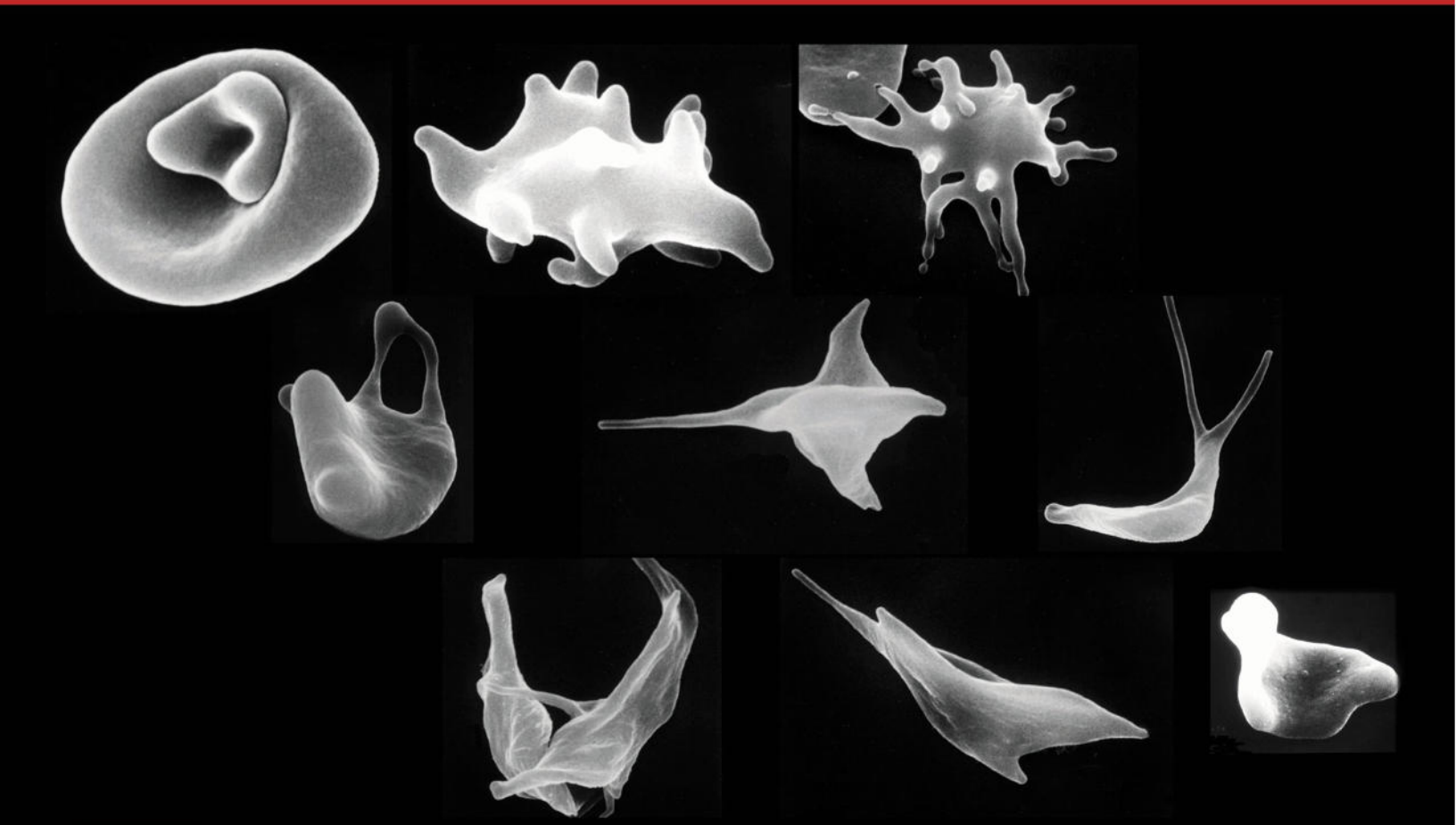
WILLIAMS HEMATOLOGY

THE Red Cell and Its Diseases



Josef T. Prchal • Marshall A. Lichtman

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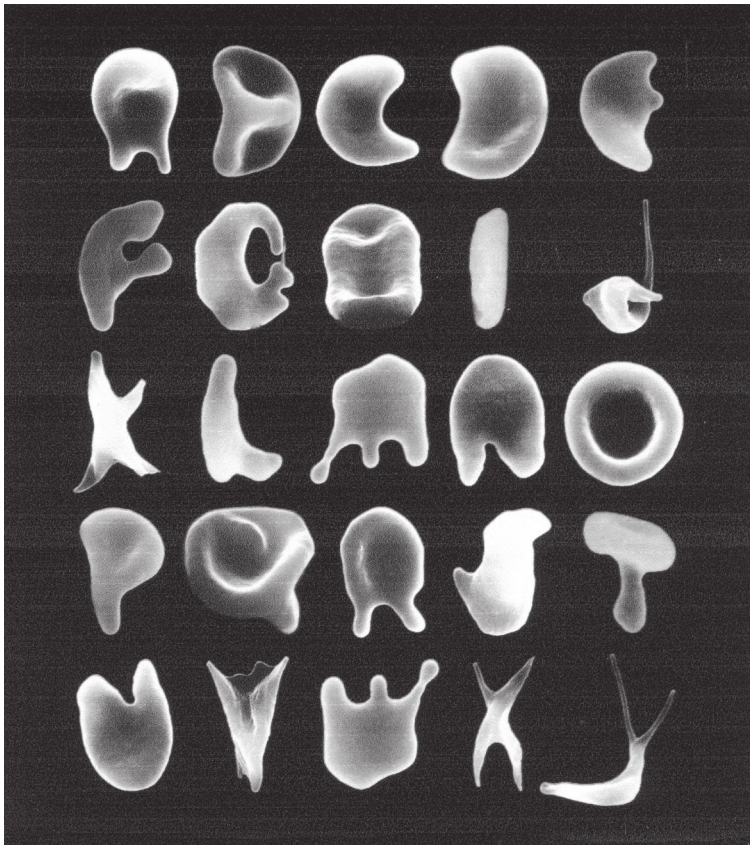

Plate 1. The alphabet made of poikilocytes from a single patient with sickle cell anemia and B-thalassemia trait. Note the electron dense areas in the cells that are in the patient who is homozygous for hemoglobin S, as a reflection of the para-crystallization of that hemoglobin in a low oxygen environment (venous blood). The patient with B-thalassemia trait from whom many of these cells were imaged was a physician whose family arrived to the United States originally from the United Kingdom with no apparent recent Mediterranean heritage. As a result of Rome's invasion of Britain on several occasions between 55 B.C.E. and 43 A.D., troops from current Italy, Spain, Egypt, and Syria garrisoned their married local Britons. *(Reproduced with permission from Lichtman MA, Shafer MS, Felgar RE, et al: Lichtman's Atlas of Hematology 2016. New York, NY: McGraw Hill; 2017.)*

