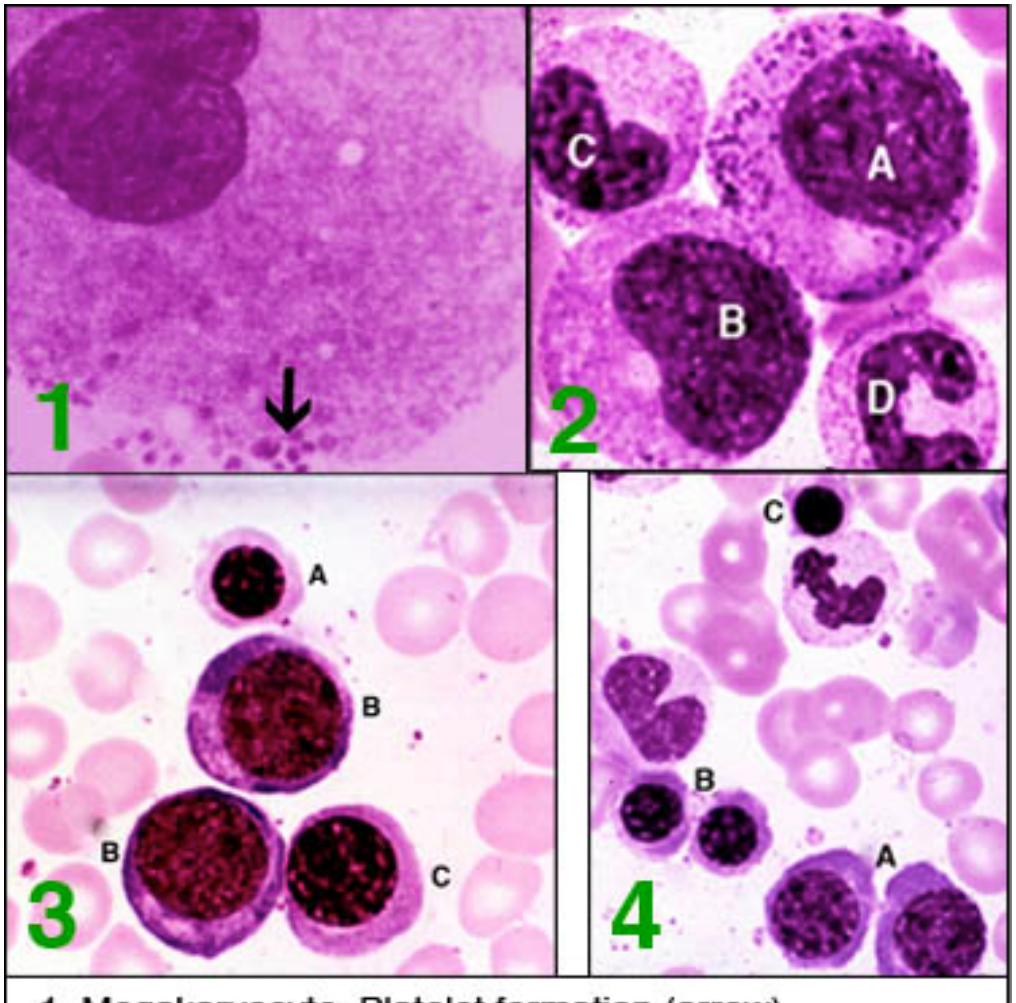
Hemopoiesis



1. Megakaryocyte. Platelet formation (arrow)
2. A: Promyelocyte: B: Myelocyte; C: Metamyelocyte; D: Neutrophil Band.
3. A: Polychromatophilic Erythroblast; B: Proerythroblast; C: Basophilic Erythroblast
4. A: Basophilic Erythroblast; B: Polychromatophilic Erythroblast; C: Normoblast.

