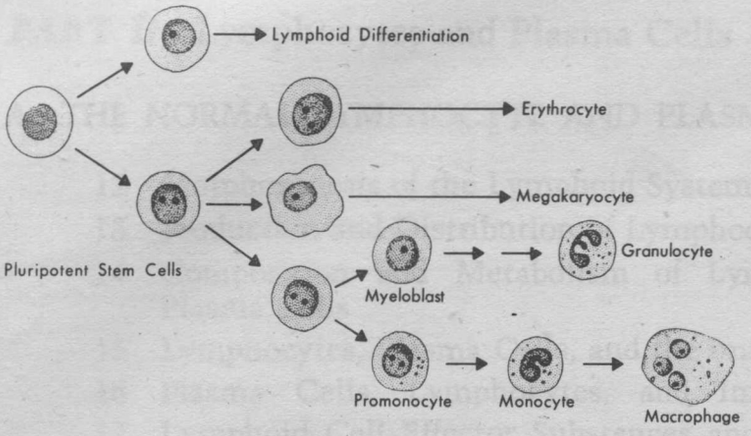

Figure I.1 Schematic representation of the relation of the major hematopoietic cell lines to a pluripotent stem cell. Erthrocytes, megakaryocytes, granulocytes, and macrophages arise from a common pluripotent stem cell different from the stem cell that gives rise to the lymphoid pathway of differentiation. However, both the lymphoid pathway and other hematopoietic cell lines are also linked to a common, more primitive progenitor cell.

cyte, and monocyte-macrophage precursors and are absent in the lymphoid series. Nevertheless, all types of leukocytes seem to have evolved for a common purpose and to function cooperatively in meeting the body's needs. Leukocytes may be regarded as the major defense against foreign invaders. *Foreignness* may be broadly applied to an invading microorganism, a multicellular parasite, an antibody-coated erythrocyte, an exogenously administered protein, or a malignant cell with surface anitgens not native to the host. The multiple aspects of the cooperation of different types of leukocytes in the performance of their defense function will be a recurrent theme in later chapters of this book. At this point, however, an illustration of leukocyte interactions may be helpful.

Let an injection of the facultative intracellular microorganism *Listeria monocytogenes* into an experimental mammal constitute the foreign invasion. Phagocytic neutrophilic granulocytes are the first line of defense, serving to immobilize, phagocytize, and kill a fraction of the inoculated organisms. Should this first line be overwhelmed by proliferating, virulent, intracellular bacteria, mononuclear phagocytes—including monocytes and tissue macrophages—ingest the released organisms and thereby contain their proliferation and spread. With the passage of time, the lymphoid system is also called into play. B lymphocytes and their progeny elaborate immunoglobulin antibody which, together with complement, "opsonizes" the invaders so that the coated bacteria attach to immunoglobulin and complement receptors on the surface of the phagocytic leukocytes. T and perhaps B lymphocytes elaborate a variety of effector substances that help to activate and direct the distribution of mononuclear phagocytes. Contrariwise, the phagocytes may localize antigen and present it to lymphocytes in a manner that facilitates their immunologic response.

During the course of infection the mononuclear phagocytes and perhaps other leukocytes may release materials that signal the bone marrow to produce more phagocytic cells; that is, the mature cells may be involved in the feedback control of leukopoiesis. Thus monocytes may influence granulopoiesis and mononuelear phagocyte production as part of a prolonged response to a foreign invader.

The leukocyte response to a somatic cell that has undergone malignant transformation appears to be fundamentally similar to the response to a foreign microorganism. The order of appearance of the leukocyte protagonists in the drama and their relative roles may differ, however, in the two situations.

From the above brief sketch it should be apparent that leukocytes possess two major defense mechanisms: phagocytosis and the elaboration of various effector substances, including immunoglobulin. Mature neutrophilic granulocytes and monocytes, and tissue macrophages, are the chief phagocytic cells. Historically, cells in the lymphoid series have been thought to be the major source of effector substances operative in defense reactions. Recently, however, it has become increasingly clear that granulocytes, including eosinophils and basophils, as well as mononuclear phagocytes may release a variety of cellular constituents that operate in defense and inflammatory reactions. These constituents may be beneficial to the host if they localize and destroy foreign invaders, or may sometimes be harmful if the inflammatory response is inappropriate or excessive.

The leukocytes thus may be considered as a widely dispersed organ comprised of three major cell types related in their embryonic origins. Most constituents of this organ are in a state of constant turnover, with cell loss and death being compensated by cell proliferation. Properly functioning, this organ serves its major purpose of defense against external invaders or internal neoplastic change. Abnormalities in organ function, however, may result in serious infection, neoplasia, or excessive inflammatory reactions that are detrimental to the host.