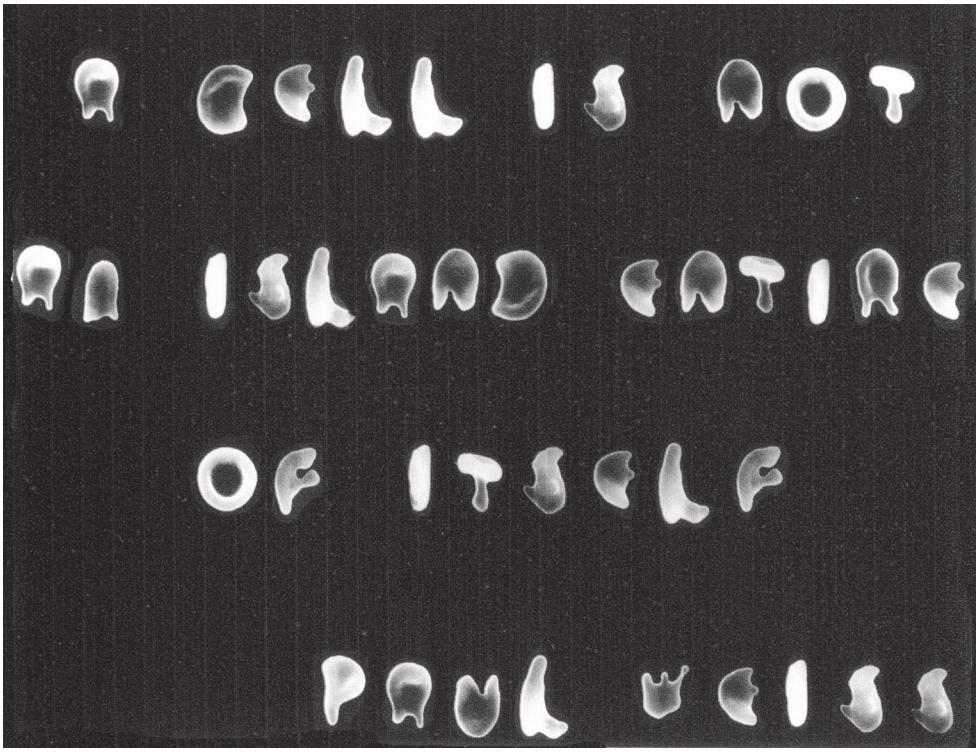

Plate 2. Paul Alfred Weiss (1898-1989) was an American cell and neurobiologist who had emigrated from Austria and specialized in morphogenesis, cell development, and differentiation. He encouraged cross-disciplinary interactions among scientist and was elected to the National Academy of Sciences. He was one of the earliest scientists to propose that the cell microenvironment (né stroma) had important influences on the parenchymal cells it held in its grasp as highlighted in this aphorism he coined, here spelled out with misshapen red cells. *(Reproduced with permission from Lichtman MA, Shafer MS, Felgar RE, et al: Lichtman's Atlas of Hematology 2016. New York, NY: McGraw Hill; 2017.)*

COVER IMAGE DESCRIPTION

The nine abnormal red cells depicted on the cover show the amazing plasticity of the red cell. Consider the membrane reorganization required to maintain these deviations from a biconcave disk.

In veins, the shear rate is low, and normal red cells remain close to a biconcave disk shape. They, also, may overlap tightly at very low flow rates into stacks (rouleaux). When subjected to increased shear rates in the arterial circulation, rouleaux would be dispersed and the red cells deform. At high flow velocities the red cell tends to elongate parallel to the direction of flow. The cytoplasm moves in what has been considered eddy flow. This eddy flow results from the shear flow of the blood being transmitted to the cytoplasm through a motion of the membrane around the elongated red cell, called *tank-tread motion* or *tank-treading*. In a blood capillary with diameters smaller than their own diameter, red cells are folded.

The markedly deformed red cells shown on the cover image were each found in the blood of a patient with a red cell disease (eg, hemoglobin SS, beta-thalassemia, or another red cell disorder). After enucleation, red cells leave the marrow through very narrow, temporary, apertures in the marrow sinus wall that separates hematopoietic cords from the marrow sinus network requiring marked deformation upon egress. Red cells navigate the confinements of capillary dimensions, squeeze through the inter-endothelial cell spaces of the splenic sinus walls, and navigate other physical constraints. Abnormal red cells may be shunted around those constricted dimensions.

The shapes of seven of the nine images approximate an animal form, if one's imagination is permitted to operate. In the upper-left corner is a slightly deformed (thickened edge) discocyte and in its biconcavity rests a triangular-shaped extremely small microcytic, but the latter, strikingly, retains its biconcavity. The other images are that of a simulated dinosaur (aka *Erythrosurus Rochesteriensis*), an octopus (although with more than eight limbs), a severely deformed red cell with a retained concavity and a large hole perforating its cytoplasm, a flying goose, a snail, dancing penguins, a shark, and a duckling, each with their own irregularities requiring extraordinary membrane adaptations. Several have para-crystallization of hemoglobin SS as a deforming force (eg, shark shape).

The distortions that can be maintained by abnormal red cells are extraordinary to consider and certain patterns, discerned on a careful examination of the blood film can be, and frequently are, important diagnostic clues to the nature of the underlying disease. In the absence of a crystallizing hemoglobin or a mutant gene that results in a membrane protein misconfiguration, it is possible that acquired alterations in the spectrin-based membrane protein network is altered so as to maintain abnormal bends and distortions of the red cell surface.

These images and those in Plates 1 and 2 were captured by Patricia A. Santillo, Senior Technologist, Electron Microscopy Laboratory, Hematology Unit at the University of Rochester Medical Center and have been used with permission from *Lichtman's Atlas of Hematology. www.accessmedicine.com*

Williams Hematology

The Red Cell and Its Diseases

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PREFACE

The discovery of the ruddy globules (red cells) is attributed to Jan Swammerdam (1637-1680) in Amsterdam; but, it was Antonj van Leeuwenhoek (1632-1723) of Delft, who as a result of his ability to grind lenses with greater magnifying power (x 275), made a more detailed description of red cells, delineating their gross structure.

The biochemistry, physiology, and biophysics of the red cell have been studied intensively over three centuries and, although considered a "simple" structure, since it is anucleate and after one day in the circulation has no cytoplasmic organelles, its mysteries have been slow to be unraveled. The process of enucleation of the erythroblast in the hematopoietic space and the movement of the anucleate cell from the hematopoietic space to the marrow sinus and from there to the systemic circulation, accomplished by a cell without an intrinsic apparatus to support amoeboid motility, and the determinants of its average life span of approximately 120 days are still being elucidated. Its structural and biophysical properties, biochemical pathways, and the relationship among those features have been of continued interest to scientists. Its absence of interfering granules, containing proteolytic enzymes, organelles, and other complexities have allowed the isolation of highly purified red cell membranes and the early exploration of the biochemical and biophysical features of cell membranes, applicable to other cells, including the characteristics of membrane transport of various molecules. The nature of the structure and function of hemoglobin and the exploration of the glycolytic pathway, the hexose monophosphate shunt, and the Luebering-Rapoport pathway were other rewards reaped from the study of red cells.

Much is known, but as our mentor, friend, and colleague, Ernest Beutler, cautioned Ph.D. graduates at a Scripps Institute doctoral graduation, one should not assume that our understanding of the biomedical sciences is so profound that what is left for us is to fill in some gaps. He argued that much fundamental biomedical knowledge was still undiscovered and waiting to be illuminated. Among his many contributions to the pathogenesis of disease and application of therapy, his contributions to understanding the red cell and anemia were notable. These observations included a classic series of papers describing the effects of oxidant stress on individuals with red cell glucose-6-phosphate dehydrogenase deficiency and a life-long interest in the enzyme's variants and epidemiology. His monograph on methods for measuring red cell enzymes was an early contribution to enhancing the specificity of the diagnosis of hemolytic anemia. Published over five decades ago, it remains an unsurpassed source of methods for the assay of red cell enzymes. Beutler, also, used red cell enzyme measurement as a surrogate for diagnosis of systemic, until then difficult to diagnose diseases, such as galactosemia, glycogen storage disorders, and others. He found that red cell glucose-6-phosphate dehydrogenase deficiency was inherited as an X chromosome-linked disorder and described the mosaicism of normal and deficient red cells in heterozygous females. This finding of mosaicism provided the basis for an intellectual jump to the hypothesis of X chromosome inactivation in humans, coincident with Mary Lyon's description of the phenomenon in mice. He, also, made seminal contributions to understanding the effects of iron deficiency in non-anemic women and the expression of iron overload in those homozygous for the *HFE* mutation and the value of additives for prolonged storage of red cells, still in current use.

With no DNA or RNA synthesis, no mitochondria and their related enzymatic biochemical energy generating pathways, and with a relatively short life span, this amitotic cell is sustained at a normal concentration in the blood by a robust daily production of new cells in the marrow, the process of erythropoiesis. This process delivers two to three million new red cells to the blood per second. Although remarkable, it is also a vulnerability should red cell production be dampened by disease or substrate insufficiency: the latter, a principal cause of anemia.

