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Ultrastructure of granulocytes

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THE ULTRASTRUCTURE OF GRANULOCYTES

by

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A THESIS

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Under the Supervision of Edward A. Holyoke, Ph.D., M.D.

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THE ULTRASTRUCTURE OF GRANULOCYTES

Historical Background

With the advent of the electron microscope, it was to be expected that this technique would be applied to the study of the cells of the peripheral blood and hematopoietic organs. The study of such cells was more difficult than the examination of thinner viruses and bacteria, and the definitive work on granulocyte ultrastructure had to await the development of techniques for thin sectioning in electron microscopy. Prior to this development, granulocytes were studied with the electron microscope after various ingenious methods of preparation, although these studies were limited in what they could hope to achieve.

What was apparently the first of these early studies to be published appeared in a brief preliminary report by Rebuck and Woods\textsuperscript{1} in 1947. These investigators made imprints of bone marrow, spleen or lymph node on glass slides coated with a Formvar film. The slides were frozen and dried in a vacuum, and the Formvar film with included cells was removed from the slides and transferred to grids for viewing. Preparations were also obtained by implanting Formvar coated grids in the dermis of human volunteers to induce an inflammatory exudate. These grids were frozen, dried and viewed directly. In 1948, Rebuck and Woods\textsuperscript{2} using these same and other similar techniques published a more extensive report. With these techniques, they were
able to clearly demarcate and measure neutrophilic granules. In 1949, Rebuck\textsuperscript{3} reported on the use of osmic acid fixation. The granulocyte layer of sedimented peripheral blood was spread on coated slides, and the slides were immersed in osmic acid rather than being frozen and dried. By this method, the granules were not as well defined, but nuclear structures were well delineated.

In 1950, Besis\textsuperscript{4} summarized in the American literature his work on cellular elements of blood which had been in progress since 1947. His work on granulocytes had been published in several French journals in 1949 and 1950. Besis used leucocytes derived from buffy coats of peripheral blood and mechanically disrupted them or allowed them to spread on slides covered with varying types of films. Shadow casting with gold enabled the observation of granules within the cytoplasm of the intact cells or the observation of free granules released from disrupted cells. In 1952, Marchant\textsuperscript{5} prepared leukocytes on Formvar films which were fixed in osmic acid or alcohol and gold shadowed.

The first three papers utilizing thin sectioning of granulocytes embedded in a supporting medium appeared in 1953 and 1954. These were the papers of Braunsteiner et al.\textsuperscript{6}, Kautz and DeMarsh\textsuperscript{7} and Watanabe\textsuperscript{3}. In 1955, seven further papers dealing with the ultrastructure of thin-sectioned granulocytes were published. The techniques were now available for definitive study of granulocytes with the electron microscope, and, as will be seen in the present paper, some
of these studies have reached a high degree of sophistication. The present paper is intended as a survey of the literature regarding the ultrastructure of granulocytes.

General Ultrastructure and Features of Maturation

The structures of the various cytoplasmic organelles of leukocytes may vary with the stage of maturation of the cell. For this reason, structures and developmental stages of these subunits will, where appropriate, be discussed together. This section will begin with a discussion of the structure and maturation of the granulocyte nucleus.

A membrane surrounding the nucleus of leukocytes has been reported by Watanabe, Grey and Bisele, Kautz and DeMarsh, Goodman et al. described the nuclear membrane as being double-walled. Each wall was 70-90 angstroms thick, and a 100-200 angstrom space separated the walls. Anderson stated that the nuclear membrane of mature human neutrophils was rarely visible.

The nucleoplasm of myeloblasts was described as homogeneous by Besis and as pale or watery with a thin layer of chromatin beneath the nuclear membrane by Anderson and Pease. Anderson reported this layer to have begun to thicken in the promyelocyte stage. The bulk of the chromatin was described as having begun to condense and form clumps in the myelocyte by Capone et al. They noted the nuclear chromatin to be heavily clumped in the metamyelocyte, and they and Besis and Thiery observed dense chromatin with
aggregation along the nuclear membrane in segmented neutrophils. The latter authors listed the condensation of nuclear chromatin as one of the basic processes of leukocyte maturation.

Nucleoli were seen to be prominent in myeloblasts by Pease\textsuperscript{17} and Watanabe\textsuperscript{3}. Capone et al.\textsuperscript{15} noted nucleoli to be less prominent as early as the promyelocyte stage. Nucleoli were described as having diminished by the myelocyte stage by Pease\textsuperscript{17} and Watanabe\textsuperscript{3}. No nucleoli were seen in metamyelocytes and bands by Bainton and Farquhar\textsuperscript{18} or in mature cells by Watanabe\textsuperscript{3} and Kautz and Demarsh\textsuperscript{19}. Watanabe\textsuperscript{3} described the nucleolus to be composed of electron-dense bands twisted together in an irregular fashion, and Capone et al.\textsuperscript{15} described a network of tightly and loosely coiled fibrils.