PART I Granulocytes

A The Normal Granulocyte

Chapter 1 Morphology and Morphogenesis of the Granulocytic Series

The granulocytic series encompasses three morphologically distinct cell lines: the neutrophil, the eosinophil, and the basophil. The cells of each series are distinguished by their cytoplasmic granules and certain other morphologic features. Examples of the mature cells of each of these lines are shown in Fig. 1.1. Occasionally the terms *granulocyte* and *neutrophil* are used synonymously. However, granulocyte generally refers to all three of the cell lines and neutrophil to one distinct line.

Controversy exists regarding the cell of origin for the three granulocytic cell lines. The traditional view, evolved at the end of the last century and still held by many hematologists, is that a primitive granule-containing precursor cell, the *promyelocyte*, is common to all three cell lines. The traditionally defined promyelocyte contains a single species of "nonspecific" azurophil granule which, with cellular maturation, differentiates into a "specific" granule characteristic of one of the cell lines. Recent evidence, based on leukocyte fine structure and cytochemical reactions, challenges this view. It suggests that *the earliest cells in which granule development can be detected are already differentiated into a particular series, either neutrophilic, eosinophilic, or basophilic.*

The modern view of granulocyte development, outlined in Fig. 1.2, is that once progenitor cells mature sufficiently to have cytoplasmic granules, they are already committed to one of the three pathways of differentiation. Recent evidence also suggests that the earliest azurophil granules are cytochemically distinct for each cell line and persist in the more mature cells of that line (11, 12, 76, 77, 92, 95). The origins of this modern concept of granulocyte morphogenesis can be found as early as 1918 (41).

Nomenclature and Morphology of Developing Granulocytes

The major morphologic techniques applied to the study of granulocyte differentiation, in order of historical development, are (*a*) observation of living cells in a transparent suspending medium; (*b*) staining of fixed or dried specimens of blood or bone marrow; (*c*) cytochemical methods; and (*d*) electron microscopy, first of dried or fixed specimens and later of thin sections of cells. These techniques have been refined over a period of time (95). Studies, by light microscopy, of fixed leukocytes stained with Romanovsky dyes have had the greatest influence on the concepts and nomenclature of cellular morphogenesis.

Studies Utilizing Light Microscopy

Since the light microscope is still the most common tool used by the hematologist to study granulocytes, we shall consider the developmental stages of the cell that can be identified with this instrument. The commonly used nomenclature is related to these identifiable stages (34, 43, 62).

Based on morphologic criteria applied to cells stained with Romanovsky dyes, a stepwise series delineating progressively more mature forms of the neutrophilic series