In 1929, 3 years after obtaining his M.D. degree at the University of Manitoba, his family having immigrated to Canada from Austria, Maxwell Myer Wintrobe, obtained his Ph.D. at Tulane University, his doctoral thesis entitled "The Erythrocyte in Man." Wintrobe is considered the father of clinical hematology having published the first comprehensive text in the English language, *Clinical Hematology,* in 1942. He introduced the technique of the hematocrit device to measure the packed red cell volume at a time when hemoglobin and red cell count measurements were neither accurate nor reproducible. The word "hematocrit" was so appealing that it became a synonym for the packed red cell volume rather than the instrument of measurement as intended by Wintrobe. Initially, the "Wintrobe" tube, as it became known, was filled by pipette with blood to the 1 mL mark etched on the tube and the gradations on the tube allowed one to read the fraction of blood that was composed of red cells after centrifugation. Later, the microhematocrit centrifuge, which reached G-forces that removed plasma trapping as a significant consideration in the measurement in capillary tubes filled with blood, could be found on every ward and clinical laboratory as the principal means to measure the packed red cell volume and, thereby, identify anemia or erythrocytosis. A chart allowed the determination of the packed cell volume when the capillary tube, regardless of the volume of blood it contained, was placed against its scales. Wintrobe institutionalized the red cell indices, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) and showed in two classic paper in 1930 and 1934 that one could classify the anemias for diagnostic purposes by distinguishing among macrocytic, normocytic, simple microcytic, and hypochromic microcytic anemias, a method of differential diagnosis still used today. After moving to the University of Utah from Johns Hopkins University, Wintrobe established one of the most esteemed hematology clinical and research training programs in the world. He also described along with his colleague George Cartwright that the average hematocrit and hemoglobin concentration was higher in residents of Salt Lake City (elevation 4300 feet) than the value observed at Johns Hopkins in Baltimore (elevation 480 feet). He deduced from that prescient observation that hypoxia, in that instance from higher altitude, is a principal regulator of normal erythropoiesis.

In 1953, F. William Sunderman and colleagues enhanced the accuracy of blood hemoglobin measurement by introducing the cyanmethemoglobin method. In 1956, Wallace Coulter introduced his high-speed, automatic blood cell counter making blood cell counting accurate, reproducible, and capable of meeting the demands of a busy clinic and hospital environment. The "Coulter Principle" held that cells are poor conductors of electricity in a salt solution. Thus, when cells are diluted in saline and are drawn through a tiny aperture carrying a current, each cell produces a slight impedance to current flow as it passes through the narrow aperture. The pulse created by this impedance can be amplified and counted. Moreover, the size of the pulse is proportional to cell volume. Thus, the number and volume distribution of red cells in a measured volume of solution can be converted to red cell count and volume electronically. Their product, red cell count and red cell volume, provided the hematocrit, now a derived value. Thousands of cells can

be counted per second. Since the red cells, leukocytes, and platelets are sufficiently different in size, they can be discriminated. The electronic particle counter's derivative technology of cell flow analysis, dependent on laser light, provided one of the most powerful diagnostic technologies in medicine, capable of measuring cell DNA content or the surface antigen array of a specific cell type. One could use the device to isolate purified, specific cell populations for analysis. The Coulter Principle and its derivative technologies revolutionized diagnostic medicine, biomedical, and industrial research and, more specifically, the diagnosis and management of red cell diseases.

A giant of studies of the red cell, perhaps little known to younger scientists, was Eric Ponder (d. 1970), an original member of the Red Cell Club (see further), whose treatise *Hemolysis and Related Phenomena* in 1948, reissued in 1971 by Grune and Stratton with a forward by Robert I. Weed, is an extraordinary compilation of his research on this cell. Many of his studies are still relevant. All scientist interested in the red cell should be familiar with this work. Weed, another gifted contributor to our understanding of the red cell, died prematurely in 1976, at the age of 48 years, of a glioblastoma. He was largely responsible for convincing the National Institutes of Health to expand the designation of the Heart and Lung Institute to the Heart, Lung and Blood Institute in 1976, facilitating research support for blood cells, especially red cell research. In 1976, in recognition of his leadership in that initiative and his contributions to research on the red cell, he was named the third recipient of the William Dameshek Award of the American Society of Hematology. At the time, the Society had two prizes, The Henry Stratton Lecture and The William Dameshek Prize. Stratton and Dameshek were very close friends. Dameshek was among the very top academic clinical hematologists in the United States and Stratton was the co-owner of Grune and Stratton Publishers. They were the prime movers of the establishment of the American Society of Hematology and started *Blood* in 1946. Dameshek was the founding editor and Grune and Stratton the publisher. Under Dameshek's editorship *Blood* became the most prestigious journal of clinical and research hematology in the world. In 1976, the journal became the official publication of the American Society of Hematology; however, the publisher still owned the title and, technically, editorial control, but some of it was ceded to the Society. In 1989, the American Society of Hematology bought the title to *Blood* from its then publisher Saunders, Inc. and it became *Blood, The Journal of the American Society of Hematology*. The purchase of title was an initiative led by H. Franklin Bunn, a distinguished hematologist at Harvard University and a world's authority on the structure and function of hemoglobin. The purchase of the Journal has provided the Society with an enormously successful economic engine to support its educational and research programs, full control of its editorial policies, and an outlet for the most impactful research in the field, including that of the red cell and its diseases.

Bob Weed's close colleagues at the University of Rochester, Claude Reed and Scott Swisher, were pioneers in forecasting the key role of a membrane protein abnormality as the primary lesion in hereditary spherocytosis, whereas others were distracted by epiphenomena, such as substrate transport. They showed that the membrane lipid composition of red cells in hereditary spherocytosis was normal but after 24 hours of incubation, lipids (cholesterol and phospholipids) were lost to the medium in their exact molar proportion as in the red cell membrane and this phenomenon could be decreased by adding glucose to the medium. This finding strongly suggested that the loss of surface area of the red cells and the disc to sphere transformation decreasing their surface area to volume ratio and moving toward their critical hemolytic volume was related to loss of pieces of membrane. This work published in 1966 was well before methods for membrane protein analysis were available. Later, the ability to characterize the protein composition of "pure" red cell membranes (ghosts) in cases of specific disorders of the red cell (eg, hereditary spherocytosis versus hereditary elliptocytosis) allowed the assignment of functional characteristics to the missing or mutant proteins. Red cell ghosts are a preparation of red cell membranes freed of their internal contents, notable hemoglobin and enzymes and substrates and colorless (ghostly pale) rather than red and are basically pure red cell membranes, a key specimen for study.

A longstanding focus on the red cell by basic and clinical investigators has been highlighted by the interactions of a group of scientists, referred to as "The Red Cell Club," which started in 1958 through the initiative of Joseph Hoffman and Daniel Tosteson, then young scientists at the National Institutes of Health. The spent their careers at Yale and Harvard, respectively. The meetings are small, informal, and an ideal milieu to focus on new science and the exchange of ideas. The Club, in its 63rd year in 2021, meets now once a year on the campus of a member to discuss new insights into the red cell and to share their current research. It is a collegial group with new "blood" being cycled in from laboratories throughout the United States and Canada as mentors introduce their acolytes to the red cell's charms. Usually, a preceding round of golf is held for those devotees of the game, weather permitting. Members, who for reasons of age or a change of interests leave the fold, are never dropped from the invitation list. Nonparticipants are tenderly referred to as "red cell ghosts." In the last several years, scientists from Europe and, occasionally Japan, have participated in these meetings. A European Red Cell Club has been established highlighting that the mysteries of the cell have not all been uncovered, confirming Beutler's admonition.

In this volume, we bring to the reader the most up-to-date consideration of the structure and function of the red cell. After two introductory chapters on the structure and biology of the red cell and erythropoiesis, the focus turns to the comprehensive set of diseases, either acquired or inherited, in which a quantitative (deficiency or excess) or qualitative (membrane, enzyme, hemoglobin) abnormality of the red cell results in disease. These chapters, also, may include important, relevant basic scientific aspects of the clinical problem under discussion. The role of certain plasma constituents, iron, folic acid, and cobalamin, critical to normal red cell production and hemoglobin synthesis, is described as well.