Regarding the surface of leukocytes, it would seem appropriate that these cells should be surrounded by a cell membrane. In 1954, Kautz and Demarsh\textsuperscript{7} reported observations of such a membrane in their early study of sectioned human neutrophils. Grey and Bisele\textsuperscript{10} could not observe a definite membrane in basophils from the peritoneal fluid of mice. Cytoplasmic projections and pinocytotic vesicles were noted at the cell surface of mature human neutrophils by Besis and Thiery\textsuperscript{16} and at the surface of human myeloblasts by Capone et al.\textsuperscript{15}

The overall appearance of the cytoplasm of leukocytes varies with the stage of maturation. The cytoplasm of the
myeloblast was observed to be thin or watery by Pease\textsuperscript{14,17} and Anderson\textsuperscript{13}. Pease\textsuperscript{17} reported increasing density of the cytoplasm with increasing cell maturation. The above authors with the addition of Capone et al.\textsuperscript{15}, Ackerman et al.\textsuperscript{20} and Watanabe\textsuperscript{9} observed the myeloblast cytoplasm to contain large numbers of freely scattered ribosomes or ribonucleoprotein particles. Pease\textsuperscript{14,17} stated that these RNA particles tended to accumulate along the membranes of the endoplasmic reticulum with further maturation. Anderson\textsuperscript{12} noted no free ribosomes in mature human neutrophils.

The character of the leukocyte cytoplasm also varies with the type of cell as well as with the stage of maturation. Both Pease\textsuperscript{17} and Winqvist\textsuperscript{21} have remarked on the loose, watery appearance of basophil cytoplasm as compared to that of other cell lines.

The endoplasmic reticulum of granulocytes varies in both amount and structure depending on the maturity of the cell in question. Few authors have referred specifically to smooth endoplasmic reticulum in studies of leukocytes, so the comments in this section will refer to rough endoplasmic reticulum unless otherwise stated. Considering the amount of endoplasmic reticulum, it has been reported to be very sparse in myeloblasts by Anderson\textsuperscript{12}, Capone et al.\textsuperscript{15}, Bainton and Parquhar\textsuperscript{13}, Braunsteiner et al.\textsuperscript{22} and Pease\textsuperscript{14,17}. Conversely, Besis\textsuperscript{23} stated that endoplasmic reticulum was abundant in blast cells, and a study by Ackerman et al.\textsuperscript{20} reported large amounts of rough endoplasmic reticulum in
the blast cells of acute granulocytic leukemia. The amount of endoplasmic reticulum has been reported to increase markedly in the progranulocyte stage by many authors including Anderson, Capone et al., Pease, Watanabe, Bainton and Farquhar and Bais. Pease observed species differences in the extent of development of the endoplasmic reticulum in the progranulocyte. There was more development in the cells of the rat as compared to those of the guinea pig. Sorensen and Winquist observed increased endoplasmic reticulum in immature granulocytes which were not specifically classified as to maturational stage. Capone et al., Bainton and Farquhar and Hudson reported persistence of the large amounts of endoplasmic reticulum into the myelocyte stage while Watanabe and Pease felt that the amount of endoplasmic reticulum had begun to fade by this stage. In metamyelocytes, Bainton and Farquhar and Capone et al. reported diminished rough endoplasmic reticulum. Decreased rough endoplasmic reticulum in segmented neutrophils was described by Anderson, Bais and Thiery, Bais, Bainton and Farquhar, Hudson and Bais, while Capone et al. and Goodman et al. reported abundant amounts. Diminished endoplasmic reticulum was observed in mature basophils by Zucker-Franklin and Pease. Anderson observed more endoplasmic reticulum in mature eosinophils than in mature neutrophils. In summary, it has been generally felt that the endoplasmic reticulum shows a temporary increase in early cells and then progressively diminishes with later
maturation. This general view has been stated by Basis\textsuperscript{23}, Basis and Thiery\textsuperscript{16} and Capone et al.\textsuperscript{15}

As mentioned previously, the structure of the endoplasmic reticulum also varies with the maturation of the cell. The endoplasmic reticulum of myeloblasts was described as being flat by Capone et al.\textsuperscript{15} and Watanabe\textsuperscript{9}. The endoplasmic reticulum of progranulocytes was reported to occur as wide canaliculi or vesicles by Pease\textsuperscript{17}. Capone et al.\textsuperscript{15} reported more round to oval forms and less flattened forms in progranulocytes than in the myeloblasts, and Anderson\textsuperscript{12} remarked on the dilated nature of the endoplasmic reticulum at this stage. Watanabe\textsuperscript{9} described the progranulocyte endoplasmic reticulum to occur as flattened or vesicular sacs. In the myelocyte, Anderson\textsuperscript{12} observed less dilated endoplasmic reticulum than in the progranulocyte. In summary, the endoplasmic reticulum appears to become most saccular in the progranulocyte and then to become less dilated with increasing maturation.

As mentioned previously, most authors have not specifically referred to smooth endoplasmic reticulum as distinguished from rough. However, Watanabe\textsuperscript{9} did describe 30-150 millimicron vesicles which were felt to represent smooth endoplasmic reticulum, and Zucker-Franklin\textsuperscript{26} noted smooth endoplasmic reticulum in basophils.