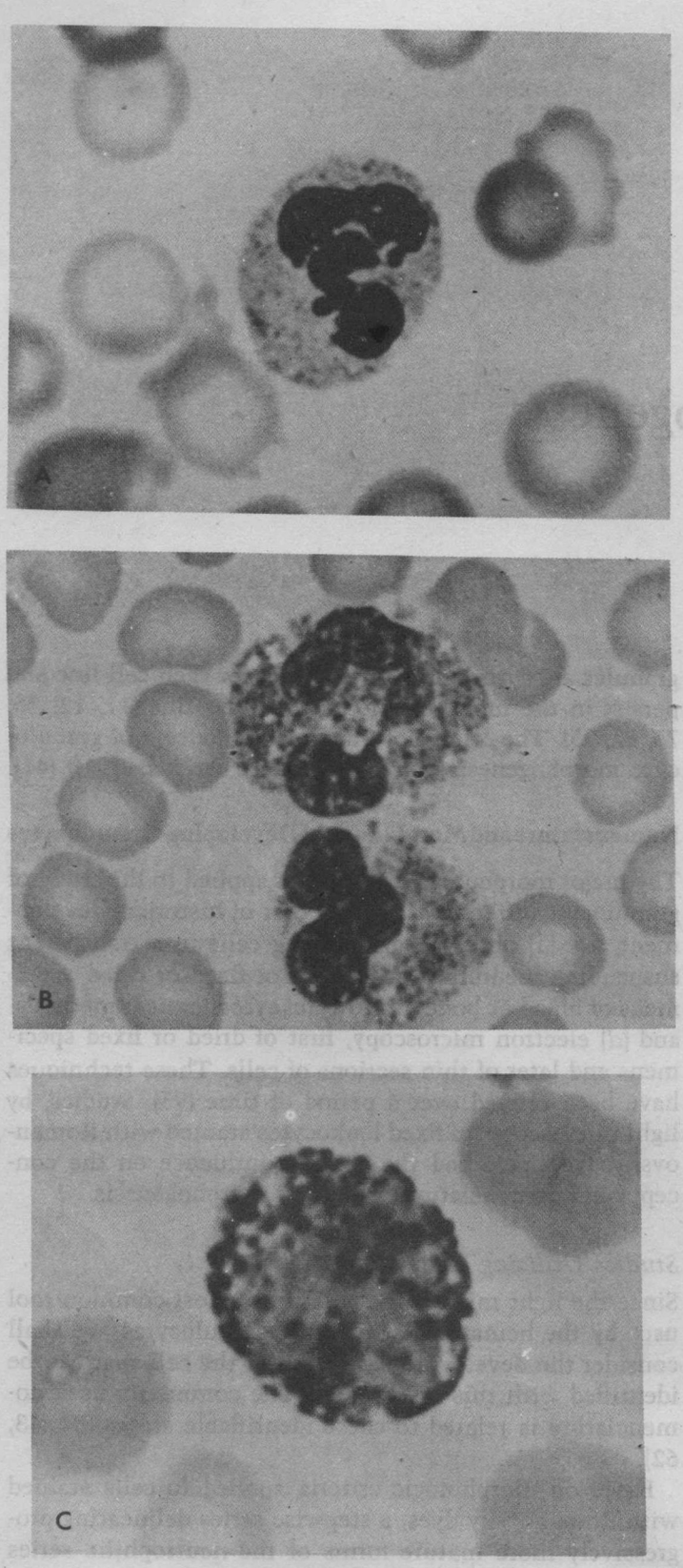

Figure 1.1 Mature granulocytes from human blood; Giemsa stain.

A. Polymorphonuclear leukocyte
B. Eosinophil
C. Basophil

has been identified by means of light microscopy. The maturational process in vivo, however, is continuous rather than stepwise. The clinical hematologist recognized the following sequence of progressively more mature cells, as shown in Fig. 1.3: *myeloblast* (*granuloblast*) → *promyelocyte* (*progranulocyte*) → *myelocyte* → *metamyelocyte* → *band form* (*stab*) → *mature neutrophil* (*polymorphonuclear or PMN leukocyte*). Similar sequences exist for the development of cells in the eosinophilic and basophilic series and will be described in Chapters 6 and 7.

The general indexes used to identify the different levels of maturation of Romanovsky-stained cells are cell size granule number and appearance, and nuclear morphology.

From the promyelocyte stage the cell progressively decreases in size as it matures. Large and prominent granules first appear at the promyelocyte stage, becoming less prominent with further maturation. In the myeloblast and promyelocyte the large round or oval nucleus has a relatively loose chromatin structure and the nucleolus, (or nucleoli) is prominent. Nuclear indentation and loss of the nucleoli are characteristics of intermediate-stage cells such as myelocytes and metamyelocytes. Nuclear elongation and lobation and increased condensation (density) define the mature granulocyte.

Briefly, the characteristics of the cells of this series are as follows.

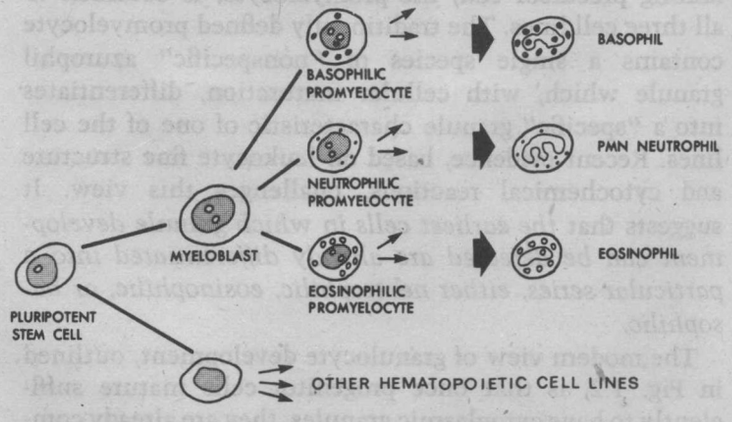

Figure 1.2 Scheme for the development of the three major granulocytic cell lines and other hematopoietic cell lines from a common pluripotent stem cell. Granulocyte differentiation occurs at the promyelocyte stage of development.

