A Study of the Morphology of the Living Cells of Blood and Bone Marrow in Vital Films with the Phase Contrast Microscope

I. Normal Blood and Bone Marrow

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INTRODUCTION

THE DIAGNOSIS OF HEMATOLOGIC DISCRASIAS is ultimately resolved to the microscopic identification and characterization of the cells of the blood and bone marrow. Often the delineation between normal and pathologic cell forms is dependent upon minute alterations in cellular morphology. The relative ease of visualization afforded by the phase contrast microscope for the study of the living cell has encouraged many laboratories to utilize this technic as an adjunct to the diagnosis of hematologic dyscrasias.

It is the purpose of this report to characterize and illustrate the cells of the normal blood and bone marrow on vital films with the phase contrast microscope and to correlate these observations with those employing the supravital staining technic. Although numerous investigators14-19 have studied the cells of the hemopoietic system with the phase microscope, few have characterized these cells in sufficient detail to provide an adjunct in the study of the cytochemistry of the cells and their components. In order to aid the experienced and to familiarize the uninitiated investigator of the cells examined in vital films, we have considered it essential to summarize briefly the salient features of the cells of the hemopoietic system to serve as a useful guide and to provide a foundation for further morphologic and cytochemical research.

MATERIALS AND METHODS

Fresh moist films prepared by touching a clean coverslip to a drop of blood and inverting it upon a clean or supravital (neutral red and janus green) prepared slide13 were examined with the phase contrast (American Optical Co., dark 4, 97X oil immersion objective) and bright field microscopes.

Normal peripheral blood was obtained from personnel in our laboratory; bone marrow specimens were obtained from patients with nonhematologic conditions, including arteriosclerosis, essential hypertension, and diabetes mellitus. Differential cell counts and the morphologic characteristics of the cellular elements of the blood and bone marrow in all instances were within normal limits.

The cells of the blood and bone marrow have been classified according to the polyphyletic doctrine of Cunningham, Sabin and Doan.13 Morphologic descriptions are based upon phase microscopic examination of these cells in supravital as well as unstained vital films.
The coloration (staining) of the cytoplasm and cytoplasmic organelles are described from supravital films following bright field microscopic examination rather than from the phase contrast microscope.

**Observations**

**Neutrophil (figs. 1–5)**

Upon immediate examination of moist films the neutrophil has a round contour with well-defined cytoplasmic borders. It measures 12–18 μ in diameter. Active ameboid movement begins within a few minutes as the cell adjusts to its new environment. A small clear cytocentrum located near the center of the cell is encompassed by granules and a lobulated or band-form nucleus. The nucleus exhibits a dense irregular chromatin pattern, a thin nuclear membrane, and fine chromatin filaments which unite its segmental lobes. The cytocentrum and adjacent hyaloplasm exhibit a greater viscosity and are optically less dense than the remainder of the hyaloplasm. The organelles oscillate (Brownian effect) to and fro within the relatively non-viscous cytoplasm and tend to follow the protoplasmic flow during the ameboid activity of the cell.

The most numerous organelle of the neutrophil is a small (0.3 μ) moderately refractile oval granule (A) which is colored tan with neutral red. These granules usually localize in the peripheral cytoplasm while smaller (0.2–0.25 μ) spherical or oval, less refractile granules (B) concentrate near the cytocentrum. The B granules stain less intensely (pink-tan) with neutral red than the A granules. A small number of highly refractile granules (C) measuring 0.5 μ in diameter in unstained moist films are scattered throughout the cytoplasm and have the capacity to segregate neutral red in the supravital films. With the segregation of the dye, these granules (vacuoles) enlarge in size and change their staining reaction from pale orange to deep red. A few fine (0.2 μ) nonrefractile spherical mitochondria which fail to stain with janus green are dispersed throughout the cytoplasm of the neutrophil.

**Eosinophil (figs. 6–8)**

The eosinophil, closely resembling the neutrophil in size and nuclear structure, exhibits a more sluggish form of ameboid activity than the neutrophil. Eosinophil nuclei are usually bilobed with a dense irregular chromatin pattern and a thin nuclear membrane. Relatively large highly refractile spherical or ovoid granules which vary in size (0.25–0.7 μ) and coloration (yellow or yellow-brown) with neutral red fill the cytoplasm. Frequently several granules may be distinguished near the clear cytocentrum which are only moderately refractile and stain deep orange or red with neutral red. Many small (0.2–0.25 μ) spherical nonrefractile mitochondria failing to stain with janus green are scattered throughout the cytoplasm of the eosinophil.