The process of red cell production is called erythropoiesis, during which the erythrocyte undergoes progressive changes that involve the cytoplasm and nucleus. The cell progressively becomes smaller, and the cytoplasm stains increasingly acidophilic as it accumulates hemoglobin and loses organelles. The nucleus shrinks and becomes more heterochromatic and condensed until ultimately it is lost from the cell. Although it is possible to describe various "stages" in the developmental sequence, the process of erythropoiesis does not occur in stepwise fashion. The process is a continuous one in which, at several points, the cells show distinctive, recognizable features. Unfortunately, the nomenclature of the red cell precursors is

confused by the multiplicity of names given by various investigators to stages in the maturational series. The terms used here are commonly used, but alternative nomenclature is provided. The *proerythroblast (pronormoblast, rubriblast)* is the earliest recognizable precursor of the red cell line and is derived from the pluripotent stem cell through a series of restricted stem cells. The proerythroblast is relatively large, with a diameter of 15 to 20 μm. The nongranular, basophilic cytoplasm frequently stains unevenly, showing patches that are relatively poorly stained, especially in a zone close to the nucleus. The cytoplasmic basophilia is an important point in the identification of this early form of red cell. Synthesis of hemoglobin has begun, but its presence is obscured by the basophilia of the cytoplasm. The nucleus occupies almost three-fourths of the cell body, and its chromatin is finely and uniformly granular or stippled in appearance. Two or more nucleoli are present and may be prominent. In electron micrographs the endoplasmic reticulum and Golgi apparatus are poorly developed, but free ribosomes are abundant and polysomes are scattered throughout the cytoplasm. The proerythroblast undergoes several divisions to give rise to basophilic erythroblasts. The *basophilic erythroblast (basophilic normoblast, prorubricyte)* generally is smaller than the proerythroblast, ranging from 12 to 16 μm in diameter. The nucleus still occupies a large part of the cell, but the chromatin is more coarsely clumped and deeply stained. Nucleoli usually are not visible. The cytoplasm is evenly and deeply basophilic, sometimes more so than in the proerythroblast. Electron microscopy shows only a few or no profiles of endoplasmic reticulum, but free ribosomes and polyribosomes are abundant. Hemoglobin can be recognized as fine particles of low electron density but, as in the proerythroblast, is not always seen by light microscopy because of the intense cytoplasmic basophilia. The basophilic erythroblast also is capable of several mitotic divisions, its progeny forming the polychromatophilic erythroblasts. The nucleus of the *polychromatophilic erythroblast (polychromatophilic normoblast, rubricyte)* occupies a smaller part of the cell and shows a dense chromatin network with scattered coarse clumps of chromatin. Nucleoli are absent, and the cell is incapable of division. Cytoplasmic staining varies from bluish gray to light slate gray, reflecting the changing proportions of ribosomes and hemoglobin. When nucleoli disappear, no new ribosomes are formed, and the change in staining characteristics is the result of a decrease in the concentration of ribosomes (which stain blue) and a progressive increase in hemoglobin (which stains red). Cell size varies considerably but generally is less than that of the basophilic erythroblast. The polychromatophilic erythroblasts encompass several generations of cells, the size reflecting the number of previous divisions that have occurred in the basophilic erythroblast. It is sometimes convenient to divide these cells into "early" and "late" stages on the basis of their size and on the intensity of the cytoplasmic basophilia. *Acidophilic erythroblasts (acidophilic normoblast, metarubricyte)* are commonly called normoblasts. At this stage the cytoplasm is almost completely hemoglobinized and takes on a distinctly eosinophilic tint. Electron micrographs show a uniformly dense cytoplasm devoid of organelles except for a rare mitochondrion and widely scattered ribosomes. The nucleus is small, densely stained, and pyknotic and often is eccentrically located. Ultimately, the nucleus is extruded from the cell along with a thin film of cytoplasm. *Reticulocytes* are newly formed erythrocytes that contain a few ribosomes, but only in a few cells (less than 2%) are they in sufficient number to impart color to the cytoplasm. After the usual blood stains, these cells have a grayish tint instead of the clear pink of the more mature forms and hence are called polychromatophilic erythrocytes. When stained with brilliant cresyl blue, the residual ribosomal nucleoprotein appears as a web or reticulum that decreases as the cell matures and varies from a prominent network to a few granules or threads. Reticulocytes are about 20% larger in volume than normal mature red cells. After loss of the nucleus the red cell is held in the marrow for 2 to 3 days until fully

mature. Unless there are urgent demands for new erythrocytes, the reticulocytes are not released except in very small numbers. These young red cells form a marrow reserve equal to about 2% of the number of cells in circulation.

Loss of Nucleus

Ordinarily, the nucleus assumes an eccentric position in the cell (late normoblast) and is lost just before the cell enters a marrow sinusoid. Active expulsion of the nucleus by the normoblast has been observed in vitro and may involve some contractile protein, possibly spectrin. Enucleation of erythrocytes also has been described as the cells pass through the pores in the sinusoidal endothelium. The flexible cytoplasm is able to squeeze through the pore, but the rigid pyknotic nucleus is held back and stripped from the cell along with a small amount of cytoplasm. Free nuclei are rapidly engulfed and destroyed by macrophages.