The Golgi apparatus is generally considered to undergo maturational changes similar to those of the endoplasmic reticulum. Anderson\textsuperscript{12}, Basis and Thiery\textsuperscript{16} and Bainton and
Farquhar have described the Golgi apparatus as consisting of vesicles or saccules which are concentrically arranged and which, according to the latter two authors, surround centriolar structures. Pease, Bessis and Anderson described a prominent Golgi apparatus in myeloblasts. Conversely, Ackerman et al. observed a small Golgi apparatus in the blast of acute granulocytic leukemia in humans. Bainton and Farquhar found the Golgi apparatus of myeloblasts to consist of three to four layers of saccules, while that of the progranulocyte consisted of four to nine layers, and that of the myelocyte consisted of three to five layers. Further decrease in the size of the Golgi apparatus was noted with further maturation, and a segmented neutrophil was described as having a minimal Golgi. Similarly, Zucker-Franklin reported scanty Golgi in mature basophils. Watanabe felt that the Golgi was most abundant in the early myelocyte stage and that it decreased thereafter. Bessis stated that the well developed Golgi of immature cells broke up into scattered vesicles with increasing cell maturation. Bessis and Thiery listed the decrease in the amount of Golgi apparatus as one of the main features of granulocyte maturation. The Golgi apparatus will be further discussed later in this paper in reference to granule formation in leukocytes.

The Golgi apparatus externally limits that part of the cell which is referred to as the centrosome and which contains the centrioles. The position of the centrioles
in the center of the concentric rings of the Golgi apparatus has been previously mentioned. The centriole of the granulocyte was described by Besis and Thiery\textsuperscript{16} as being a cylindrical structure measuring 150 millimicrons across and 500 millimicrons long. Nine longitudinally oriented tubules made up the wall of this cylinder. The tubules were sometimes twisted to form a helix. Around the cylinder were two annularly arranged layers of small dense structures which were connected to the tubules of the cylinder by bridge-like attachments. Canalicular structures were observed to radiate from the centrioles outward toward the limiting Golgi apparatus. Bainton and Farquhar\textsuperscript{13} observed a system of microtubules radiating outward in spoke-like fashion from the centriole. These authors did not observe centrioles in myeloblasts, but they observed two centrioles to lie in the center of the Golgi apparatus in progranulocytes. They saw centrioles less often in segmented neutrophils. This indicated a maturational sequence somewhat like that of the endoplasmic reticulum and Golgi apparatus. Besis\textsuperscript{23} and Besis and Thiery\textsuperscript{16} reported two centrioles in the centrosome of neutrophils. Zucker-Franklin\textsuperscript{26} found centrioles frequently in human basophils, while Winqvist\textsuperscript{21} saw them only occasionally.

The size and number of mitochondria in granulocytes vary with maturation similarly to the variability in the endoplasmic reticulum and Golgi apparatus. Large numbers of mitochondria were observed in myeloblasts by Bainton
and Farquhar\textsuperscript{13}, Watanabe\textsuperscript{3,9} and Kautz and DeMarsh\textsuperscript{19}. Anderson\textsuperscript{12} reported them to be fairly numerous in the blast of acute granulocytic leukemia but to be less frequent in the blast of the chronic variety. Mitochondria were seen to become less frequent in promyelocytes by Capone et al.\textsuperscript{15} and Anderson\textsuperscript{12}, while Watanabe\textsuperscript{3} noted large numbers in these cells. Watanabe\textsuperscript{3,9}, Anderson\textsuperscript{12} and Capone et al.\textsuperscript{15} stated that there were small numbers in the myelocyte stage, while Bainton and Farquhar\textsuperscript{13} described increased numbers of mitochondria in these cells as compared to progranulocytes. These latter authors did report the numbers to decrease in the metamyelocyte stage. Small numbers of mitochondria in mature neutrophils were seen by Watanabe\textsuperscript{3,9}, Goodman et al.\textsuperscript{11} and Anderson\textsuperscript{12}. Kautz and DeMarsh\textsuperscript{7} saw no definite mitochondria in mature neutrophils, although they did observe them in comparable stages of basophils and eosinophils. A differing view was expressed by Besis\textsuperscript{23} who stated that there were large numbers of mitochondria in mature neutrophils. Zucker-Franklin\textsuperscript{26} reported greater numbers of mitochondria in mature basophils than in neutrophils. Anderson\textsuperscript{12} made similar observations regarding the mitochondria of eosinophils as compared to those of neutrophils. The general view that mitochondria decrease in number with maturation of granulocytes has been stated by Anderson\textsuperscript{12} and Watanabe\textsuperscript{3,9}.

Considering the size and shape of the mitochondria, Kautz and DeMarsh\textsuperscript{19}, Besis\textsuperscript{13} and Pease\textsuperscript{14,17} described the mitochondria of the myeloblast to be large. An opposite
observation was made by Capone et al.\textsuperscript{15} who described smaller mitochondria in blast cells. Pease\textsuperscript{17} observed the mitochondria to become smaller with maturation to the myelocyte stage. The mitochondria of mature neutrophils were stated to be small, long and slender by Besis\textsuperscript{23} and Besis and Thiery\textsuperscript{16}. Similar mitochondria were observed in mature basophils by Winqvist\textsuperscript{21}, while Pease\textsuperscript{17} described larger forms resembling those of myeloblasts to occur in mature basophils. Besis\textsuperscript{23} and Besis and Thiery\textsuperscript{16} stated that the mitochondria were large and rounded in early cells and became smaller and elongated as part of the maturational process.