**Basophil (figs. 9, 10)**

The basophil, the smallest of the granulocytes, measured 10–14 μ in diameter. It exhibits a characteristic sluggish type of motility in which the nucleus is advanced, rather than cytoplasmic pseudopodia as seen in the neutrophil and eosin-
ophil. The basophil has a band or bilobed nucleus with a dense irregular chromatin pattern and a thin nuclear membrane. The granules are nonrefractile, oval, spherical, or irregular in shape. They vary in size between 0.3 and 0.8 μ and stain pale pink to brick red with neutral red. A few small (0.2–0.3 μ) nonrefractile, non-

PLATE 1.

All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

Fig. 1.—Normal band form neutrophil of the peripheral blood. Marker indicates 10 μ.
Fig. 2.—Four lobed neutrophil of the peripheral blood.
Fig. 3.—Four lobed neutrophil of the peripheral blood.
Fig. 4.—Four lobed neutrophil of the peripheral blood.
Fig. 5.—Five lobed neutrophil of the peripheral blood.
Fig. 6.—Normal band form eosinophil of the peripheral blood.
Fig. 7.—Motile bi-lobed eosinophil of the peripheral blood.
Fig. 8.—Bi-lobed eosinophil of the peripheral blood.
Fig. 9.—Motile bi-lobed basophil of the peripheral blood.
Fig. 10.—Motile band form basophil of the peripheral blood.
Fig. 11.—Old lymphocyte of the peripheral blood.
Fig. 12.—Mature small lymphocyte of the peripheral blood.
staining ovoid mitochondria are scattered diffusely throughout the cytoplasm of the basophil.

**Lymphocytes** (figs. 11–17)

The nucleus advances ahead of the cytoplasm during the slow movement of the lymphocyte which begins within a few minutes after the preparation of the moist film. Lymphocytes have a round or ovoid contour with well-defined borders and a small amount of cytoplasm surrounding a slightly indented or oval nucleus. The nucleus has a dense coarse chromatin pattern with a prominent nuclear membrane and usually contains a small nucleolus. Several small (0.4–0.7 µ) slightly refractile spherical granules, which stain deep red with neutral red, localize near the small nuclear hof and cytocentrum. A highly refractile small nonstaining lipoid droplet is seen occasionally in the cytoplasm near the cytocentrum. Small nonrefractile elongate (0.4–1.5 µ) or spherical (0.2–0.5 µ) blue-green staining mitochondria are scattered throughout the cytoplasm concentrating near the nuclear hof. The perinuclear zone and cytocentrum is less basophilic and opaque than the remaining homogenous hyaloplasm.

Marked variations occur in the morphology of the lymphocytes in peripheral blood which may reflect the degree of cell maturity. Younger cell forms exhibit a more vesicular nucleus with more abundant and basophilic cytoplasm containing a greater number of mitochondria and granules than the mature and older lymphocytes. Cell size *per se* does not appear to be the most reliable criteria for estimating lymphocyte maturity although most intermediate and large sized lymphocytes are in the young age group as judged by the characteristics mentioned above.

**Monocyte** (figs. 18–24)

Large film-like pseudopods are slowly extended from the delicate cytoplasm of the monocyte during its motility in moist films. The large oval or reniform nucleus has a delicate chromatin network and a thin nuclear membrane and usually contains one or two small nucleoli. Many small (0.3–0.6) nonrefractile spherical or slightly irregular granules staining orange-pink with neutral red are scattered throughout the cytoplasm and tend to localize near the nuclear hof and cytocentrum. Monocytes in unstained moist films exhibit a few granules within their cytoplasm which have a greater optical density and are slightly larger (0.4–0.6 µ) than the granules previously described. These organelles are capable of segregating neutral red increasing in size (0.7–1.3 µ) and represent the so-called segregation vacuoles characterizing the monocyte stained by the supravital technic. The rosette of vacuoles seen in the monocytes of various laboratory animals is not well delineated in the human monocyte. Many nonrefractile small (0.2–0.3 µ) spherical mitochondria are scattered diffusely throughout the cytoplasm and stain green-blue with janus green. Occasionally several small (0.6–0.9 µ) clear nonstaining spherical areas may be seen in the hyaloplasm of the monocyte in unstained moist, supravital and Wright's stained films. An increase in the contrast of the various organelles occurs during supravital staining without alteration in morphologic characteristics (with the exception of the cytoplasmic vacuoles). Variations in the morphology of the monocytes in peripheral blood may
be attributed to differences in the age of the cell. The older monocytes have deeply indented nuclei, more abundant, less basophilic cytoplasm with fewer mitochondria, and more numerous cytoplasmic granules and vacuoles than the young monocytes.