We believe the authors have brought to our reader an insightful exposition of the red cell and its disorders to enlighten the clinicians faced with their challenges and to the benefit of the care of their patients. In addition, we hope this text provides scientists a clear delineation of the remaining mysteries of the cell and provides them with new foundations for development of therapy of red cell diseases. We hope that this text will fill the vacuum that has existed since the monograph published in 1970 devoted to the red cell by John W. Harris, and Robert W. Kellermeyer: *The Red Cell: Production, Metabolism, Destruction: Normal and Abnormal.*

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Part I **Structure and Physiology of the Red Cell**

CHAPTER 1
STRUCTURE AND COMPOSITION OF THE ERYTHROCYTE*

Mohandas Narla

SUMMARY

Collectively, the erythroid progenitors, terminally differentiating erythroblasts (precursors), and adult red cells are termed the *erythron* to reinforce the idea that they function as an organ. The widely dispersed cells comprising this organ arise from pluripotential hematopoietic stem cells. Following commitment to the erythroid lineage (unipotential progenitor), further maturation gives rise to the erythroid progenitors, burst-forming unit–erythroid (BFU-E) and, subsequently, colony-forming unit–erythroid (CFU-E), that can be identified by their development into representative clonal colonies of red cells in vitro. The CFU-E then undergoes terminal differentiation, progressing through four to five morphologic stages, each having characteristic light microscopic and ultrastructural features. During terminal erythroid differentiation, there is an increasing amount of hemoglobin synthesis accompanied by nuclear chromatin condensation, and at the final stage of differentiation, there is nuclear extrusion to generate an anucleate polychromatophilic macrocyte (reticulocyte with supravital staining). The human polychromatophilic macrocyte (reticulocyte) matures over 2 to 3 days, first in the marrow and then in circulation into the discoid erythrocyte. During reticulocyte maturation, cytoplasmic inclusions, including residual mitochondria and ribosomes, are degraded, and the reticulocyte loses surface area to achieve the mean cell volume and surface area of a discoidal erythrocyte. Mature erythrocytes are approximately 7 to 8 μm in diameter and undergo extensive deformation to pass through 3-μm-diameter capillaries and the 1-μm-wide and 0.5-μm-thick endothelial slits in the red pulp of the spleen. The ability of the red cell to undergo extensive reversible deformation is essential for both its function and its survival. Red cell deformability is a function of its geometry, the viscosity of the cytoplasm, largely determined by the concentration of hemoglobin. Decreased deformability is a feature of red cells in various pathologic states. The erythrocyte is unique among eukaryotic cells in that its principal physical structure is its cell membrane, which encloses a concentrated hemoglobin solution. Thus, all structural properties of this cell are in some way linked to the cell membrane. In contrast to other cells, the erythrocyte has no cytoplasmic structures or organelles. Among human cells, only red cells and platelets do not have a nucleus.

Acronyms and Abbreviations: BFU-E, burst-forming unit–erythroid; CFU-E, colony-forming unit–erythroid; cP, centipoise; DIC, disseminated intravascular coagulation; EMP, erythroblast macrophage protein; ICAM-4, intercellular adhesion molecule-4; IL, interleukin; MCH, mean cell hemoglobin content; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; MDS, myelodysplastic syndrome; SA:V, surface area-to-volume ratio; TTP, thrombotic thrombocytopenic purpura.

*This chapter contains text written for previous editions of this book by Brian Bull, Paul Herrmann, and Ernest Beutler.

ERYTHRON

The mass of circulating erythrocytes constitutes an organ responsible for the transport of oxygen to tissues and the removal of carbon dioxide from tissues for exhalation. Collectively, the progenitors, precursors, and adult red cells make up an organ termed the *erythron*, which arises from pluripotential hematopoietic stem cells. Following commitment to the erythroid lineage, unipotential progenitors mature into the erythroid progenitors, the burst-forming unit–erythroid (BFU-E) and, subsequently, the colony-forming unit–erythroid (CFU-E), which then undergoes further maturation to generate anucleate polychromatophilic macrocytes (reticulocytes on supravital staining). The BFU-E and CFU-E are identified by their development into morphologically identifiable clonal colonies of red cells in vitro. The reticulocyte further matures, first in the marrow for 2 to 3 days and, subsequently, in the circulation for approximately 1 day, to generate discoid erythrocytes.[1-5] The proerythroblast, the first morphologically recognizable erythroid precursor cell in the marrow, typically undergoes 5 mitoses (range 4-6) before maturation to an orthochromatic erythroblast, which then undergoes nuclear extrusion. A feature of erythropoiesis is that after each cell division, the daughter cells advance in their state of maturation with significant changes in gene and protein expression compared with the parent cell and, ultimately, become functional as mature erythrocytes.[4] In this process, they acquire the human blood group antigens, transport proteins, and all components of the erythrocyte membrane.[4,6]

In the adult stage of development, the total number of circulating erythrocytes is in a steady state, unless perturbed by a pathologic or environmental insult. This effect does not hold during growth of the individual in utero, particularly in the early stages of embryonic development and during neonatal development as the total blood volume increases markedly. Consequently, erythrocyte production in the embryo and fetus differs markedly from that in the adult.

THE EARLIEST ERYTHRON

In the very early stages of human growth and development, there are two forms of erythroid differentiation: primitive and definitive.[7-10] Chapters 2 and 17 provide detailed information of embryonic and fetal hematopoiesis. The primitive erythron supplies the embryo with oxygen during the phase of rapid growth before the definitive form of maturation has had a chance to develop and seed an appropriate niche. The hallmark of this primitive erythron is the release of nucleated erythroid precursors containing embryonic hemoglobin. Although primitive in the sense that the cells contain nuclei when released into the circulation, this form of maturation differs from avian and reptilian erythropoiesis in that the nucleus is eventually expelled from the mammalian cells as they circulate. The transient presence of a nucleus in the cells of the circulating primitive erythron can decrease the efficiency of gas exchange in the lungs and microvasculature because the nucleus

prevents the red cell from behaving as a fluid droplet.[11] The definitive stage of maturation makes its appearance around week 5 of embryogenesis when multipotential stem cells develop and seed the liver, which maintains the erythron for most of fetal life. In later fetal life, skeletal development provides marrow niches to which erythropoiesis relocates, being sustained in the form of erythroblastic islands, a central macrophage with circumferential layers of developing erythroid cells.[12] The definitive stage of erythroid maturation predominates during the remainder of fetal development and is the only type of erythroid maturation present through childhood and adult life. All normal human erythropoiesis occurs in the marrow in the form of erythroblastic islands.[13]

ERYTHROID PROGENITORS

Burst-Forming Unit–Erythroid

The earliest identifiable progenitor committed to the erythroid lineage is the BFU-E (Chap. 2, Fig. 2-1). A BFU-E is defined in vitro by its ability to create a "burst" on semisolid medium, that is, a colony consisting of several hundred to thousands of cells by 10 to 14 days of growth, during which time smaller satellite clusters of cells form around a larger central group of erythroid cells, giving rise to the designation of a "burst." The generation of BFU-E from hematopoietic stem cells requires interleukin (IL)-3, stem cell factor, and erythropoietin for differentiation, proliferation, prevention of apoptosis, and maturation (Chap. 2).[5,13]