 Several authors have remarked on the presence of various vesicles and vacuoles in the cytoplasm of granulocytes in addition to the organelles previously discussed. The existence of pinocytotic vesicles at the cell surface has been previously mentioned. In 1954, Kautz and DeMarsh\textsuperscript{7} remarked on the existence of cytoplasmic vacuoles but did not mention endoplasmic reticulum or Golgi apparatus, so one might assume that some of their vacuoles represented these organelles. Later authors have observed vacuoles in the cytoplasm of granulocytes in addition to endoplasmic reticulum and Golgi. Capone et al.\textsuperscript{15} observed what they felt were pinocytotic vesicles in myeloblasts and pro-granulocytes. Braunsteiner et al.\textsuperscript{22} observed large numbers of vesicles in addition to a small amount of endoplasmic
reticulum in the blast cells of acute granulocytic leukemia. Vesicles and vacuoles were reported in the cytoplasm of mature neutrophils by Besis and Thiery\textsuperscript{16}, Besis\textsuperscript{23}, Anderson\textsuperscript{12} and Goodman et al.\textsuperscript{11} and in the cytoplasm of basophils by Rinehart\textsuperscript{27}. Besis and Thiery\textsuperscript{16} postulated that the cytoplasmic vacuoles may be derived from endoplasmic reticulum or Golgi apparatus, may be of pinocytotic origin, may represent a stage in specific granule formation or may represent areas of dissolved glycogen.

Glycogen particles have been reported in the cytoplasm of granulocytes by several authors. Rinehart\textsuperscript{27}, as early as 1955, observed what were felt to be glycogen particles. The reported measurements of these glycogen particles by several investigators are quite consistent. Anderson\textsuperscript{12} reported the particles in neutrophils to be 200-250 angstroms across, and Zucker-Franklin\textsuperscript{26} reported figures of 250-300 angstroms in basophils. Bainton and Farquhar\textsuperscript{18} described the particles in neutrophils to be 250 angstroms in greatest diameter and to be composed of subunits of 25 angstroms. The number of glycogen particles in basophils was observed to be less than that of neutrophils by Anderson\textsuperscript{12}.

Ackerman et al.\textsuperscript{20} referred to osmiophilic structures seen in cells from a patient with acute granulocytic leukemia which were felt to be fat droplets. Similar osmiophilic droplet-like particles were seen in normal human basophils by Kautz and DeMarsh\textsuperscript{7}. These investigators also felt the
particles represented lipid droplets.

Other cytoplasmic elements of granulocytes not yet discussed have been primarily reported in papers on leukemic cells and will be discussed in a later section. Zucker-Franklin\textsuperscript{26} did observe fibrils and microtubules in the cytoplasm of normal human basophils, but the other reports of fibrillar structures have been in leukemic cells. The specific granules themselves will be discussed in separate sections.

Theories of Granule Formation

The method of formation of leukocyte granules has been investigated with the electron microscope using two different techniques. The first involves observations on the contiguity of developing granules with cellular elements or the observation of transitional forms between granules and cellular elements. The second method involves tracing a labeled molecule with electron microscope autoradiography. Theories as to the source of leukocyte granules have included mitochondria, cytoplasmic matrix, endoplasmic reticulum and the Golgi apparatus.

Mitochondria were implicated as a source of granule formation by Rinehart\textsuperscript{27} who described transitional forms between mitochondria and specific granules in observations on human and rat peripheral blood and marrow. The transitional forms of mitochondria were noted to lose their internal structure and to become rounded. To further support this idea, he cited parallels in size between the granules and
the mitochondria within one cell line such as the neutrophilic series. The alternative was stated by Pease\textsuperscript{17}, Goodman et al.\textsuperscript{11} and Sorenson\textsuperscript{24} who saw no evidence for granule formation from mitochondria in studies of rat and guinea pig marrow, human peripheral blood and the cells of fetal rabbits respectively.

Cytoplasmic ground substance was postulated as a source of basophilic granule formation by Winqvist\textsuperscript{21} in observations on guinea pig marrow. He observed granule formation to begin in the Golgi region as areas of cytoplasmic substance which were larger than definitive granules and were either surrounded by a fine membrane unrelated to the endoplasmic reticulum or by small vesicles. With maturation, these areas were noted to become more electron dense and to have a structure resembling mature granules. Winqvist assumed that the granule development was in the cytoplasm closely associated with the Golgi apparatus but not in the Golgi vacuoles themselves.

The endoplasmic reticulum was proposed as a source of granule formation by Capone et al.\textsuperscript{15} who noted continuity of the membrane of a developing eosinophil granule with the rough endoplasmic reticulum. Besis and Thiery\textsuperscript{16} proposed that the clear vacuole in which an electron dense substance condensed during the formation azurophilic granules may have been derived from either the rough endoplasmic reticulum or the Golgi apparatus. Other workers have not commented on the endoplasmic reticulum as a source
of granule formation.

