Electron Microscope Studies on Normal Human Myeloid Elements

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The publication of Low and Freeman's Electron Microscopic Atlas of Normal and Leukemic Human Blood in 1958 served as a stimulus for additional studies on the ultrastructure of blood and bone marrow cells. A recent review of the development of human blood cells has demonstrated many of the cellular organelles and described nuclear and cytoplasmic features of developing cells.

The present study was undertaken to trace the development of myeloid elements in greater detail with particular reference to changes in cellular organelles and their relation to granulogenesis. The cytoplasmic changes coincident with maturation can be clearly followed in the accompanying electron micrographs. This study is the basis for further correlative work on fine structure and cytochemistry in normal and pathologic material. Simultaneously, an endeavor was made to improve technics of blood preservation. Previous studies have usually employed methacrylate as the embedding medium. This synthetic resin, although generally useful for other tissues, is not satisfactory for blood, as it causes clumping of nuclear components and obscuring of cytoplasmic detail necessary for organelle study.

Materials and Methods

Samples of bone marrow were obtained from patients, clinically free of hematologic disorders, by aspiration of the iliac crest. Smear preparations were made for whole cell study by light microscopy and treated with Wright's blood stain. Samples for electron microscopy were fixed in cold 1 per cent osmium tetroxide in modified Michaelis' acetate-veronal buffer, pH 7.4, for 1 hour, washed in buffer and pre-stained for 30 minutes in buffer containing 0.5 per cent uranyl acetate. The usual graded ethanol series was employed for dehydration, followed by embedding in Epon 812. Polymerization was accomplished at 60°C for 12-18 hours. Silver to gold sections, cut on a Porter-Blum microtome, were stained with uranyl acetate for study with an RCA EMU-21 microscope.

Observations

The myeloblast, the most immature marrow cell found, is identified in light microscopy by its deeply basophilic cytoplasm and its large nucleus (fig. 1). The cytoplasm may appear reticular or spongy and small cytoplasmic tags may be present. No granules are seen at this stage. The nucleus contains loose, diffuse chromatin and several nucleoli. The nuclear membrane is often very indistinct; the nucleus exhibits no chromatin condensation.

Electron microscopy of the myeloblast (fig. 2) reveals that the deep baso-
Fig. 1.—Light micrograph of myeloblast showing the reticular pattern of the cytoplasm (C) and several small cytoplasmic tags (T). The nuclear membrane is poorly defined (N). X2000.

Fig. 2.—Electron micrograph of myeloblast demonstrating small mitochondria (M), indistinct Golgi apparatus (G), flattened, elongate endoplasmic reticulum (ER) and abundant ribonucleoprotein particles (RNP) throughout the cytoplasm. Pinocytotic vesicles (PV) are present at the surface. Portions of two nucleoli (n₁, n₂) are visible; the compact fibrillar aggregates (arrows) are noted. X13,000.
Fig. 3.—Light micrograph of an early neutrophilic promyelocyte showing the concentration of non-specific granules (A). X2000.

Fig. 4.—A thin section of an early neutrophilic promyelocyte revealing decrease in cytoplasmic ribonucleoprotein (RNP) and mitochondria (M), with increase in vesicles of endoplasmic reticulum (ER). The cytoplasm is filled with non-specific granules (A) and early neutrophilic granules (N). The nucleus demonstrates increased clumping of chromatin (C) and fading nucleolus (n). X8900.
Fig. 5.—Light micrograph of a late neutrophilic promyelocyte showing promyelocytic granulation (A) in cytoplasm and over nucleus. The early paling of the cytoplasm is noted (H). X2000.

Fig. 6.—Electron micrograph of a later neutrophilic promyelocyte demonstrating the increase in endoplasmic reticulum vesicles (ER) of greater size and granular content. Azurophilic granules show degenerative signs (A). Elongate neutrophilic granules (N) are present. Ribonucleoprotein particles (RNP) are more diffuse. Marginal condensation of chromatin (C) is increased. X10,800.