All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

**Plate 2**

*Fig. 13.*—Mature small lymphocyte of the peripheral blood.

*Fig. 14.*—Young small lymphocyte of the peripheral blood.

*Fig. 15.*—Young slightly motile small lymphocyte of the peripheral blood.

*Fig. 16.*—Young small lymphocyte of the peripheral blood.

*Fig. 17.*—Intermediate young lymphocyte of the peripheral blood.

*Fig. 18.*—Monocyte of the peripheral blood.

*Fig. 19.*—Monocyte of the peripheral blood.

*Fig. 20.*—Monocyte of the peripheral blood.

*Fig. 21.*—Monocyte of the peripheral blood.

*Fig. 22.*—Motile monocyte of the peripheral blood.

*Fig. 23.*—Monocyte of the peripheral blood several hours after preparation of the film.

*Fig. 24.*—Living unstained motile monocyte in the peripheral blood.
Morphology of Living Blood and Bone Marrow Cells. I

Myeloid Cells (figs. 25–36)

The earliest neutrophil progenitor, the myelocyte A, found in normal bone marrow is a round or ovoid cell measuring 14–20 \( \mu \) with a large slightly indented or oval nucleus possessing a delicate chromatin network, a thin nuclear membrane, and several (2–5) small oval nucleoli. The cytoplasm is homogenous, moderately opaque (phase microscope) and has a clear yellow-gray appearance (bright field). Small (0.2–0.3 \( \mu \)) spherical nonrefractile mitochondria which stain pale green-blue (janus green) are scattered throughout the cytoplasm concentrate near the nuclear hof. One to 20 small (0.3 \( \mu \)) spherical deep red staining granules localize about the cytocentrum. A more primitive potential granulocyte, the myeloblast, exhibits the same morphologic characteristics as the myelocyte A except that it is devoid of granules and rarely seen in the bone marrow.

The number of granules and the physical state (gel or sol) of the cytoplasm serve as the most reliable index for estimating the degree of maturation of the myeloid cells. Following the myelocyte A stage, the cell is designated as a myelocyte B until it contains a full complement of granules when it is then termed myelocyte C. As the granules begin to exhibit Brownian motion, the cell is considered a metamyelocyte reaching the mature form with the initiation of ameboid motion and the formation of a banded or lobulated nucleus.

The neutrophil enlarges during the first part of its development, attaining its maximum size (16–27 \( \mu \)) at the mid B myelocyte level and then decreases, reaching its adult size during the metamyelocyte or band-form stage. As the nucleus enlarges, it assumes an indented contour with a thinning of the nuclear membrane, a condensation of the chromatin network and a diminution in number and size of the nucleoli. The mitochondria are reduced in number and alter their staining properties with janus green changing from a light green-blue to a deeper blue-green and usually lose their staining capacity during the myelocyte C and metamyelocyte stages. As the cytoplasmic granules increase in number, their coloration with neutral red changes from dark red to orange-brown, appearing yellow-brown and tan at the myelocyte C and metamyelocyte stages. The granules form near the cytocentrum and enlarge (0.3–0.7 \( \mu \)) slightly as they are forced peripherally by the newly elaborated granules. The type A, B, and C granules of the neutrophil may be distinguished during the middle and late portion of the myelocyte B stage, although their coloration with neutral red suggests a more acidic nature of the granules in these immature cells.

Developing basophils and eosinophils differ from the adult cells in the following ways. Mitochondria are stainable with janus green in the young cells and are unstained in the mature cells. The cytocentrum is larger in the eosinophil and basophil myelocytes than in the mature forms. Eosinophil myelocytes contain a greater number of red staining granules near the cytocentrum and also have larger highly refractile granules in the periphery of the cytoplasm than the adult cell. Basophil myelocytes are smaller than the cells of the neutrophil and eosinophil series. In other respects the general morphologic changes occurring in the developing eosinophils and basophils correspond with those described for the neutrophil.
All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

Fig. 25.—Early neutrophilic myelocyte A of the normal bone marrow. A number of small mitochondria are scattered about the cytoplasm and one darker specific granule may be distinguished at eight o’clock in the cytoplasm.

Fig. 26.—A. Neutrophilic myelocyte A of the normal bone marrow. Note small cluster of darker specific granules near the cytocentrum. B. Neutrophilic myelocyte C. C. Neutrophilic metamyelocyte.

Fig. 27.—A. Young neutrophilic Myelocyte B with a large cluster of granules near the cytocentrum. B. Neutrophilic myelocyte C. A small lymphocyte and normoblast are also present.