The origin of granules from the Golgi apparatus has been proposed by several authors and has received the most study and support. Early proponents of this theory were Besis\textsuperscript{13} and Watanabe\textsuperscript{9}. Besis\textsuperscript{13} noted the first appearance of specific granules in the area of vacuoles around the centrosome. Watanabe\textsuperscript{9} observed vesicular bodies with intermediate forms leading to mature granules to be aggregated in the Golgi area of human neutrophils. Besis\textsuperscript{23} later described azurophilic granule formation as the condensation of dense material in vacuoles related to the Golgi apparatus. In observations on the blast cell of acute granulocytic leukemia, Anderson\textsuperscript{12} noted Golgi vesicles containing dense material and considered these to be transitional to the granules of promyelocytes. Sorenson\textsuperscript{24}, in studying granulocytes of fetal rabbits, observed small granules in the Golgi zone and suggested this as an area of granule formation.

Bainton and Farquhar\textsuperscript{13} studied the origin of leukocyte granules in rabbit marrow and stated that the first indication of transformation from myeloblast to promyelocyte involved the appearance of vacuoles with a dense core in the area of the Golgi apparatus. These vacuoles were most frequent along the central or concave side of the Golgi apparatus, and the membranes of the vacuoles were sometimes continuous with the membranes of the inner Golgi saccules. The vacuoles representing developing azurophilic granules
were felt to be pinched off from the Golgi saccules. Larger vacuoles with several dense cores were also noted in the Golgi region and were thought to represent the fusion of smaller vacuoles. In the myelocyte, these authors observed specific granules which were thought to arise from the opposite or outer side of the Golgi complex. Outpocketings off the outer saccules of the Golgi apparatus were noted to have a similar content to that of granules in the area. The authors concluded that azurophilic granules developed from the inner face of the Golgi apparatus and that specific granules developed from the outer.

Autoradiographic studies with the electron microscope have also supported the Golgi apparatus as the source of granule formation. This method as used by Fedorko and Hirsch28 involved incubation of marrow cells with tritiated lysine followed by varying periods of incubation in unlabelled lysine. Suitable emulsions and fixatives applied to thin sections of these tissues allowed visible grains representing the lysine label to be counted on electron-microscopic images of the cells. The grains were counted and categorized as to their association with cytoplasmic organelles and as to the time of incubation. This allowed the flow of lysine through the cellular compartments to be studied. These authors, in a study of rabbit myelocytes, reported a peak of the label in the Golgi apparatus soon after exposure to the tritiated lysine. The level in the Golgi apparatus then began to fall simultaneously with a
rise in the level in the granules. This was taken to represent a flow of lysine through the Golgi apparatus before incorporation into granules. Fedorko \(^2\text{9}\) demonstrated similar findings in human eosinophils.

In considering methods of granule formation, it is necessary to consider the role of the azurophilic or non-specific granules of light microscopy in the later formation of specific granules. This topic has not been dealt with extensively with the electron microscope. Capone et al. \(^1\text{5}\) noted the nonspecific granules of the promyelocyte to lose density and undergo degenerative changes with maturation, and Besis and Thiery \(^1\text{6}\) and Besis \(^2\text{3}\) reported no evidence for the transformation of azurophilic to specific granules.

Bainton and Farquhar \(^1\text{8}\), as previously noted, reported that azurophilic granules and specific granules developed from opposite sides of the Golgi complex and were unrelated from the point of origin on through the maturation of the cell. They described the azurophilic granule as being irregularly spherical, ovoid or angular and consisting of an approximately 70 angstrom wide triple-layered limiting membrane enclosing a dense, finely particulate homogeneous material. The specific granules which they began to observe in the myelocyte were smaller, more variable in size, more regularly spherical and less dense than the azurophilic granules. In the metamyelocytes, bands and mature polymorphonuclear leukocytes, almost all of the granules were noted to be of this specific type. These authors noted no
evidence for the transformation of azurophilic granules to specific granules, for their degeneration or for their discharge from the cell. They explained the diminishing numbers of azurophilic granules occurring with maturation on the basis of no further formation after the promyelocyte stage coupled with reduction in their numbers by mitotic divisions during the myelocyte stage. The authors further stated that the azurophilic granules of neutrophil precursors could be distinguished from those of eosinophil and basophil precursors and were, therefore, more specific than previously thought.

Additional descriptions of the azurophilic granule resemble the one given in the above discussion. The granules have been described as being spherical and as having a limiting membrane by Besis, Besis and Thiery and Capone et al. The former two papers listed the size of the granule as varying between .5 and 1 micron. Both Capone et al. and Anderson described the inner structure of the granule as dense and homogeneous. Anderson also noted less homogeneous granules filled with a pale granular material.