Theophilia of the cytoplasm is due to an abundance of uniformly distributed ribonucleoprotein particles. No granulation is noted. The endoplasmic reticulum appears infrequently and as profiles of greatly flattened, elongated vesicles (fig. 22). Small mitochondria of typical structure are abundant throughout the cytoplasm. Small cytoplasmic extensions and pinocytotic vesicles project...
Fig. 7.—Electron micrograph of early neutrophilic myelocyte demonstrating increase in specific granules (N), vesicles of endoplasmic reticulum (ER), ring form of Golgi complex (G), centriole (L) and sparse mitochondria (M). Increased clumping of chromatin (C) is noted. X17,000.

from the cell surface. Profiles of Golgi vesicles are noted in the thicker cytosomal area opposite the eccentric nucleus. The smoothly outlined nucleus contains a diffuse nucleoplasm of nearly uniform density distribution. The nucleoli visible in the immature nucleus (fig. 2) are in close association with the nuclear membrane, the nucleolar components extending up to the nuclear membrane. The nucleolar substance also appears continuous with chromatin material. The nucleolus is composed of an aggregation of coiled fibrils arranged in two fashions: the tightly aggregated fibrils suggesting a network.
Figs. 8 (inset) and 9

Fig. 8.—Light micrograph of neutrophilic myelocyte clearly demonstrating acidophilic "hof" (H) and neutrophilic granulation (N). Eccentric nucleus shows early indentation. X1850.

Fig. 9.—A later myelocyte illustrating concentration of neutrophilic granules (N) and degenerative azurophilic granules (arrows). Granular endoplasmic reticulum vesicles (ER) are prominent. Progressive chromatin (C) clumping is noted. X16,200.

Maturation of the myeloblast is evidenced by distinct cytoplasmic and nuclear changes observable in the next (promyelocyte) stage. The main feature of the promyelocyte, observed by light microscopy, is the abundance of non-specific granules which fill the cytosome (figs. 3 and 5). The nucleoplasm tends to become more coarse and the nucleoli become less prominent. Study of thin sections shows that the cytoplasm now has become vacuolated, containing vacuoles continuous with the endoplasmic reticulum and vacuoles
Fig. 10.—Light micrograph of neutrophilic metamyelocyte exhibiting faint specific granulation and deeply indented sausage-shaped nucleus (N) with clearly demarcated nuclear membrane. X1850.

Fig. 11.—Electron micrograph of metamyelocyte revealing concentration of neutrophilic granules (N) opposite sausage-shaped nucleus. Occasional azurophilic granules (A) are seen. Golgi complex (G) is prominent, whereas vesicles of endoplasmic reticulum (ER) are less numerous. Chromatin is heavily clumped at margin and interior of nucleus. X16,300.

Profiles of granular endoplasmic reticulum are more numerous and are round to oval in contrast to the flattened, elongated cisternae of the myeloblast. Although still abundant in the cytoplasm, the ribonucleoprotein particles are more diffusely distributed. The mitochondria become progressively less numerous. Round, dense, homogeneous, membrane-enclosed granules appear. These azurophilic granules fill the cytoplasm and
Fig. 12.—Increased maturity of segmented neutrophil is noted in increased neutrophilic granulation (N) and segmentation of nucleus with advanced peripheral clumping of chromatin (C). X16,200.

constitute the first granules to appear in the developing granulocyte (figs. 4 and 6). At this early stage, specific neutrophilic granule formation indicates the direction of development from the stem cell (fig. 4). These granules appear to be round or slightly elongated and contain a material of greater density than the substance of the azure granule (fig. 23). In a later stage of development the number of specific granules increases as the azurophilic granules become less prominent. Loss in density and indications of degeneration of the azure granule are noted (figs. 6 and 23). Large round or irregular vesicles, probably part of the endoplasmic reticulum complex, contain varying amounts of granular material with a density similar to that of the cytoplasm.

In the early promyelocyte stages, the nucleoplasm still appears diffuse (fig. 4). In the later promyelocyte, however, the coarsening of the nucleoplasm is observed. Condensation of chromatin takes place around the periph-
Fig. 13.—Electron micrograph of mature neutrophil revealing abundant specific granulation (N), Golgi complex (G), abundant small endoplasmic reticulum profiles (ER) and peripheral chromatin condensation in the nuclear lobes (arrows). X13,000.

ery of the nucleus and extends inward resulting in areas of clumped chromatin (fig. 6). The nucleolar structure appears to fade and the more compactly aggregated fibrils tend to loosen, and thereby exhibit less electron density. The fibrils still appear to be continuous with the chromatin material (fig. 4).

As the number of specific granules increases to a maximum, the myelocyte stage is identified. Characteristically, the cytoplasm, having lost much of its basophilia, now exhibits a palely acidophilic "hof." It is in this region that the specific granules tend to aggregate (fig. 8). The eccentric nucleus is obscured by the concentration of granules. Electron micrographs of the early neutro-
philic myelocyte (fig. 7) demonstrate the increased density of the neutrophilic granule. The cytoplasm contains abundant vesicles of endoplasmic reticulum, mainly of the granular variety (fig. 24). A few filamentous mitochondria are present. The vesicles of the Golgi complex are organized in the form of a ring, at this sectioning angle, in the region of nuclear indentation. A section through a centriole is noted in the center of the Golgi complex (figs. 7 and 24). The nucleoplasm exhibits progressive condensation with concomitant clumping of the chromatin.