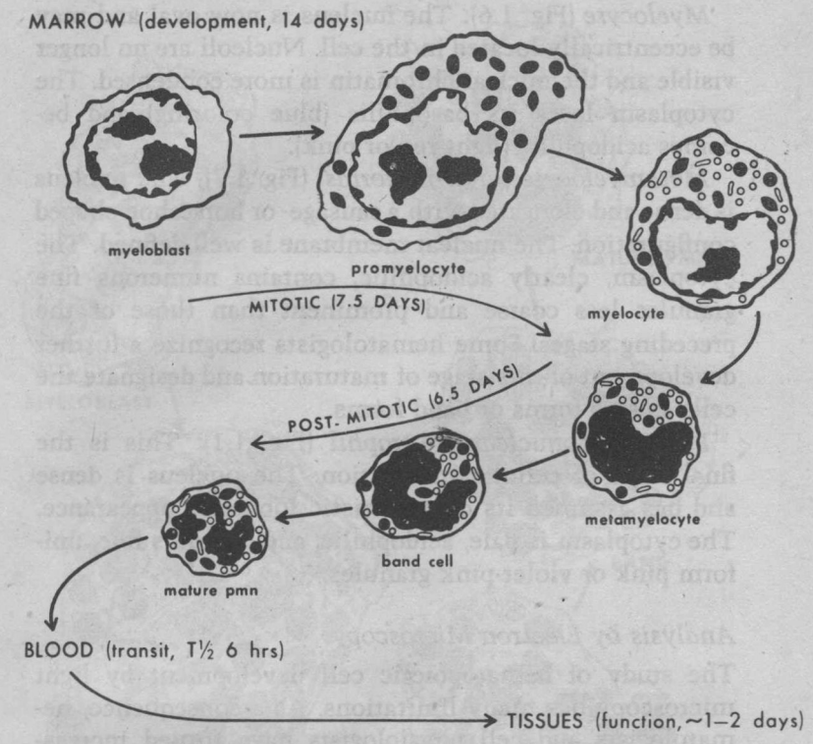

Figure 1.3 Diagrammatic representation of PMN life cycle and stages of PMN maturation. During each of the secretory stages a distinct type of secretory granule is produced: azurophils (*solid black*) are formed only during the promyelocyte stage, and specific granules (*light forms*) are produced during the myelocyte stage. The metamyelocyte and band forms are nonproliferating, nonsecretory stages that develop into the mature PMN. The times indicated for the various compartments were determined by isotope labeling techniques. (From D. F. Bainton, J. L. Ullyot, and M. G. Farquhar, *J. Exp. Med.* 134:907, 1971. Reprinted by permission of authors and publisher.)

Myeloblast (Fig. 1.4): This is a primitive cell normally restricted to the bone marrow. The nucleus, large in relation to the volume of cytoplasm, is round or slightly oval and has one or more prominent nucleoli. The nuclear membrane is smooth without condensation of chromatin. The cytoplasm is deep blue and has no perinuclear clear zone or cytoplasmic inclusions. A foamy appearance is sometimes observed (see Chapter 11). Myeloblasts from patients with leukemia may show considerable variation from the prototype cell.

Promyelocyte (Fig. 1.5): At this stage of maturation, granules ("azurophilic") appear in the cytoplasm and apparently cover the nucleus. Nuclear outline is similar to that of the myeloblast, but the nucleoli may be more difficult to see. The chromatin structure may be more coarse. Cytoplasmic basophilia is still prominent.

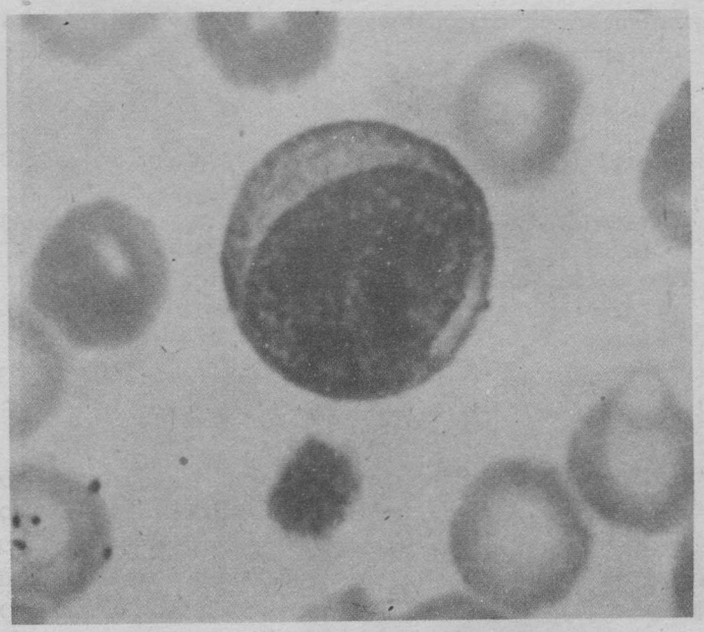

Figure 1.4 Human myeloblast; Wright's stain. Nucleoli are prominent, nuclear chromatin is fine, and cytoplasmic granulation is absent.