The Eosinophil Granule

The ultrastructure of the eosinophilic leukocyte has been of interest to many investigators primarily because of its unique granular architecture. The granule is round to oval, and varying reports as to its size are listed in Table 1.
Table 1. Largest Diameter of Eosinophil Granules in Varying Species.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Size of Granule</th>
<th>Species Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Besis and Thiery</td>
<td>.5-1.0 microns</td>
<td>human</td>
</tr>
<tr>
<td>Goodman et al.</td>
<td>.7-1.3 microns</td>
<td>human</td>
</tr>
<tr>
<td>Watanabe</td>
<td>.4-.3 microns</td>
<td>human</td>
</tr>
<tr>
<td>Miller et al.</td>
<td>.3-1.2 microns</td>
<td>human, rat, mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and guinea pig</td>
</tr>
<tr>
<td>Anderson</td>
<td>.5-1.0 microns</td>
<td>human</td>
</tr>
<tr>
<td>Zucker-Franklin</td>
<td>.2-.9 microns</td>
<td>human</td>
</tr>
<tr>
<td>Watanabe</td>
<td>.3-1.0 microns</td>
<td>guinea pig</td>
</tr>
<tr>
<td>Rebuck and Woods</td>
<td>.78-1.15 microns</td>
<td>human</td>
</tr>
<tr>
<td>Grey and Biesele</td>
<td>.3-.7 microns</td>
<td>mouse</td>
</tr>
<tr>
<td>Sheldon and Zetterquist</td>
<td>.47 microns</td>
<td>mouse</td>
</tr>
<tr>
<td>Marchant</td>
<td>.3-1.7 microns</td>
<td>human</td>
</tr>
</tbody>
</table>

The early study by Rebuck and Woods in 1948 described the eosinophil granule as a clear sphere, but a more complex structure has since been demonstrated. As seen by the conventional methods of preparation and staining for electron microscopy, the granule has been observed to consist of a dense inner structure or internum of varying shape contained in a less dense matrix. Hudson using phosphotungstic acid stain demonstrated a reversal of the electron densities by which the inner structure appeared electron-lucent and was surrounded by a dense matrix, but this is not the usual picture. The inner structure in the eosinophil granule was described by Sheldon and Zetterquist as a dense band which
was .07 microns wide. The dense inner structure has additionally been described as an angular disc by Pease\textsuperscript{14,17}, as a trapezoidal or tapered crystal by Besis and Thiery\textsuperscript{16}, as a rod-like crystallloid by Capone et al.\textsuperscript{15}, as a lobulated cylinder by Kautz and DeMarsh\textsuperscript{7} and as a rod, trapezoid or triangle by Goodman et al.\textsuperscript{11} Miller et al.\textsuperscript{36} reported rectangular forms to be more prevalent in rodent species, while human eosinophils were more likely to have squares and needle-like forms. Watanabe\textsuperscript{9} prepared agar models of eosinophil granules and cut them in various planes of section to demonstrate that eosinophil granule ultrastructure was consistent with a lens-shaped disc containing a quadrilateral or round plate in its equatorial plane. In the guinea pig eosinophil, Miller et al.\textsuperscript{30} observed the dense inner bar to be sometimes divided lengthwise by a lighter strip of material resembling the matrix. More than one dense inner structure per eosinophil granule has been described by Miller et al.\textsuperscript{30}, Watanabe\textsuperscript{9}, Pease\textsuperscript{14,17}, Besis\textsuperscript{23} and Hudson\textsuperscript{25}.

A substructure within the dense central bar of the eosinophil granule has been reported and characterized in various species. Dark lines about 50 angstroms thick alternating with light areas were seen in the internal bar of the eosinophil granule of the mouse by Sheldon and Zetterquist\textsuperscript{32} in 1955. In 1957, Watanabe\textsuperscript{9} observed an alternating arrangement of light and dark bands within the central bar of the cat eosinophil granule. In some
granules, the bands were roughly parallel, while in others they were arranged in a concentric fashion. In 1966, Miller et al.\textsuperscript{30} reported alternating light and dark bands to comprise the substructure of the central bar of rat, mouse, guinea pig and human eosinophil granules. The dense bands further appeared to be composed of a linear series of dots. In some granules, perpendicular crossing of the dense bands formed a structure resembling a lattice. The spacings from the center of one dense band to the center of the adjacent band varied from a low of 23 angstroms in mouse and human cells up to 40 angstroms in other human specimens. Widths of the dark bands themselves were about 15 angstroms in rodents and 20 angstroms in man. From this work, they suggested that the central bar of the eosinophil granule represented a crystal composed of a cubic lattice.

Although a substructure of the internum of the eosinophil granule was noted by some relatively early workers, it was not noted by all. In 1955 and 1956, Pease\textsuperscript{14,17} stated that the internum of guinea pig cells was homogeneous. In 1957, Goodman et al.\textsuperscript{11} could not demonstrate an internal structure in the central bars of human eosinophil granules. In 1964, Besis\textsuperscript{23} stated that such an internal structure had not yet been demonstrated in human eosinophils. This latter statement was prior to the demonstration of such a structure in human cells by Miller et al.\textsuperscript{30} in 1966.