The signs of further maturation observed with light microscopy are confirmed by electron microscopy (fig. 9).

Increased indentation of the nucleus inaugurates the appearance of the
Fig. 15.—Light micrograph of eosinophilic myelocyte demonstrating specific granulation (E) and eccentric nucleus (N). X1750.

Fig. 16.—Thin section of early eosinophilic myelocyte revealing non-specific granules (A), enclosed by serrated membrane (arrow), and specific eosinophilic granules (E), containing dense inclusions, enclosed by a smooth membrane. Vesicles of endoplasmic reticulum (ER) and Golgi complex (G) are noted. The limiting membrane of the "developing" granule appears continuous with adjacent endo-
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_metamyelocyte_. During this stage the nucleus becomes sausage-shaped. The nuclear outline is sharp, the chromatin coarse, and the nucleoli are no longer visible (fig. 10). Cytoplasmic granulation in the neutrophilic metamyelocyte, observed by light microscopy, appears quite fine and neutral, or slightly acidophilic, with the common blood stains. Electron microscopy clearly demonstrates the changes in both nuclear shape and density of chromatin. Few mitochondria are seen at this stage. A well-developed Golgi apparatus is still visible and the endoplasmic reticulum is now limited to sparse vesicles almost obscured by the abundant granulation. A concentration of neutrophilic granules fills the cytoplasm opposite the nuclear indentation (fig. 11). The granules vary in shape and, depending on the sectioning angle, appear round to elongate.

During maturation the nuclear changes observed in the electron microscope again confirm light microscope observations mentioned above and noted in the segmented neutrophil (fig. 12).

The neutrophil (fig. 13) demonstrates the heavy peripheral condensation of chromatin in the nuclear lobes, characteristic of the mature cell. Golgi vesicles form the complex ring at the probable junction of the nuclear lobes. Small profiles of endoplasmic reticulum are very abundant in the mature cell. The granulation appears to be less dense and homogeneous than in earlier developmental stages.

Development of the eosinophil from the myeloblast and early promyelocyte parallels that described for the neutrophil. The eosinophilic promyelocyte is identifiable by the presence of developing eosinophilic granules (fig. 14) which are distinguished from the early neutrophilic granules (fig. 4) by the presence of crystallloid inclusions. The azurophilic granules, which fill the cytoplasm, are similar to those described above. A few elongate granules, which are the developing specific granules, contain the characteristic rod-like inclusions in addition to the homogeneous matrix (fig. 25).

The eosinophilic myelocyte, as seen in light microscopy, exhibits abundant specific granulation filling the cytoplasm opposite the region of nuclear indentation (fig. 15). The picture in electron microscopy is similar, revealing both the non-specific and specific granules, and clearly demonstrates the internal structure of the latter (figs. 16 and 17). The granule inclusions noted in the specific granules take a variety of geometric shapes and sizes, but are mainly rectangular or trapezoidal (fig. 26). They are of greater electron density than the surrounding matrix and tend to fill the granule, occasionally distorting the limiting membrane. The membrane enclosing the developing granules appears serrated, whereas the outline of the specific granules is

plasmic reticulum cisternae and outer membrane of nearby mitochondrion (inset). Remnants of two nucleoli are visible. X10,500.

_Fig. 16i._—Higher magnification of fig. 16 showing apparent continuity of membrane (arrows) enclosing granule (G), endoplasmic reticulum cisternae (ER) and mitochondrion (M). Granular deposits (R) are noted on the external surface of the cisternae. X43,700.
smoof. The limiting membrane of one granule appears to be continuous with
the adjacent rough-surfaced cisternal element and a nearby mitochondrial
membrane (fig. 16). Figure 16i presents these relationships at higher magnifi-
ication.

The eosinophilic metamyelocyte (fig. 18), seen in light microscopy, exhibits
Fig. 18.—Light micrograph of eosinophilic metamyelocyte exhibiting eosinophilic granulation (E) and eccentric nucleus (N). X2000.