Colony-Forming Unit–Erythroid

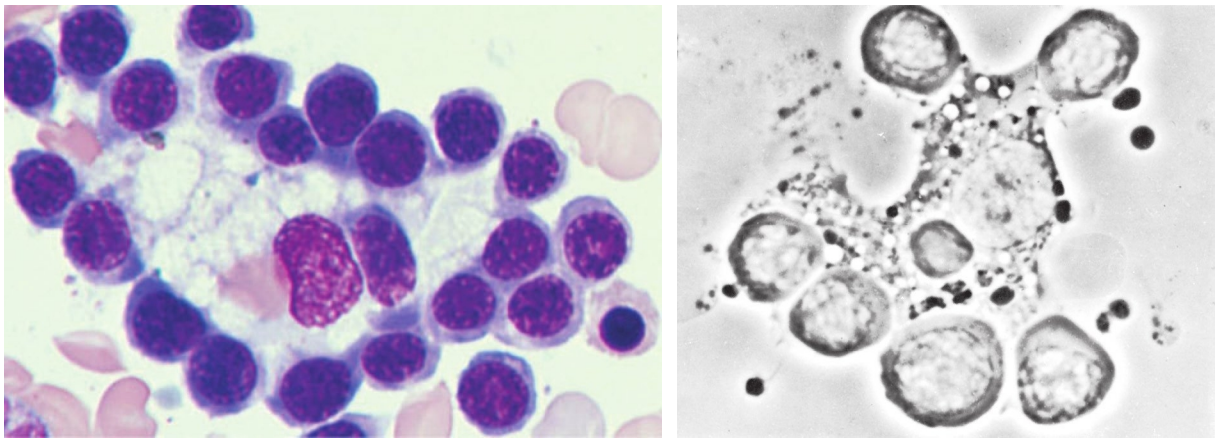
As erythroid maturation progresses, a later progenitor, the CFU-E, derived from the BFU-E, can be defined in vitro. The CFU-E is dependent on erythropoietin for its development and can undergo only a few cell divisions.[5,14,15] Thus, the CFU-E forms a smaller colony of morphologically recognizable erythroid cells in 5 to 7 days (see Chap. 2, Fig. 2-1). Adhesion between erythroid cells and macrophages occurs at the CFU-E stage of maturation.

Using cell-surface markers, IL-3 receptor, CD34, and CD36, highly purified populations of BFU-E and CFU-E can be isolated from human marrow.[5] Gene expression profiling shows distinctive changes in gene expression profiles in hematopoietic stem cells, BFU-E, and CFU-E.[5] Some of the marrow failure syndromes are the result of defects in differentiation of stem cells into erythroid progenitors.

ERYTHROBLASTIC ISLAND

The anatomical unit of erythropoiesis in the normal adult is the erythroblastic island or islet.[13,16,17] The erythroblastic island consists of a centrally located macrophage surrounded by maturing terminally differentiating erythroid cells (Fig. 1-1A). Several binding proteins are implicated in the cell–cell adhesions important to this process. These include $\alpha_4\beta_1$ integrin, erythroblast macrophage protein (EMP), and intercellular adhesion molecule-4 (ICAM-4) on the erythroblasts and vascular cell adhesion molecule (VCAM-1) EMP, α_V integrin on macrophages.[16] Additional macrophage receptors include CD69 (sialoadhesin) and CD163, but the counterreceptors for these on erythroblasts remains to be defined.[16] Phase-contrast microcinematography reveals that the macrophage is far from passive or immobile. Evidence suggests that either the erythroblastic islands migrate or that erythroid precursors move from island to island, because islands near sinusoids are composed of more mature erythroblasts, whereas islands more distant from the sinusoids are composed of proerythroblasts.[18] The macrophage's pseudopodium-like cytoplasmic extensions move rapidly over cell surfaces of the surrounding wreath of erythroblasts. On phase-contrast micrographs, the central macrophage of the erythroblastic island appears spongelike, with surface invaginations in which the erythroblasts lie (Fig. 1-1B). As the erythroblast matures, it moves along a cytoplasmic extension of the macrophage away from the main body. When the erythroblast is sufficiently mature for nuclear expulsion, the erythroblast makes contact with an endothelial cell, passes through a pore in the cytoplasm of the endothelial cell, and enters the circulation as a polychromatophilic macrocyte (reticulocyte).[19-21] The nucleus is ejected before egress from the marrow, phagocytized, and degraded by marrow macrophages.[22] In addition to the unique cytologic features just described, the macrophage of the erythroblastic island is also molecularly distinct as demonstrated by a unique immunophenotypic signature.[23] In addition, the macrophage of the erythroblastic island appears to play a stimulatory role in erythropoiesis; independent of erythropoietin. The anemia of chronic inflammation and of the myelodysplastic syndrome (MDS) may result partly from inadequate stimulation of erythropoiesis by these macrophages (Chaps. 2 and 6).

Despite the central role of erythroid islands in erythropoiesis in vivo, morphologically normal development of erythroid cells can be



A **B**

Figure 1–1. Erythroblastic island. **A.** Erythroblastic island as seen in Wright-Giemsa–stained marrow. Note central macrophage surrounded by a cohort of attached erythroblasts. **B.** Erythroblastic island in the living state examined by phase-contrast microscopy. The macrophage shows dynamic movement in relation to its surrounding erythroblasts. *(A, reproduced with permission from Lichtman MA, Shafer MS, Felgar RE, et al:* Lichtman's Atlas of Hematology *2016. New York, NY: McGraw Hill; 2017.)*

recapitulated in vitro without these structures, assuming developing cells are provided with supraphysiologic concentrations of appropriate cytokines and growth factors. Such growth in vitro, however, is much less optimal than when erythroblasts form erythroblastic islands.[24] The erythroblastic island is a fragile structure. It is usually disrupted in the process of obtaining a marrow specimen by needle aspiration but can be seen in marrow biopsies.

Macrophages in erythroblastic islands not only affect erythroid differentiation and/or proliferation but also perform other functions, including rapid phagocytosis (<10 min) of extruded nuclei as a result of exposure of phosphatidylserine on the surface of the membrane surrounding the nucleus.[22] This phagocytosis is the reason for the inability to find extruded nuclei in marrow aspirates despite the fact that 2 million nuclei are extruded every second during steady-state erythropoiesis. A protective macrophage function linked to efficient phagocytosis has been described. In normal mice, DNase II in macrophages degrades the ingested nuclear DNA, but in DNase II-knockout mice, the inability to degrade DNA results in macrophage toxicity, with a resultant decrease in the number of marrow macrophages and in conjunction with severe anemia.[25] Macrophages can play both positive and negative regulatory roles in human erythropoiesis, but the mechanistic basis for these regulatory processes are not completely understood.[16,24] These processes may play a role in the ineffective erythropoiesis in disorders such as MDS, thalassemia, and malarial anemia.

Another potentially important role originally proposed for the central macrophage is direct transfer of iron to developing erythroblasts mediated by ferritin exchange between macrophages and erythroblasts (Chap. 10).[13] This is an interesting evolving concept with identification of various transport proteins involved in this exchange.

ERYTHROID PROGENITORS AND PRECURSORS

Early Progenitors

A "progenitor" in the hematopoietic system is defined as a marrow cell that is a derivative of the pluripotent hematopoietic stem cell through the process of differentiation, and is antecedent to a "precursor" cell, the latter being identifiable by light microscopy by its morphologic characteristics. In erythropoiesis, the earliest precursor is the proerythroblast. Erythroid progenitor cells are identified as marrow cells capable of forming erythroid colonies in semisolid medium in vitro under conditions in which the appropriate growth factors are present. Progenitor cells also may be identified by characteristic profiles of surface CD antigens using flow cytometry. Numerically, erythroid progenitors, BFU-E, and CFU-E represent only a minute proportion of human marrow cells. BFU-E range from 300 to 1700 × 10^6 mononuclear cells and CFU-E range from 1500 to 5000 × 10^6 mononuclear cells.[5] In vitro cultures using CD34+ cells from blood, cord blood, and marrow as the starting material have identified the critical cytokines required for erythroid differentiation and maturation and have enabled the identification and isolation of pure cohorts of erythroid progenitors and erythroblasts at all stages of terminal erythroid maturation.[4,5]

Precursors

Figure 1-2 shows the sequence of precursors as seen in marrow films. Figure 1-3 shows the marrow precursors as isolated by flow cytometry.