The matrix containing the dense internum has been described as being finely granular by Miller et al.\textsuperscript{30} and
Anderson\textsuperscript{12}. Ghidoni and Goldberg\textsuperscript{33} demonstrated the presence of acid phosphatase activity in this matrix but not in the dense portion of the granule. After incubation of eosinophils in glycerophosphate substrate, the reaction product resulting from acid phosphatase activity could be localized within the matrix with the electron microscope as lead phosphate.

The eosinophil granule has been reported as being membrane bound by Anderson\textsuperscript{12}, Kautz and DeMarsh\textsuperscript{19}, Besis and Thiery\textsuperscript{16}, Besis\textsuperscript{23} and Ghidoni and Goldberg\textsuperscript{33}. This limiting membrane was measured as being 50 angstroms across in the Swiss mouse eosinophil by Sheldon and Zetterquist\textsuperscript{32} and as being 95 angstroms across in rat, mouse, guinea pig and human eosinophils by Miller et al.\textsuperscript{36}

Other eosinophil granules have been observed which do not contain the typical internal dense bar. Besis and Thiery\textsuperscript{16}, Fedorko\textsuperscript{29} and Pease\textsuperscript{17} made general observations on the existence of granules of this type. Zucker-Franklin\textsuperscript{31} reported in eosinophils the presence of dense inclusions .2-.9 microns in diameter which were irregular in outline and lacked a membrane as well as an internal structure. Anderson\textsuperscript{12} observed similar .2-.3 micron granules which had a dense outer margin and a pale center. Hudson\textsuperscript{25} studied stages in the development of eosinophils and reported that the fraction of granules with the characteristic internum increased with increasing maturity of the cell. The internum-lacking granules were thus considered
to be immature forms.

An allied topic to the ultrastructure of the eosinophil granule involves the role of this granule in the formation of the Charcot-Leyden crystal seen with light microscopy in the exudates of patients with various allergic disorders. This was studied with the electron microscope by Welsh\textsuperscript{34} who treated blood from allergic patients with wetting agents and incubated it. He observed the eosinophil granules to swell with this treatment and leave the central bar in a clear space surrounded by the disrupted limiting membrane of the granule. He observed early forms of Charcot-Leyden crystals which appeared to represent coalescence of the central bars of the altered granules. Electron micrographs showed the internal structure of the developing crystals and that of the altered granules to be similar. It was assumed, therefore, that the Charcot-Leyden crystals were derived from the internum of the eosinophil granule.

The Basophil Granule

Like the eosinophil granule, the granule of the basophil has been of interest because of its substructure. This substructure is equally as intricate as that of the eosinophil granule, although it is less striking. The granules have been generally described as being round to oval, although Zucker-Franklin\textsuperscript{26} reported angular forms, and Besis and Thiery\textsuperscript{16} and Besis\textsuperscript{23} stated that some of the granules had an irregular shape suggesting the condensation of
smaller granules. Varying reports as to the size of basophil granules are listed in Table 2.

Table 2. Largest Diameters of Basophil Granules in Varying Species.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Size of Granule</th>
<th>Species Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker-Franklin</td>
<td>.2-1.2 microns</td>
<td>human</td>
</tr>
<tr>
<td>Anderson</td>
<td>.4-1.0 microns</td>
<td>human</td>
</tr>
<tr>
<td>Kautz and DeMarsh</td>
<td>1.0 micron</td>
<td>human</td>
</tr>
<tr>
<td>Winqvist</td>
<td>1.0-2.0 microns</td>
<td>guinea pig</td>
</tr>
<tr>
<td>Watanabe</td>
<td>1.0 micron</td>
<td>guinea pig</td>
</tr>
<tr>
<td>Fedorko and Hirsch</td>
<td>.5-1.0 microns</td>
<td>guinea pig</td>
</tr>
<tr>
<td>Goodman et al.</td>
<td>.8-1.0 microns</td>
<td>human</td>
</tr>
<tr>
<td>Winqvist</td>
<td>.5-1.0 microns</td>
<td>guinea pig</td>
</tr>
</tbody>
</table>

Watanabe observed that basophil granules appeared less dense than those of other granulocytes, and Besis and Thiery described clear granules. Kautz and DeMarsh and Fedorko and Hirsch remarked on the difficulty of fixing basophil granules, and Pease and Grey and Bisele mentioned the solubility of basophil granules in water. This may contribute to the pale appearance observed by some investigators.

Grey and Bisele observed the basophil granules to be heavily outlined, and Rinehart described a dense periphery with a clear center. A distinct limiting membrane of the basophil granule was seen by Kautz and DeMarsh, Besis and Thiery, Fedorko and Hirsch and Zucker-Franklin. Goodman et al. described the membrane as
being double-walled with a width of 30-150 angstroms. Winqvist\textsuperscript{21,36} did not observe a definite limiting membrane around the basophil granule. Winqvist\textsuperscript{21} did describe a clear space containing material of low electron density which surrounded the granule and which, in turn, was surrounded by a discontinuous membrane-like structure.