Granular Leukocytes

The maturational process that leads to production of mature granular leukocytes is called granulocytopoiesis. During this process the cells accumulate granules and the nucleus becomes flattened and indented, finally assuming the lobulated form seen in the mature cell. During maturation, several stages can be identified, but as in red cell development, the maturational changes form a continuum, and cells of intermediate morphology often can be found. The stages commonly identified are myeloblast, promyelocyte, myelocyte, metamyelocyte, band form, and polymorphonuclear or segmented granulocyte. An alternative nomenclature substitutes the stem granulo for myelo, and the series of stages becomes granuloblast, progranulocyte, granulocyte, metagranulocyte, band form, and polymorphonuclear granulocyte. *Myeloblasts* are the first recognizable precursors of granular leukocytes and represent a restricted stem cell committed to granulocyte and monocyte production. It is present in bone marrow only in low numbers. The myeloblast is relatively small, ranging from 10 to 13 μm in diameter. The cytoplasm is rather scant and distinctly basophilic, but much less so than in the proerythroblast, and lacks granules. Electron microscopy reveals abundant free ribosomes but relatively little granular endoplasmic reticulum; mitochondria are numerous and small. The round or oval nucleus occupies much of the cell, stains palely, and presents a somewhat vesicular appearance. Multiple nucleoli are present. The *promyelocyte* is somewhat larger, measuring 15 to 22 μm in diameter. The nucleus may be slightly flattened, show a small indentation, or retain the round or oval shape. Chromatin is dispersed and lightly stained, and multiple nucleoli still are present. The basophilic cytoplasm contains purplish red azurophil granules, which increase in number as the promyelocyte continues its development.

Electron micrographs show abundant granular endoplasmic reticulum, free ribosomes, numerous mitochondria, and a well-developed Golgi apparatus. Azurophil granules are formed only during the promyelocyte stage and are produced at the inner (concave) face of the Golgi complex by fusion of dense-cored vacuoles. Divergence of granulocytes into three distinct lines occurs at the myelocyte stage with the appearance of specific granules. Thus neutrophil, eosinophil, and basophil myelocytes can be distinguished. *Myelocytes* are smaller than promyelocytes and measure 12 to 18 μm in diameter. The nucleus may be round or indented, and the chromatin is more condensed. Some myelocytes still may show a nucleolus outlined by a condensation of chromatin, whereas in others the nucleolus is poorly defined. The myelocyte is the last stage capable of mitosis. The cytoplasm of the neutrophil myelocyte

contains three populations of granules: azurophil granules produced at the promyelocyte stage and smaller specific granules formed by the myelocyte. Specific granules also arise by fusion of dense-cored vacuoles but are produced at the outer (convex) face of the Golgi complex rather than the concave face. As the myelocyte undergoes successive divisions, the number of azurophil granules in each cell is progressively reduced, and specific granules soon outnumber the azurophil type. The cytoplasm becomes less basophilic, and free ribosomes and granular endoplasmic reticulum are decreased. Specific neutrophil granules stain lightly in routine blood smears, taking up a delicate lilac-pink color, and are too small to be resolved individually with the light microscope. Azurophil granules are larger and stain purplish red with the usual bloodstains but are less numerous. With the electron microscope, azurophil granules appear more dense than neutrophil granules. The contents of the granules differ: azurophil granules are lysosomes and possess a complex of enzymes, among which aryl-sulfatase, acid phosphatase, beta-galactosidase, beta-glucuronidase, esterase, and nucleotidase have been identified. Specific neutrophil granules contain alkaline phosphatase and proteins with antibacterial properties. Myelocytes eventually reach a state at which they no longer can divide and then mature into *metamyelocytes*. These cells show most of the features of the myelocyte except that the nucleus is deeply indented to form a horseshoe or kidney shape, nucleoli are lacking, and the chromatin forms a dense network with many well-defined masses of chromatin. Specific granules make up more than 80 to 90% of the granules present; the remainder are azurophil. The neutrophil band has the same general morphology as the mature polymorphonuclear cell except that the nucleus forms a variously curved or twisted band. It may be irregularly segmented but not to the degree that definite nuclear lobes and filaments have formed. The stages of maturation of eosinophil granulocytes are the same as for the neutrophil. Specific eosinophil granules appear at the myelocyte stage and usually are identifiable soon after they appear. Occasionally, the granules may have a slightly purple-blue color, becoming progressively more orange as the cell matures. A rare eosinophil granule may be present as early as the promyelocyte stage. The granules are much larger than the neutrophil type, stain brilliantly with eosin, and in electron micrographs are only slightly less dense and smaller than azurophil granules. As the cells mature, the cytoplasm becomes less basophilic and the nucleus more and more indented to form lobes. In the late myelocyte and the metamyelocyte, the contents of the eosinophil granules crystallize. Some granules show a crystal of variable shape occupying the center of the granule, surrounded by a matrix of lower density; others remain dense and homogeneous. Eosinophil granules are lysosomes and contain the usual battery of lysosomal enzymes and myeloperoxidase. The crystalloid core is rich in major basic protein. The nuclei of a number of eosinophilic metamyelocytes segment into two portions that remain interconnected by a thin nuclear filament. These become eosinophils that exhibit bilobed nuclei. The remainder of the eosinophilic metamyelocytes become *band forms* and their nuclei segment into three or four lobes interconnected by nuclear filaments. Basophil granulocytes also pass through the same maturational sequences. The definitive granules usually appear at the myelocyte stage and rarely are seen in the promyelocyte. Initially, the granules are truly basophilic but become metachromatic and with toluidine blue or methylene blue stain violet rather than blue-black.