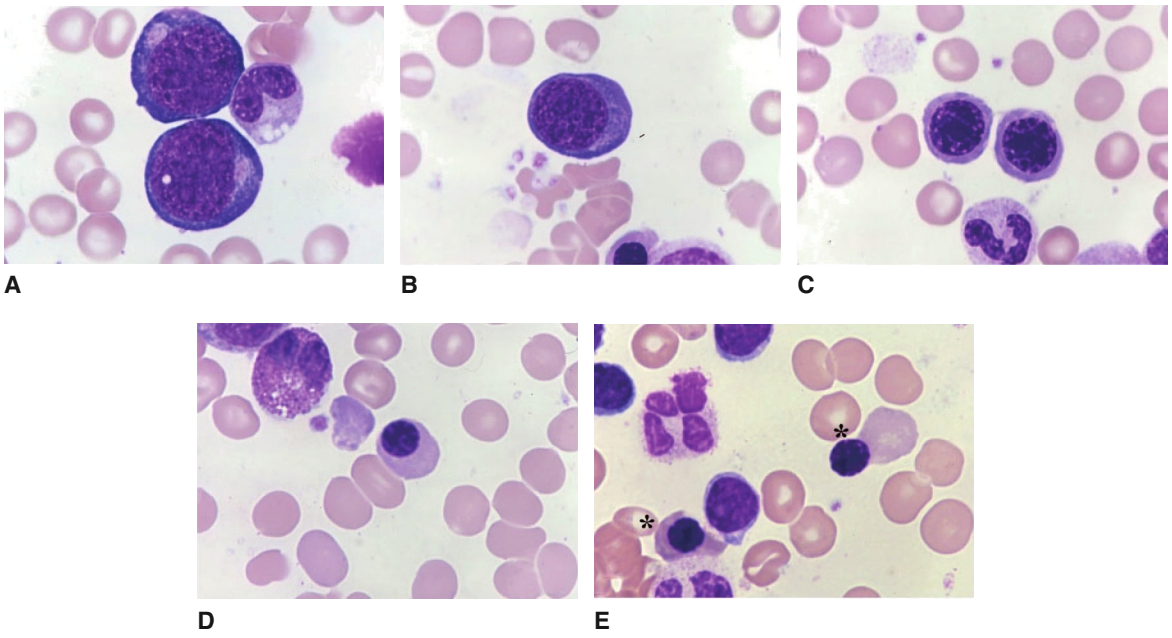


Figure 1–2. Human erythrocyte precursors. Light microscopic appearance. Marrow films stained with Wright stain. There are five stages of erythroblast development recognizable by light microscopy. **A.** Proerythroblasts. Two are present in this field. They are the largest red cell precursor, with a fine nuclear chromatin pattern, nucleoli, basophilic cytoplasm, and often a clear area at the site of the Golgi apparatus. **B.** Basophilic erythroblast. The cell is smaller than the proerythroblast, the nuclear chromatin is slightly more condensed, and cytoplasm is basophilic. **C.** Polychromatophilic erythroblasts. The cell is smaller on average than its precursors. The nuclear chromatin is more condensed, with a checkerboard pattern that develops. Nucleoli are usually not apparent. The cytoplasm is gray, reflecting the staining modulation induced by hemoglobin synthesis, which adds cytoplasmic content that takes an eosinophilic stain, admixed with the residual basophilia of the fading protein synthetic apparatus. **D.** Orthochromic normoblast. Smaller on average than its precursor, increased condensation of nuclear chromatin, with homogeneous cytoplasmic coloration approaching that of a red cell. **E.** Late orthochromatic erythroblasts *(asterisks)*. The orthochromatic erythroblast to the right is undergoing apparent enucleation. The other three mononuclear cells are lymphocytes. A degenerating four-lobed neutrophil is also present. *(Reproduced with permission from Lichtman MA, Shafer MS, Felgar RE, et al:* Lichtman's Atlas of Hematology *2016. New York, NY: McGraw Hill; 2017.)*

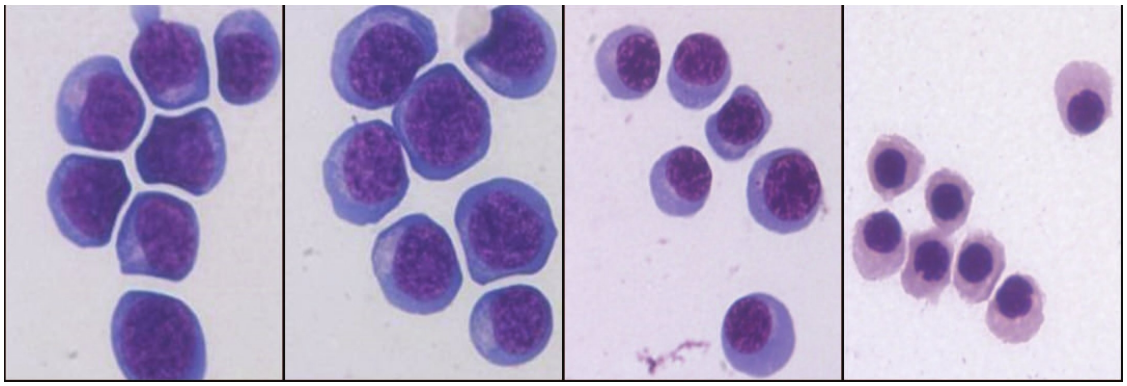


Figure 1–3. Human erythroblast precursors as isolated by cell flow cytometry. Images are of populations of human erythroblast precursors at stages of erythroid maturation when sorted from human marrow by flow cytometry. **A** and **B.** Proerythroblasts and early basophilic erythroblasts; **(C)** polychromatic erythroblasts; and **(D)** orthochromatic erythroblasts.

Proerythroblasts On stained films, the proerythroblast appears as a large cell, irregularly rounded or slightly oval.[13] The nucleus occupies approximately 80% of the cell area and contains fine chromatin delicately distributed in small clumps. One or several well-defined nucleoli are present. The high concentration of polyribosomes gives the cytoplasm of these cells its characteristic intense basophilia. At very high magnification, ferritin molecules are seen dispersed singly throughout the cytoplasm and lining the clathrin-coated pits on the cell membrane (Figs. 1-2 and 1-4). Diffuse cytoplasmic density on sections stained for peroxidase indicates that hemoglobin is already present. Dispersed glycogen particles are present in the cytoplasm.

Basophilic Erythroblasts Basophilic erythroblasts are smaller than proerythroblasts. The nucleus occupies three-fourths of the cell area and is composed of characteristic dark violet heterochromatin interspersed with pink-staining clumps of euchromatin linked by irregular strands.[13] The whole arrangement often resembles wheel spokes or a clock face. The cytoplasm stains deep blue, leaving a perinuclear halo that expands into a juxtanuclear clear zone around the Golgi apparatus. Cytoplasmic basophilia at this stage results from the continued presence of polyribosomes (Figs. 1-2 and 1-5).

Polychromatophilic Erythroblasts After the mitotic division of the basophilic erythroblast, the cytoplasm changes from deep blue to gray as hemoglobin dilutes the polyribosome content. Cells at this stage are smaller than basophilic erythroblasts. The nucleus occupies less than half of the cell area. The heterochromatin is located in well-defined clumps spaced regularly about the nucleus, producing a checkerboard pattern. The nucleolus is lost, but the perinuclear halo persists.[13] It is at this point that erythroblasts lose their mitotic potential. Electron microscopy of the polychromatophilic erythroblast reveals increased aggregation of nuclear heterochromatin.[13] Active ferritin transport across the cell membrane is always evident, and siderosomes along with dispersed ferritin molecules can be identified within the cytoplasm (Figs. 1-2 and 1-6).

Orthochromic (syn. Orthochromatic) Erythroblasts After the final mitotic division of the erythropoietic series, the concentration of hemoglobin increases within the erythroblast. Under the light microscope, the nucleus appears almost completely dense and featureless. It is measurably decreased in size. This cell is the smallest of the erythroblastic series.[13] The nucleus occupies approximately one-fourth of the cell area and is eccentric. Cell movement can be appreciated under the phase-contrast microscope. Round projections appear suddenly in different parts of the cell periphery and are just as quickly retracted.[13] The movements probably are made in preparation for ejection of the nucleus. The cell ultrastructure is characterized by irregular borders, reflecting its motile state. The heterochromatin forms large masses. Mitochondria are reduced in number and size (see Figs. 1-2, 1-7, and 1-8).