Fig. 28.—Early neutrophilic myelocyte B in the bone marrow.

Fig. 29.—Large neutrophilic myelocyte B in the bone marrow.

Fig. 30.—Neutrophilic myelocyte B in the bone marrow.
Erythroid Series (figs. 37–43)

The earliest erythrocyte progenitor, the early erythroblast, found in normal bone marrow is a large cell (18–24 μ) with a round or oval nucleus, extremely vesicular chromatin network and a thin nuclear membrane. It contains several round or irregular nucleoli which vary in size and optical density. A few small (0.3–0.6 μ) spherical, nonrefractile granules staining deep orange-red with neutral red localize near the cytocentrum and may form a rosette pattern about the centrosphere. Spherical or slightly elongate (0.2–0.4 μ) nonrefractile mitochondria which color light green-blue with Janus green and are less dense optically than the cytoplasmic granules concentrate about the nucleus and cytocentrum. The cytoplasm is deeply basophilic, exhibiting a yellow-gray cast under bright field microscopy while hemoglobin, which first appears about the cytocentrum, has a more deep yellow color. These two substances cannot be readily differentiated with phase microscopy.

The hemoglobin content of the developing erythroid cells varies inversely with cell size and cytoplasmic basophilia. The large early erythroblast containing only a faint trace of hemoglobin develops into a late erythroblast as the hemoglobin content increases and the size is reduced to between 14 and 20 μ. The cell is classified as a normoblast when it measures less than 14 μ and has a high hemoglobin content. During the maturation, the nucleus decreases in size, the chromatin becomes coarse and more compact, and the nuclear membrane becomes thickened. Mitochondria assume an elongate contour, enlarge slightly (0.3–0.5 μ), stain more deeply with Janus green and gradually decrease in number as the cell becomes more mature. Only a few mitochondria and cytoplasmic granules remain within the cell, reticulocyte, following the extrusion of the pyknotic nucleus from the normoblast. These organelles soon disappear and the erythrocyte reaches its adult form.

Megakaryocyte (fig. 48)

The megakaryocyte, which may reach 80 μ in diameter, possesses an irregular cytoplasmic contour, a large lobulated nucleus with a coarse chromatin pattern and a thin nuclear membrane. The cytoplasm is packed with small granules and mitochondria, giving a ground glass appearance to the cytoplasm. The mitochondria are nonrefractile and rarely stain with Janus green. Platelets appear to be actively formed from the fragmentation of the peripheral portions of the cytoplasm of the megakaryocyte. The cytoplasmic granules are slightly larger than the mitochondria, moderately refractile, and infrequently stain with neutral red. Platelets are ovoid or irregular in contour and usually measure between 2–7 μ in diameter. A small number of fine (0.2–0.3 μ) nonrefractile, nonstaining mitochondria and one to several larger (0.3–0.7 μ) moderately refractile granules, which frequently stain with neutral red, may be distinguished within the platelet.

Plasma Cell (fig. 44)

The plasma cell measures 16–20 μ in diameter, is nonmotile and has an ovoid contour. The nucleus is eccentrically placed within the cell and has blocks of chromatin aggregated about the thin nuclear membrane and near the center of the nucleus. The cytocentrum and surrounding hyaloplasm is less opaque and
All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

Fig. 31.—Late neutrophilic myelocyte B in the bone marrow.

Fig. 32.—A. Motile band form neutrophil. B. Plasma cell. C. Neutrophilic myelocyte C. D. Neutrophilic metamyelocyte of the bone marrow.

Fig. 33.—Late neutrophilic myelocyte B in mitosis; also a neutrophilic myelocyte C, and a motile b1-labeled neutrophil in the bone marrow.

Fig. 34.—A. Basophilic myelocyte C. B. Neutrophilic myelocyte B in the bone marrow.

Fig. 35.—Eosinophilic myelocyte B in the bone marrow.

Fig. 36.—Eosinophilic metamyelocyte in the bone marrow.
All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

Fig. 37.—Two early erythroblasts and a cluster of platelets in the bone marrow.

Fig. 38.—A. Early erythroblast. B. Early neutrophilic myelocyte A in the bone marrow.

Fig. 39.—Two late erythroblasts in close proximity to one another in the bone marrow.

Fig. 40.—Two early forms of normoblasts in the bone marrow.

Fig. 41.—Four normoblasts in the bone marrow in addition to a small lymphocyte in the lower right hand corner.

Fig. 42.—A group of normoblasts in the bone marrow. One normoblast is extruding its nucleus.
All figures are photographed from supravital films with the phase contrast microscope. Magnification 1275X.