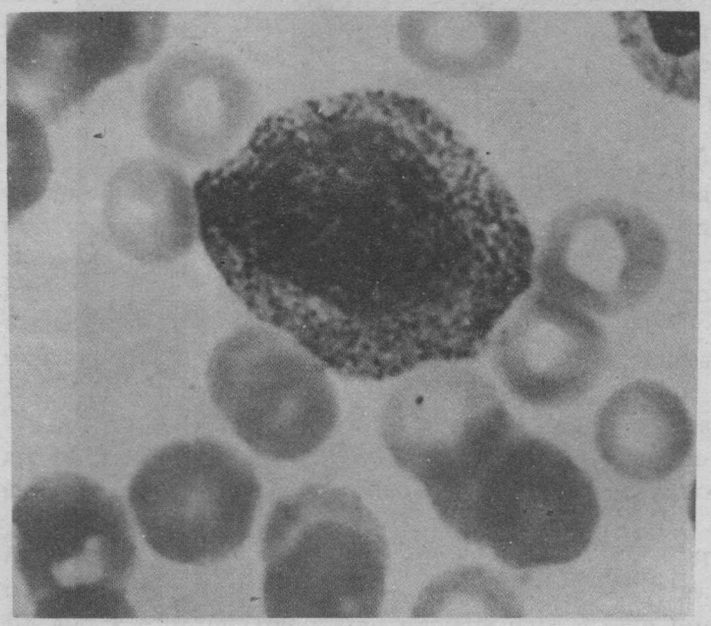

Figure 1.5 Human promyelocyte; Wright's stain. Note prominent nucleoli and cytoplasmic granulation.

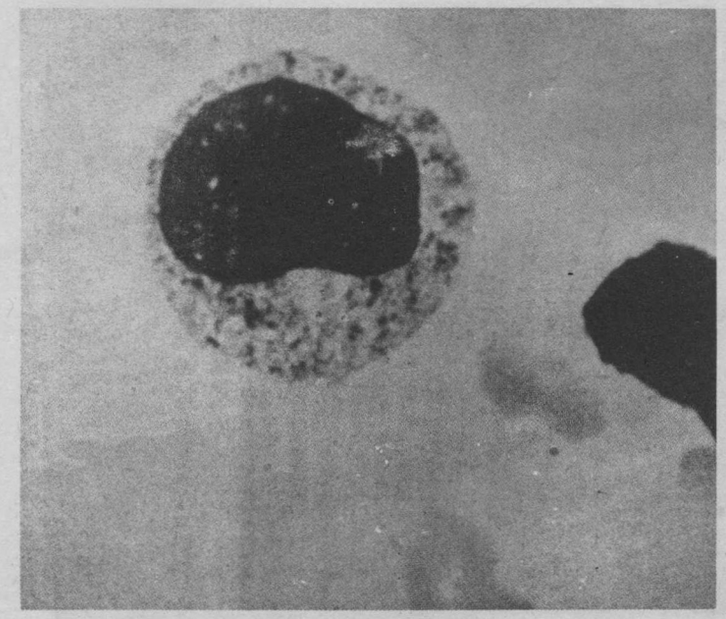

Figure 1.6 Human metamyelocyte (early); Wright's stain. Note dense nuclear chromatin and indented nuclear configuration.

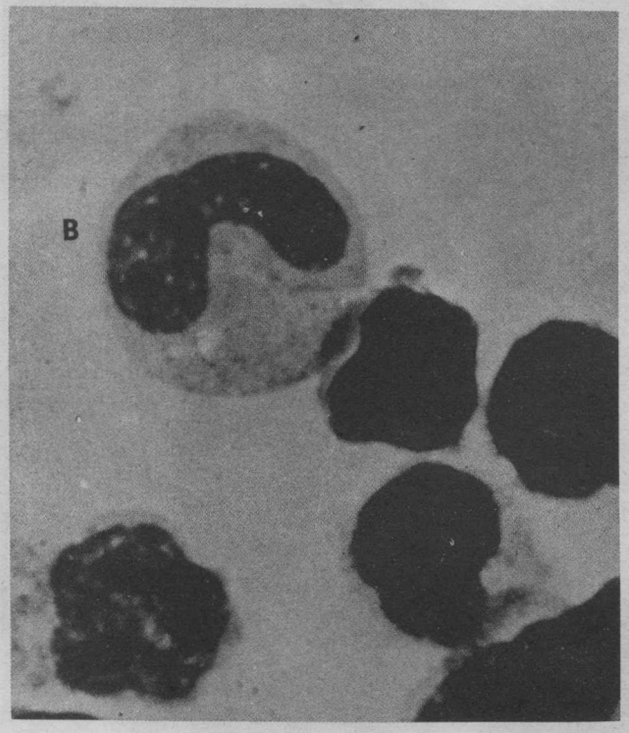

Figure 1.7 Band form (*B*); Wright's stain.