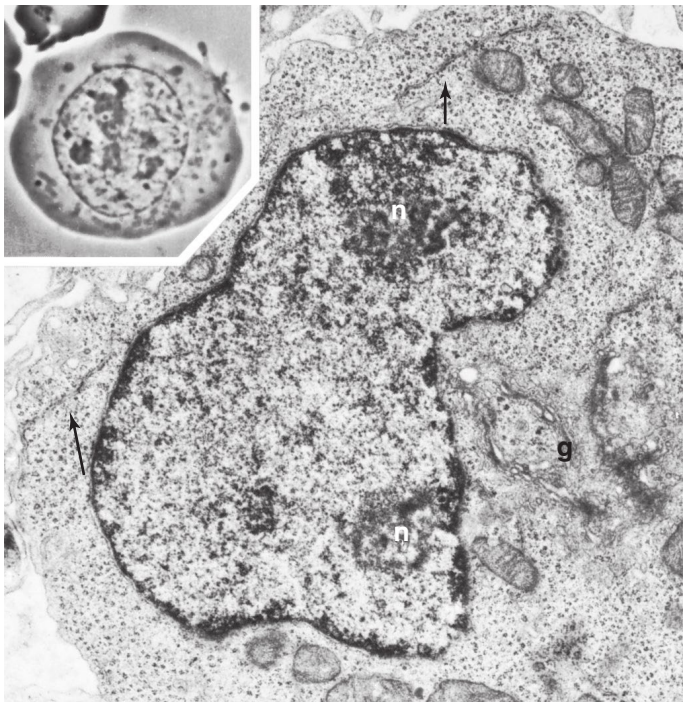


Figure 1–4. Proerythroblast. Phase-contrast micrograph *(inset)* of a proerythroblast showing the immature nucleus with nucleoli and finely dispersed nuclear chromatin. The centrosome (juxtanuclear clear zone) is apparent with its dense accumulation of mitochondria. Electron microscopic section of the proerythroblast shows nucleoli *(n)* in contact with the nuclear membrane. Chromatin is finely dispersed and forms small aggregates in the fixed nuclear membrane. The perinuclear canal is narrow but well defined. Polyribosome groups, many in helical configuration, are dispersed throughout the cytoplasm. The Golgi apparatus *(g)* is well developed, and regions of endoplasmic reticulum *(arrows)* are seen.

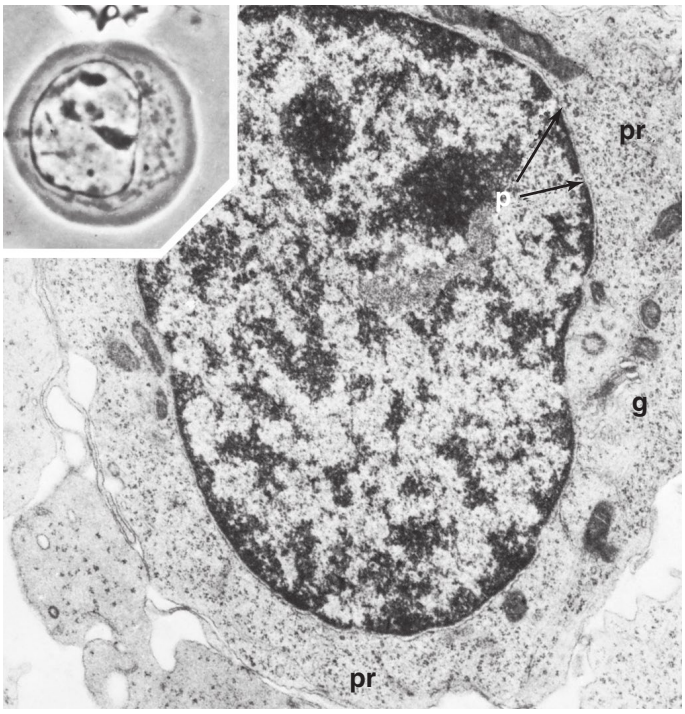


Figure 1–5. Basophilic erythroblast. Phase-contrast photomicrograph *(inset)* shows increased clumping of the nuclear chromatin and further rounding of the cell, with aggregation of the mitochondria and centrosome into the regions of nuclear indentation. The electron microscopic section shows clumping of the nuclear chromatin, nuclear pores *(p)*, organization of the nucleoli, increased density of polyribosomes *(pr)*, well-developed Golgi apparatus *(g)*, and a decrease in smooth endoplasmic reticulum.

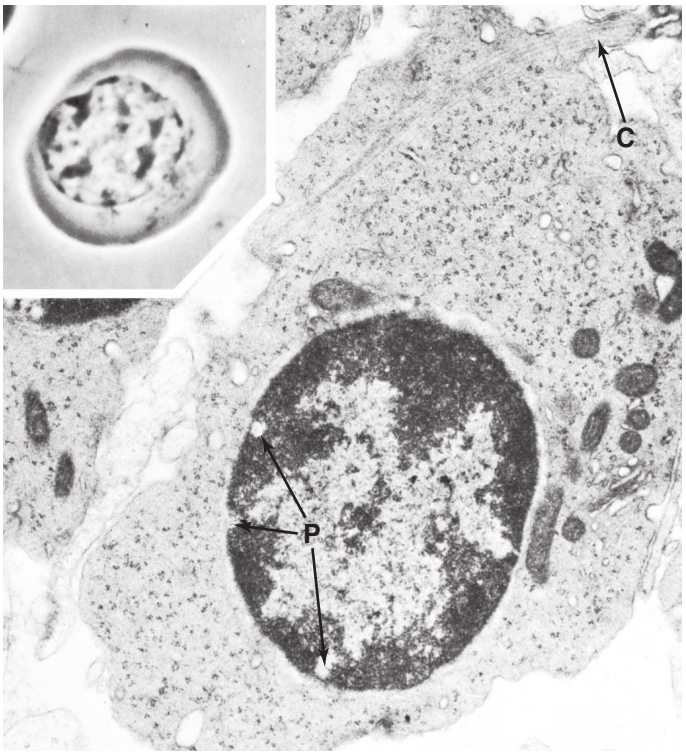


Figure 1–6. Polychromatophilic erythroblast. Phase-contrast micrograph *(inset)* demonstrates diminished size of this cell compared with its precursor. Further clumping of nuclear chromatin gives the nucleus a checkerboard appearance. The centrosome is condensed, and a perinuclear halo has developed. The electron microscopic section demonstrates relative reduction of the density of polyribosomes and dilution by the moderately osmiophilic hemoglobin in the cytoplasm. Nuclear chromatin shows a marked increase in clumping, and nuclear pores *(P)* are enlarged.

Normal Sideroblasts All normal erythroblasts are sideroblasts in that they contain iron in structures called *siderosomes*, as evident by transmission electron microscopy. These structures are essential for the transfer of iron for heme (hemoglobin) synthesis. By light microscopy, under the usual conditions of Prussian blue staining for iron, a minority of normal erythroblasts (approximately 15%-20%) can be identified as containing siderosomes, and those that can be so identified have very few (1-4) small Prussian blue–positive granules.

Pathologic Sideroblasts A heterogeneous group of erythrocyte disorders is accompanied by ineffective erythropoiesis, abnormal erythroblast morphology, and hyperferremia. These disorders include acquired megaloblastic anemia (Chap. 9), congenital dyserythropoietic anemias (Chap. 14), thalassemias (Chap. 17), the inherited and acquired sideroblastic anemias, pyridoxine-responsive anemia, alcohol-induced sideroblastic anemia, and lead intoxication (Chaps. 20 and 23). Some of these conditions are characterized by the presence of pathologic sideroblasts. Pathologic sideroblasts are of two types. The first is an erythroblast that has an increase in number and size of Prussian blue–stained siderotic granules throughout the cytoplasm. The second is the erythroblast that shows iron-containing granules that are arranged in an arc or a complete ring around the nucleus (Fig. 1-8). These pathologic sideroblasts are referred to as *ring* or *ringed sideroblasts*.[26,27] Electron microscopic studies show that granules in ringed sideroblasts are iron-loaded mitochondria. In cells with iron-loaded mitochondria, many ferritin molecules are deposited between adjacent erythroblast membranes.