In addition to chemotactic and platelet factors, the granules contain heparin, histamine, and several enzymes including diaphorase, dehydrogenases, peroxidases, and histidine carboxylase, which converts histidine to histamine.

Platelets

Thrombopoiesis (thrombocytopoiesis) refers to the formation of platelets. Platelets are derived from giant cells, the megakaryocytes, which measure 100 μm or more in diameter. Megakaryocytes normally are found only in bone marrow. The nucleus of the megakaryocyte is large and convoluted and contains multiple irregular lobes of variable size interconnected by constricted regions. The coarsely patterned chromatin stains deeply. The cytoplasm is abundant and irregularly outlined and often has blunt pseudopods projecting from the surface. In smears, the cytoplasm appears homogeneous and contains numerous azurophil granules. Ultrastructurally, a variable degree of zoning is apparent. Immediately around the nucleus a narrow perinuclear zone contains a few mitochondria, the Golgi complex, granular endoplasmic reticulum, numerous polyribosomes, and some granules. A large intermediate zone is indistinctly separated from the perinuclear zone and contains granules, vesicles of different sizes and shapes, mitochondria, ribosomes, and components of the Golgi element. Depending on the degree of development of the megakaryocyte, the granules may be distributed uniformly or in small clusters outlined to a variable degree by a system of membranes. The outer most marginal zone is finely granular, varies in width, and lacks organelles; it does contain packets of microfilaments. Platelets are formed by segmentation of the megakaryocyte cytoplasm via a system of demarcation membranes. The azurophil granules form small clusters, and simultaneously, small vesicles appear that become aligned in rows between the groups of granules. The vesicles initially are discontinuous but subsequently elongate and fuse to create a three-dimensional system of paired membranes. The narrow spaces between the membranes form clefts that surround each future platelet; as they are shed, the platelets separate along the narrow clefts. Demarcation membranes are continuous with the plasmalemma, and thus each platelet is bounded by a typical cell membrane. The megakaryocyte delivers platelets through openings in the walls of the sinusoids, either as individual platelets or as ribbons of platelets that separate into individual elements within the sinusoidal lumen. After shedding its platelets, the megakaryocyte consists only of a nucleus surrounded by a thin rim of cytoplasm with an intact cell membrane. It generally is assumed that such megakaryocytes are unable to restore their cytoplasm and degenerate, with new generations of megakaryocytes being formed to replace them. Degenerate megakaryocytes can be found in the circulation, especially in the capillaries of the lung, where they may remain for some time. Megakaryocytes arise from stem cells, the first recognizable precursor being a large cell, 25 to 45 μm in diameter, with a single round or oval nucleus. The chromatin has a finely granular pattern, and the basophilic cytoplasm is free of granules. These cells, which are rare, have been called megakaryoblasts. The cell undergoes a series of divisions in which the nucleus is replicated but the cytoplasm does not divide (endomitosis). At metaphase, the chromosomes become aligned in several planes on an increasingly complex multipolar spindle. With subsequent reconstitution, groups of chromosomes are incorporated into a huge lobulated nucleus. Thus, a series of polyploid cells arises that may reach 64n in some megakaryocytes, although 16n nuclei are the more common. In general, cell and nuclear size are proportional to the degree of ploidy. The intriguing suggestion has been made that segmentation of the cytoplasm by demarcation membranes represents a delayed and modified cytokinesis. Cells of 4n and 8n ploidy, measuring 30 to 40 μm in diameter, frequently are called promegakaryocytes. The fully formed but not yet functional megakaryocyte consistently shows a clear marginal zone, whereas in platelet-forming megakaryocytes this zone disappears.