Myelocyte (Fig. 1.6): The nucleus is now oval and may be eccentrically located in the cell. Nucleoli are no longer visible and the nuclear chromatin is more condensed. The cytoplasm loses its basophilia (blue coloring) and becomes acidophilic (light red or pink).

Metamyelocyte (*juvenile forms*) (Fig. 1.7); The nucleus is dense and elongated with a sausage- or horseshoe-shaped configuration. The nuclear membrane is well defined. The cytoplasm, clearly acidophilic, contains numerous fine granules less coarse and prominent than those of the preceding stages. Some hematologists recognize a further development of this stage of maturation and designate the cells as stab forms or band forms.

Polymorphonuclear neutrophil (Fig. 1.1): This is the final stage of cellular maturation. The nucleus is dense and has assumed its characteristic lobulated appearance. The cytoplasm is pale, acidophilic, and contains fine, uniform pink or violet-pink granules.

Analysis by Electron Microscopy

The study of hematopoietic cell development by light microscopy has many limitations. As a consequence, hematologists and cell physiologists have turned increasingly to electron microscopy for analysis of the fine structure (ultrastructure) of cells.

The most comprehensive studies of the fine structure in developing granulocytes have utilized bone marrow obtained from rabbits (9, 11, 12, 84, 95, 99). A more limited number of comprehensive studies of human marrow is available (5, 26, 63, 74). We shall consider first the fine structure and cytochemistry of the developing rabbit granulocyte; and second, the relatively minor additional features that characterize granulopoiesis in man and other species.

In electron microscopic studies of developing rabbit granulocytes several types of morphologic criteria have been used to identify cells at different levels of maturation. The sequence of events in granulocyte maturation is schematically outlined in Fig. 1.8. As with Romanovsky-strained cells, the important ultrastructural criteria of maturity are nuclear shape, chromatin density, presence of nucleoli, and cytoplasmic organelles. The relative importance of these criteria is indicated in Table 1.1.

Large round or oval nuclei with prominent nucleoli are characteristic of the young myeloblast and early promyelocyte cells. With progressive maturation (15) the large and highly differentiated nucleoli of early cell forms diminish in size and complexity (Fig. 1.3 and 1.8). Maturation involves a progressive increase in the degree of condensation (electron density) of the interphase nuclear chromatin of granulocytes similar to that found in devel-