Fig. 43.—A. Large normoblast in mitosis. B. Normoblast in the bone marrow.

Fig. 44.—A. Neutrophilic metamyelocyte. B. Plasma cell in the bone marrow.

Fig. 45.—Clasmatocyte containing a partially digested neutrophil and cellular debris. The ingested material stains deeply with neutral red.

Fig. 46.—Clasmatocyte containing ingested erythrocytes in the bone marrow.

Fig. 47.—Endothelial cell in the bone marrow.

Fig. 48.—Megakaryocyte in the bone marrow which is not actively producing platelets. A small cluster of platelets is seen at the top of the field. Mag. X640.
basophilic than the peripheral deeply basophilic hyaloplasm. Mitochondria and cytoplasmic granules form a large rosette about the cytocentrum of the plasma cell. Mitochondria are nonrefractile, spherical to filliform in shape (0.2–1.5 μ in length), and stain blue-green with janus green. The cytoplasmic granules are spherical (0.3–0.7 μ), have a greater optical density than the mitochondria, and stain orange-red with neutral red. A small (0.7 μ) highly refractile, nonstaining lipoid droplet is seen frequently in the cytoplasm of the plasma cell near the centrosphere.

Clasmatocyte (figs. 45, 46)

The clasmatocyte is a rather large cell (19–30 μ) with irregular indistinct cytoplasmic borders, a vesicular oval nucleus with a thin nuclear membrane, and one or two small indistinct nucleoli. The cytoplasm has a granular appearance and is filled with amorphous debris, including platelets, erythrocytes and leukocytes, which vary in size, shape and coloration (orange, pink or red), with neutral red as a result of partial enzymatic digestion. The clasmatocyte contains a few spherical (0.2–0.3 μ) mitochondria which are extremely difficult to differentiate in the cytoplasm due to their failure to stain with janus green. Intrinsic cytoplasmic granules (0.3–0.6 μ) are present but cannot always be distinguished from the phagocytized material.

Endothelial Cell (fig. 47)

Endothelial cells occasionally seen in the bone marrow specimens are large elongate (40–60 μ), nonmotile cells with indistinct cytoplasmic borders and an oval nucleus. The nucleus is eccentrically placed within the cell and has a delicate chromatin network and a well-defined nuclear membrane and usually contains a small indistinct nucleolus. The cytoplasm is faintly basophilic and contains a large number of mitochondria and small granules; the cytocentrum is small and indistinct. Mitochondria are nonrefractile with a spherical or elongate (0.2–0.5 μ) contour and usually fail to stain with janus green. The granules stain orange-brown or tan with neutral red and are relatively nonrefractile and spherical (0.25–0.7 μ) in shape. A few highly refractile nonstaining lipoid droplets (0.5–1.2 μ) are scattered throughout the cytoplasm of the endothelial cell.

A table summarizing the characteristics of the cells of normal blood and bone marrow studied with the phase and bright field microscopes employing supravital and unstained vital films is available upon request.

Discussion

The morphology of the cells of the blood and bone marrow have been characterized and illustrated as they are observed by the phase contrast microscope. Supravital and unstained vital films have been employed in this investigation. The findings do not differ essentially from those already reported by other investigators. A comparison and correlation of features observed by both the supravital bright field and phase microscope have been described which aid in the interpretation and evaluation of the cells of the blood and bone marrow studied with the phase microscope.
The phase microscope permits a more clear visualization of the fine structure of the cell in addition to facilitating its photography, although it is seldom that the phase microscope has revealed more detail than that already described by skilled microscopists employing the supravital bright field microscope method. Ideally, phase contrast studies of the living cell should be combined with the bright field examination of supravital preparations, since the latter method affords aid in the identification of the cell’s organelles by the selective staining with the vital dyes. This combined approach would enhance interpretation of the phase studies.

Cytochemical localization of substances within the cell must have its foundation in morphology and must strive for exact localization within the structural elements of the intact living cell. The precise morphologic characterization of the cell and its components is essential in a study of their composition and function, as well as for the ultimate diagnosis of the hematologic dyscrasias.

**SUMMARY**

The cells of normal human blood and bone marrow have been examined in the living condition by means of the phase contrast microscope employing both supravital and unstained moist films. The morphologic characteristics of the cells studied in this manner have been carefully described and illustrated.

**SUMMARIO IN INTERLINGUA**

Le cellulas de normal sanguine e medulla ossee human esseva examine in stato vive per medio del microscopio a contrasto phasic. Esseva usate films supravital e films humide non-coborate. In le presente reporto le caracteristicas del cellulas assi studiate es describite e illustrate in grande detalio.

**REFERENCES**


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