Monocytes

Monocytes originate from a pool of precursors in the bone marrow; most likely the same restricted stem cells that give rise to granular leukocytes. In cultures, cells from granulocytic spleen colonies yield monocytes as well as granulocytes. Immature monocytes of the bone marrow (promonocytes) are rare and difficult to distinguish. They range from 8 to 15 μm in diameter and possess large round to oval nuclei with evenly dispersed chromatin and several nucleoli. The cytoplasm is fairly abundant and contains numerous free ribosomes but only scant endoplasmic reticulum. The prominent Golgi complex is associated with numerous small granules that represent the formative stages of azurophil granules. The mature monocytes of the bone marrow closely resemble those of the blood, are somewhat smaller than promonocytes (9 to 11 μm in diameter), and contain fewer ribosomes and larger and more abundant azurophil granules. Azurophil granules contain a variety of hydrolytic enzymes and are primary lysosomes. Following release into the blood, the monocyte continues its maturation in the circulation, and additional azurophil granules are formed. Monocytes migrate into various tissues where they complete their maturation by transforming into macrophages, such as those of the peritoneum, alveoli of the lungs, or Kupffer cells of the liver. At these sites they receive further molecular programming from the surrounding environment to carry out specialized functions.

Marrow Lymphocytes

Lymphocytes arise from pluripotent stem cells in the marrow and then emigrate to populate the lymphatic organs and tissues. Restricted stem cells that reach the thymus proliferate there and are released as T-lymphocytes (T-cells). After being released from the thymus, the T-cells migrate to the spleen, where they complete their maturation and are released as long-lived lymphocytes. B-lymphocytes (B-cells) also originate in the marrow. In birds they migrate to the bursa of Fabricius, where they differentiate into B-cells. The mammalian equivalent of the bursa has not been identified, but the bone marrow itself may be the organ for differentiation of B-cells. These cells also appear to complete their development in the spleen. B- and T-cells circulate and recirculate through the blood and lymphatic organs. In humans, B-cells have a life span of at least several months and T-cells may exist for several years.

Control of Blood Formation

Erythropoiesis is under humoral control and is regulated mainly by a specific erythropoiesis-stimulating factor called erythropoietin. However, the fundamental stimulus for red cell production is hypoxia, since the rate of production of erythropoietin is inversely related to the oxygen supply of the tissues. Erythropoietin is a glycoprotein hormone mainly produced by the kidney, though this is not the sole source of erythropoietin, since it can be produced in animals whose kidneys have been removed. The sites of extrarenal production of erythropoietin are not known, but small amounts of an erythropoietic-active substance have been detected in liver perfusates. The liver is the primary source of erythropoietin in the fetus. Erythropoietin increases synthesis of ribonucleic acid (RNA) and protein, followed by the initiation of hemoglobin synthesis and differentiation of the restricted stem cell. Erythropoietin also appears to accelerate hemoglobin synthesis in those more differentiated cells, which still are capable of synthesizing RNA, and promotes release of reticulocytes from the marrow. Hormones other than erythropoietin have been implicated in blood formation. Testosterone stimulates red cell

development and appears to be important for normal maintenance of red cell formation; estrogen has the opposite effect. Whether there are individual hemopoietic hormones for each of the cell lines in bone marrow is unknown. There is evidence for circulating proteins that control the differentiation of granulocytes and megakaryocytes. These factors, granulopoietin and thrombopoietin, have not been characterized fully, nor is their tissue of origin known.
Agents capable of controlling the proliferation and maturation of specific CFUs have been demonstrated in T-lymphocytes.