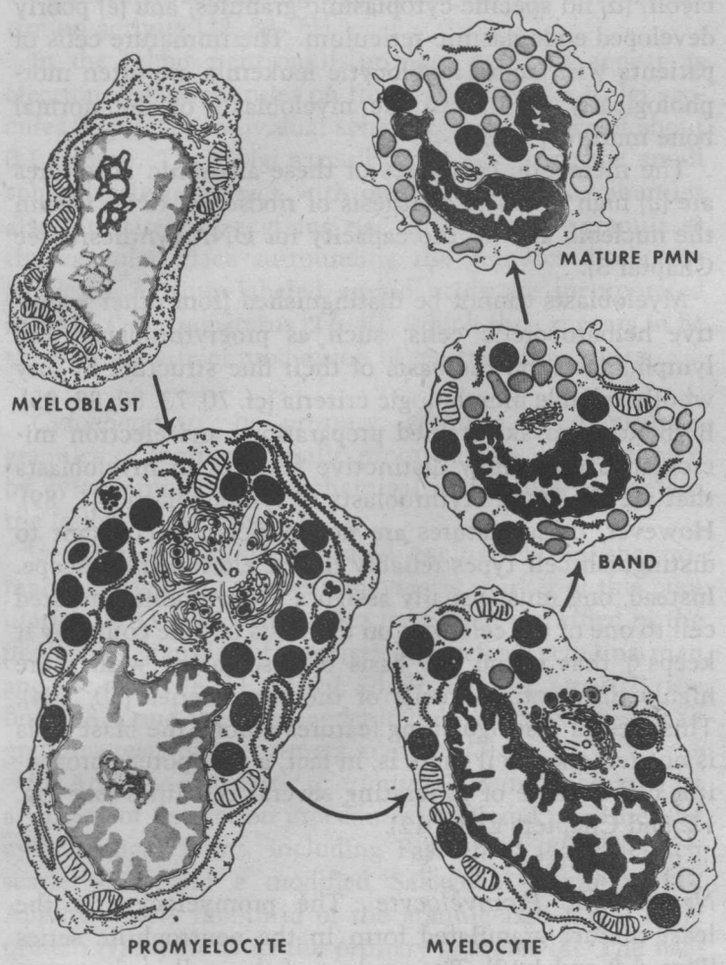

Figure 1.8 Diagrammatic representation of stages in the maturation of PMN leukocytes. Granules are shown 1.5× scale, and only half the average number are shown. The myeoblast lacks granules but contains abundant ribosomes, mitochondria, and a small rudimentary Golgi complex. The promyelocyte (progranulocyte) and myelocyte are stages of intense secretory activity and show elaborate development of cytoplasmic organelles involved in protein synthesis, segregation, and concentration (that is, ribosomes, rough-surface endoplasmic reticulum, and Golgi complex). The larger azurophil granules are formed by condensation of secretory material along the proximal or concave face of the Golgi complex of the progranulocyte. Smaller, less dense specific granules are formed by a similar process occurring along the distal or convex face of the Golgi complex of the myelocyte. The metamyelocyte (not shown) and band cell are nonsecretory stages showing a gradual diminution in most cytoplasmic organelles. The mature PMN has a multilobulated nucleus and a cytoplasm containing primarily glycogen and granules. (From D. F. Bainton and M. G. Farquhar, *J. Cell Biol.* 28:277, 1966. Reprinted by permission of authors and publisher.)

Table 1.1 Morphologic criteria for judging granulocyte maturity.

Reliable nuclear signs	Less reliable nuclear signs	Cytoplasmic signs
Chromatin condensation	Nuclear indentation and lobation	Organization of granular reticulum
Nucleolus	Nuclear shape	Numbers and organization of ribosomes
	Nuclear size	Size and numbers of mitochondria
		Volume of cytoplasm

oping red cells (80) and thymocytes (70). Progressive nuclear indentation and lobation, although generally concomitant with maturation, are probably the least reliable nuclear signs. Nuclei, immature by other fine structural criteria, sometimes may show indentation. Nuclear shape also may vary considerably in more mature cell forms (23).

The cytoplasmic features useful in determining the maturational level of the cell include: organization of the granular reticulum, amount and organization of ribosomes, size and number of mitochondria, and cytoplasmic volume.

The granular reticulum increases transiently in early cells and decreases during subsequent cell maturation. A parallel increase and succeeding decrease occurs in the numbers of ribosomes attached to the endoplasmic reticulum of the cytoplasm. Presumably this diminution in endoplasmic reticulum and ribosomes reflects a decrease in diversity and capacity of the protein-synthesizing apparatus with cell maturation.

In all the granulocytic lines, and particularly in the neutrophilic series, the size and number of mitochondria decrease as the granulocytes mature (Fig. 1.8). Changes in the aerobic metabolism of these cells reflect this morphologic alteration (see Chapters 3 and 4). Finally, as noted in Romanovsky-stained fixed cells, the volume of the cell cytoplasm at the promyelocyte stage transiently increases and, with further maturation, progressively decreases.

The size, number, and density of cytoplasmic granules are additional guides to cell maturity. They will be considered in detail in subsequent sections of this chapter.

Myeloblast Known also as a granuloblast, the myeloblast is characterized by the following cytologic features (Figs. 1.8 and 1.9): (*a*) a large round nucleus with a high nucleus/cytoplasm ratio; (*b*) an "open" or dispersed pattern of nuclear chromatin; (*c*) large and highly developed nu-