Fig. 19.—Electron micrograph of eosinophilic metamyelocyte containing specific granules with characteristic crystalloid inclusions (arrows). Limiting membranes of two granules (A₁, A₂) appear to open into cytoplasm; membrane on granule A₂ also appears continuous with endoplasmic reticulum vesicles (arrows). The nucleus is now so deeply indented it appears lobed. X18,000.
Fig. 20.—Electron micrograph of monoblast demonstrating flattened, elongate vesicles of endoplasmic reticulum (ER), numerous mitochondria (M) and abundant ribonucleoprotein particles (RNP) throughout the cytoplasm. Golgi complex (G) is located at the nuclear indentation. Diffusely arranged loose chromatin is indicative of nuclear immaturity. X17,000.

the eccentric metamyelocytic nucleus previously described, and the specific eosinophilic granulation. The electron micrograph (fig. 19) reveals the typically heavily clumped chromatin and sausage shape of the metamyelocytic nucleus and the internal structure of the fully formed granulation. The apparently crystalloid inclusions usually appear singly in the specific granule, although multiple inclusions have been seen. The limiting membrane enclosing some non-specific granules appears to open into the cytoplasm, and one such
membrane appears continuous with adjacent vesicles of endoplasmic reticulum. As previously noted in the neutrophilic metamyelocyte, the endoplasmic reticulum complex appears to decrease in amount with the increase in specific granulation.

The monocyte is the developing agranulocyte usually found in bone marrow samples. An electron micrograph of the monoblast (fig. 20) shows many round, conspicuous mitochondria in the cytoplasm. The profiles of endoplasmic reticulum are similar to those in the myeloblast, flattened and elongate. Particles of ribonucleoprotein, indicative of the basophilia, are abundantly scattered throughout the cytoplasm. Vesicles of the Golgi complex assume a circular pattern of distribution in the region of nuclear indentation. The diffuse, loosely arranged chromatin of low electron density is characteristic of the immature nucleus.

Fig. 21.—A more mature monocyte exhibiting many large vesicles of endoplasmic reticulum (ER), abundant mitochondria (M) and ribonucleoprotein (RNP). Some small non-specific granulation (A) is present. Increased clumping of chromatin (C) is associated with nuclear maturity. X14,300.
Figs. 22–24.—See legends, facing page.
In the monocyte (fig. 21), mitochondria are still very prominent, and the vesicles of endoplasmic reticulum are more numerous. The endoplasmic reticulum exhibits both elongate and round profiles, mainly of the granular type. Ribonucleoprotein appears in increasing amounts throughout the cytoplasm. A few non-specific granules of the azurophilic variety may be present. The characteristic large nucleus of irregular outline occupies most of the cell.

**DISCUSSION**

Maturation of bone marrow cells is evidenced by the cytoplasmic and nuclear changes which occur synchronously in the normal cell. The cytosomal development observed in the neutrophil and eosinophil is fundamentally the same. Unfortunately, the small sampling techniques of electron microscopy greatly minimize the chances of demonstrating the basophilic series, which constitute less than 0.5 per cent of a myelogram.

The transition of the endoplasmic reticulum from the few flattened vesicles in the myeloblast to numerous round, distended profiles in the promyelocyte and myelocyte parallels the development of the non-specific granules, and the appearance of specific granulation in the myelocyte. The formation of leukocyte granules by accumulation or condensation of material within the vesicles of endoplasmic reticulum has been suggested. Further, the variation in endoplasmic reticulum may be associated with the processes of cell differentiation, and the appearance of the cisternae (as noted in the promyelocyte) attributed to cells actively engaged in a secretory process as in granulogenesis. Following accumulation of granular material, with distention of the vesicles of endoplasmic reticulum, these vesicles may pinch off to form the granules which become increasingly prominent with the conspicuous decrease in the vesicles. Possible "transitional" forms between endoplasmic reticulum profiles and granules have been noted.

Continuity of membrane systems has been demonstrated in several cell types, including some found in Rous sarcoma and leukocytes, indicating a single interconnected system within the cytoplasm. The continuity noted in the myelocyte is reminiscent of the hypothetical cell proposed by Robertson. Although this arrangement may be present in the developing blood cell where active exchange or transport of material is occurring, the same arrangement may not exist in the mature, motile cell. If the origin of the granule is associated with the cisternae of the endoplasmic reticulum and/or mitochondria.

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Fig. 22.—Higher magnification of a portion of the myeloblast (fig. 2) showing the flattened vesicles of endoplasmic reticulum (ER) and the nucleolus composed of tightly aggregated fibrils (arrows) and ground substance of more loosely coiled fibrils (LF). X30,900.