RETICULOCYTE

Birth

Before enucleation at the late orthochromatic erythroblasts stage, intermediate filaments and the marginal band of microtubules disappear. Enucleation is a highly dynamic process that involves coordinated action of multiple mechanisms.[28-30] Tubulin and actin become concentrated at the point where the nucleus will exit. These changes, accompanied by microtubular rearrangements and actin polymerization, play a role in nuclear expulsion. Expulsion of the nucleus in vitro is not an instantaneous phenomenon; it requires a period of 6 to 8 minutes. The process begins with several vigorous contractions around the midportion of the cell, followed by a division of the cell into unequal portions. The smaller portion consists of the expelled nucleus surrounded by a thin ring of hemoglobin and plasma membrane (Fig. 1-9). In vivo, expulsion of the nucleus may occur while the erythroblast is still part of an erythroblastic island and the outer leaflet of the bilaminar membrane surrounding the expelled nucleus is high in phosphatidylserine, a signal for macrophage ingestion (Fig. 1-10).[22] Two hypotheses have been proposed to explain how the reticulocyte exits the marrow.[19-21] The reticulocyte may actively traverse the sinus epithelium to enter the lumen. More likely, however, the reticulocyte may be driven across by a pressure differential because it appears incapable of directed amoeboid motion. In vitro experimental evidence favors the hypothesis that pressure differential is likely the driver for reticulocyte release.[21]

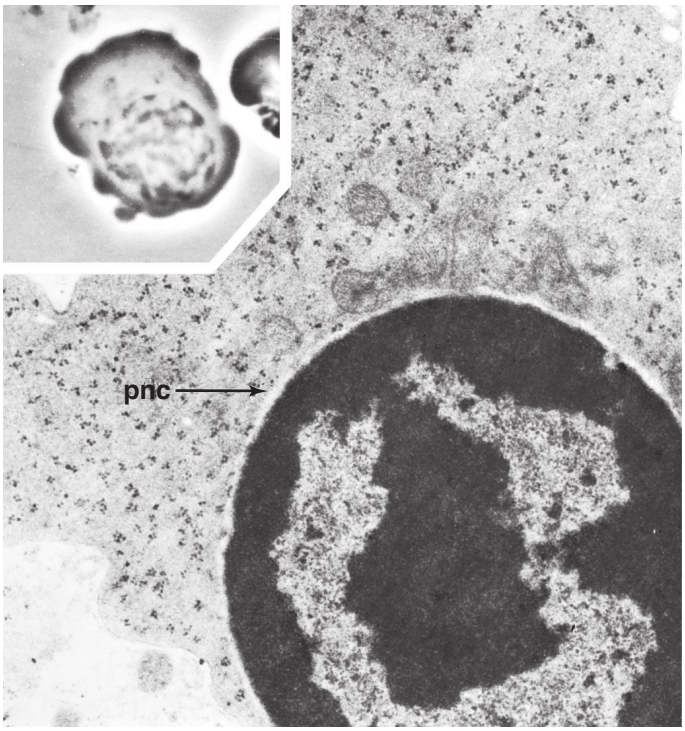


Figure 1–7. Orthochromic erythroblast. Phase-contrast appearance of this cell in the living state *(inset)* shows the irregular borders indicative of its characteristic motility, the eccentric nucleus making contact with the plasmalemma, further pyknosis of the nuclear chromatin, and condensation of the centrosome. The electron microscopic section shows further dilution of polyribosomes, some of which appear to be disintegrating into monoribosomes, by the increasing hemoglobin. The number of mitochondria is decreased, and some mitochondria are degenerating. Nuclear chromatin is clumped into large masses, and a perinuclear canal *(pnc)* is seen.

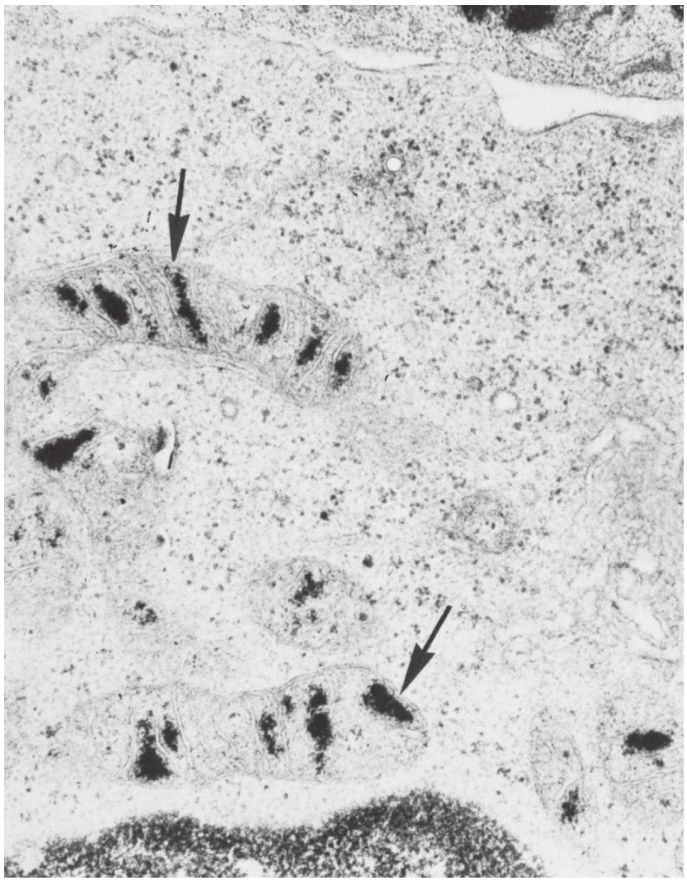


Figure 1–8. Pathologic sideroblast is an erythroblast characterized by the presence of mitochondrial deposits of iron-containing ferruginous micelles *(arrows)* between the cristae.

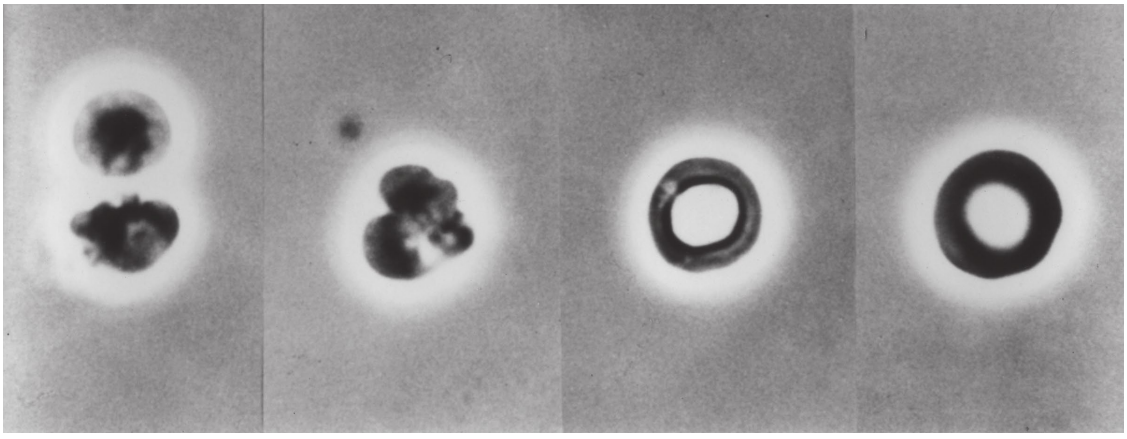


Figure 1–9. Morphology of cells during reticulocyte maturation. **A.** Orthochromatic erythroblast extruding its nucleus. **B.** Multilobular, motile reticulocyte generated after nuclear extrusion. **C.** The cup-shaped, nonmotile reticulocyte at a later stage of maturation. **D.** Mature discoid red cell.