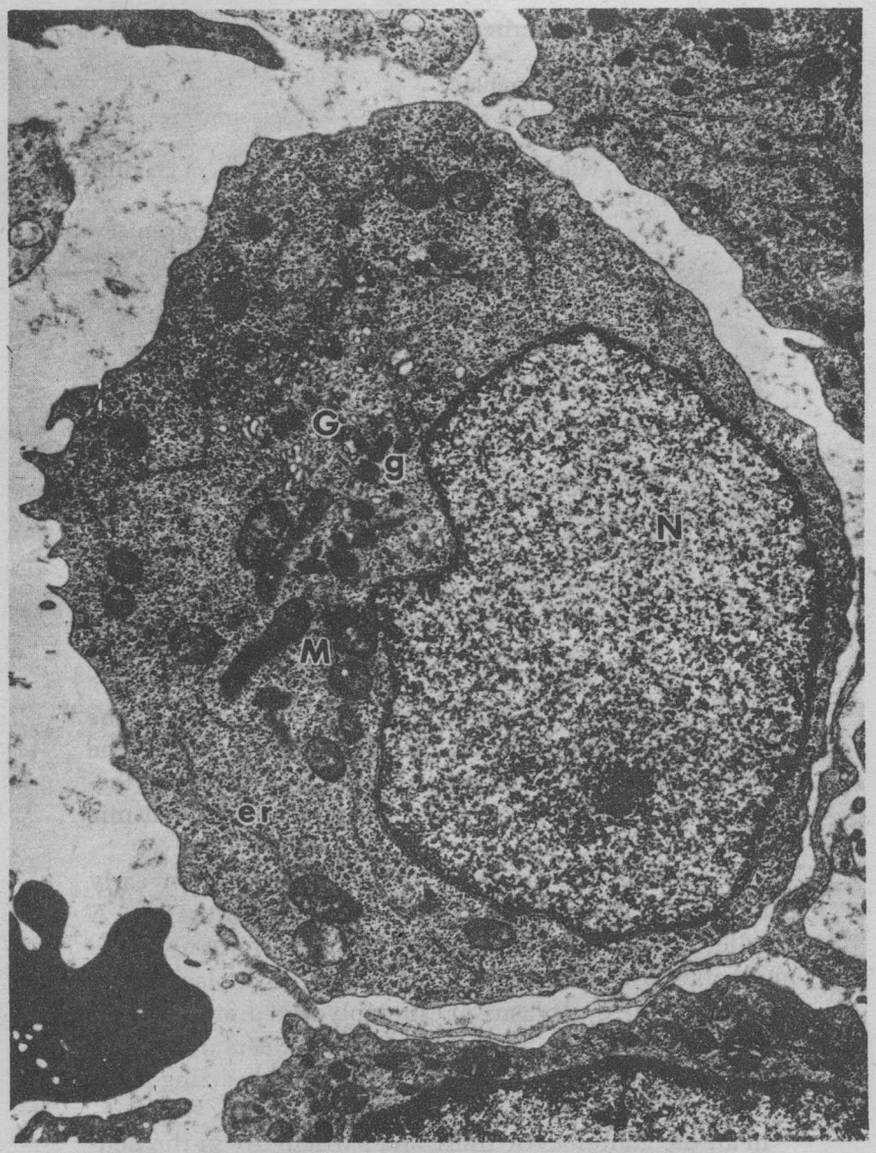

Figure 1.9 Blast cell from a patient with granulocytic hyperplasia. The large, ovoid nucleus (*N*) has finely dispersed chromatin and a prominent nucleolus. The Golgi apparatus (*G*) is well developed. A few dense granules (*g*), mitochondria (*M*), polyribosomes, and some development of endoplasmic reticulum (*er*) are seen in the cytoplasm. (From Y. Tanaka and J. R. Goodman, Electron Microscopy of Human Blood Cells, Harper & Row, New York, 1972. Reprinted by permission of authors and publisher.)

cleoli; (*d*) no specific cytoplasmic granules; and (*e*) poorly developed endoplasmic reticulum. The immature cells of patients with acute myelocytic leukemia are often morphologically similar to the myeloblasts of the normal bone marrow (5, 63, 74).

The metabolic correlates of these anatomic structures are (*a*) high levels of synthesis of ribosomal RNA within the nucleoli, and (*b*) the capacity for DNA synthesis (see Chapter 3).

Myeloblasts cannot be distinguished from other primitive hematopoietic cells, such as proerythroblasts and lymphoblasts, on the basis of their fine structure by any wholly reliable morphologic criteria (cf. 70, 73, 80, 88, 95). Both Romanovsky-stained preparations and electron micrographs may show distinctive features in myeloblasts that are absent in erythroblasts or lymphoblasts (41, 89). However, these features are not sufficiently constant to distinguish cell types reliably in the elctron microscope. Instead, one must usually assign a given undifferentiated cell to one of the cell lines on the basis of "the company it keeps"; that is, on the basis of association with more highly differentiated cells of the same series (50, 104). This lack of distinguishing features among the blast cells is not remarkable if there is, in fact, a pluripotent progenitor cell capable of generating several cell lines (see Fig. 1.2 and Chapters 2 and 12).

Neutrophilic Promyelocyte The promyelocyte is the least mature granulated form in the neutrophilic series (Figs. 1.8 and 1.10). The nucleus of this cell is characterized by diffuse chromatin and persistent complex nucleoli. Typically, the nucleus is indented opposite the Golgi zone. The nucleus/cytoplasm ratio is lower than that of the antecedent myeloblast.

Observed within the cytoplasm are occasional mitochondria and lipid droplets, extensive cisternae of granular reticulum, free polyribosomes, and a population of dense granules each about 0.4 micron in diameter (Figs. 1.10 and 1.11). The granules are usually spherical and homogeneously dense after fixation in glutaraldehyde-osmium tetroxide. In the neutrophilic series these granules correspond to the "azurophil granules" of the promyelocyte in Romanovsky-stained preparations and are best designated as *primary granules*, denoting their early appearance in cell maturation (11, 12, 95). In hematologic literature they have been called by a variety of other terms, including type 1, alpha, and A granules. The granules of the neutrophilic leukocyte have many features in common with the lysosomes of other tissues such as the liver and kidney. Lysosomes are membrane-bound