Fig. 23.—Higher magnification of maturing neutrophilic granules (N), degenerating azure granules (A) and enlarged vesicles of endoplasmic reticulum (ER) shown in neutrophilic promyelocyte (fig. 6). X20,800.

Fig. 24.—Higher magnification of portion of neutrophilic myelocyte (fig. 7) showing increasing density of neutrophilic granules (N), vesicles of granular endoplasmic reticulum (ER), Golgi complex (G) and centriole (L). X41,700.
Fig. 25.—Portion of eosinophilic promyelocyte (fig. 14) shown at higher magnification illustrating non-specific granule (A) and early eosinophilic granules (E) containing the characteristic elongate inclusion (arrow). Two degenerating non-specific granules are noted in lower right. X62,500.

Fig. 26.—Four eosinophilic granules in eosinophilic myelocyte (fig. 16) shown at higher magnification demonstrating dense crystalloid inclusions (arrows). X54,000.

dria, then the transport of granular material may take place within the endoplasmic reticulum serving as a "circulatory system."13

The endoplasmic reticulum appears to function in a dual capacity during cell maturation: as a means of transport during the active stages of maturation and as a possible origin of the granules.

In addition to changes in the endoplasmic reticulum complex, the mitochondria become progressively less numerous, with cell maturation and granule formation, until there is little evidence of their presence in the metamyelocyte. Changes in mitochondria during leukocyte development were reported by Sabin et al.14 in supravital studies on human leukemic cells. The decrease in mitochondria during leukocyte maturation was found to precede granule differentiation in the neutrophil. A further correlation between the mitochondria and granulogenesis has been suggested more recently on the basis of ultrastructure similarities;15 however, evidence is lacking for the mitochondrial origin of granules. The trends noted in the endoplasmic reticulum and mitochondria coincide with those previously reported in the guinea pig neutrophilic series.16,17

Nuclear changes may also be correlated with cytosomal maturation. The
diffuse, loosely arranged chromatin becomes progressively more condensed while the nucleoli fade. The loss of nucleolar material appears to involve a loosening of the more compact fibrils with consequent loss of electron density. The fading of the nucleolus corresponds to the culmination of granule formation and decrease in mitochondria.

The evidence presented thus far suggests close morphologic and developmental interrelationships among the cytosomal organelles and the nucleus. The ultimate description of granulogenesis will require further correlated cytochemical and morphologic study.

**Summary**

The development of representative myeloid elements is traced by correlated light and electron microscopy. Cytoplasmic changes during maturation of granulocytes from the myeloblast include loss of basophilia, development of the endoplasmic reticulum complex, decrease in number of mitochondria, and granule formation. The endoplasmic reticulum vesicles increase in size and number during the promyelocyte and myelocyte stages, accompanied by the appearance of non-specific and specific granules, and decrease again during the cytosomal maturation of the metamyelocyte. A reduction in number of mitochondria is noted through the metamyelocyte stage. The apparent continuity of the limiting membranes of both the granules and mitochondria with those of the cisternae of endoplasmic reticulum suggests a direct connection among cytosomal organelles. The role of the endoplasmic reticulum in granulogenesis is discussed. Maturation of the nucleus involves a loss of nucleolar differentiation by a loosening of the compact fibrillar aggregates, and progressive chromatin condensation.

**Summario in Interlingua**

Le disveloppamento de representative elementos myeloide es traciate a base de correlationate studios de microscopia luminari e electronic. Le obse-vate alterationes cytoplasmic occurrente in le curso del maturation de granulocytos ex le myeloblasto include le perdita de basophilia, le disveloppamento del endoplasmatic complexo reticular, le declino del numero de mitochondrios, e le formation de granulos. Le endoplasmatic vesiculas reticular cresce in magnitude e numero durante le stadios promyelocytic e myelocytic, accompaniante del apparition de non-specific e specific granulos. Le magnitude e numero del endoplasmatic vesiculas reticular declina de novo durante le maturation cytosomal del metamyelocyto. Un declino del numero de mito-chondrios es a notar durante le stadio metamyelocytic. Le apparente continu-itate del membranas limitante (tanto del granulos como etiam del mitochondrios) con illos del cisternas de reticulo endoplasmatic suggestiona un directe connexion inter le organellas cytosomal. Le rolo del reticulo endoplasmatic in le granulogenese es discutite. Le maturation del nucleo es associate con un perdita de differentiation nucleolar in consequentia de un relaxation del compacte aggregatos fibrillar e de un progressive condensation de chromatina.
REFERENCES


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