Concerning the inner structure of the basophil granule, several authors have reported a lamellated pattern. On thin section, the lamellae appear as roughly parallel fine lines. This type of structure has not been reported in species other than the guinea pig. The lamellated substructure has been studied in greatest detail by Winqvist\textsuperscript{21,36} who described two varieties of lamellae in basophil granules. The two types could coexist in the same granule and occur at varying angles to each other or could be found in different granules. One type was characterized by lamellae which were straight, regularly spaced and ran perpendicular or oblique but not parallel to the long axis of the granule. These lamellae were 7 millimicrons wide and separated by spacings of 9 millimicrons. The second type of lamellae were often wavy rather than straight, tended to be more irregularly spaced than the first type and often appeared to run in pairs. Lamellae of this variety were 7-9 millimicrons across. This type could be found in any orientation with regard to the long axis of the granule. The latter type was the one most commonly observed. Pease\textsuperscript{14,17} also observed a lamellated substructure. In the first paper,
he also remarked on the presence of both regularly arranged and less regularly spaced layers. Fedorko and Hirsch described the substructure as being composed of a honey-combed arrangement of dense walls surrounding a core of less dense material. Spacings of 140 angstroms separated the dense walls from each other. Fedorko and Hirsch also observed a granule with parallel lamellae separated by the same 140 angstroms. Watanabe did not observe the lamellated substructure in guinea pig basophils.

In species other than the guinea pig, variable structures of basophil granules have been observed, but lamellae have not been reported. Grey and Bisele reported the granules of mouse basophils to consist of a fine reticular substance. Kautz and DeMarsh referred to the granules of human basophils as homogeneous. Fine granular material intermixed with pale areas was observed to comprise the internal structure of human basophil granules by Anderson. Zucker-Franklin reported similar particles measuring up to 200 angstroms in diameter in human granules. She observed some granules which contained concentric arrangements of membranes resembling myelin figures. Winqvist observed similar structures in guinea pig basophils which he felt were in the process of degeneration. These structures consisted of concentric membranes surrounding disintegrating granules.

Goodman et al. reported a striking appearance of the granules of basophils from a case of chronic granulocytic
leukemia. They observed concentric layers of materials of differing densities in sections of the granules. Most commonly seen was a layer of relatively electron-lucent material just beneath the limiting membrane. Inside of this was a layer of denser material, and in the center was a relatively lucent core. Other granules were seen with only a dense core in a matrix of the more lucent material. Mottled dense and lucent areas were seen in other granules, and some granules had a vesicular appearance without dense inner material.

The Neutrophil Granule

Last to be considered are the neutrophil granules. These are quite variable in shape with rod-like, oval and rounded forms being described. Varying reports as to the size of these granules are listed in Table 3.

Table 3. Largest Diameters of Neutrophil Granules in Varying Species

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Size of Granule</th>
<th>Species Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rebuck and Woods²</td>
<td>465-650 milli-microns</td>
<td>human</td>
</tr>
<tr>
<td>Marchant⁵</td>
<td>.6 microns</td>
<td>human</td>
</tr>
<tr>
<td>Goodman et al.¹¹</td>
<td>.5 microns</td>
<td>human</td>
</tr>
<tr>
<td>Anderson¹²</td>
<td>.5-.8 microns</td>
<td>human</td>
</tr>
<tr>
<td>Bainton and Farquhar¹³</td>
<td>500 milli-microns</td>
<td>rabbit</td>
</tr>
<tr>
<td>Besis and Thiery¹⁶</td>
<td>.5 microns</td>
<td>human</td>
</tr>
<tr>
<td>Watanabe⁹</td>
<td>.5 microns</td>
<td>human</td>
</tr>
<tr>
<td>Watanabe³</td>
<td>.5 microns</td>
<td>guinea pig</td>
</tr>
</tbody>
</table>
Basis and Thiery described a dark border around the neutrophil granule, and a definite limiting membrane was observed by Watanabe and Kautz and DeMarch in human neutrophils and by Bainton and Farquhar in rabbit cells. Watanabe did not observe this limiting membrane in a study of guinea pig cells, and Anderson saw it only rarely around the granules in human cells.

A well defined substructure such as that mentioned for the eosinophil and basophil granules has not been found in neutrophil granules. Several authors have grouped the granules in two to three different categories depending on their electron densities. Anderson grouped the granules into high, medium and low density forms. The largest granules were in the medium density group, and smallest were the least dense. Watanabe grouped the granules into high and low density varieties. The low density granules were the most numerous in mature forms and were smaller. The high density granules were more numerous very early in the maturational sequence, but their formation ceased, and the numbers of less dense granules began to increase with further maturation. Pease divided the granules in an identical manner to Watanabe. His less dense category also included the smaller and the more numerous of the two granule varieties. His larger, dense granule appeared to be formed in a vacuole with the dense material occupying the center of the structure. Goodman et al. also observed a dense granule and a less dense, more vesicular form. This latter
type was thought possibly to represent cross sections of endoplasmic reticulum rather than a granule variety. Grey and Bissele\textsuperscript{10} reported variable densities of neutrophil granules with some forms appearing vesicular. The smaller, less dense granule of Pease\textsuperscript{14} was observed to be homogeneous in its inner structure. Similar descriptions were made by Besis\textsuperscript{23}, Besis and Thiery\textsuperscript{16}, and Kautz and DeMarsh\textsuperscript{7}. A finely particulate, homogeneous interior of the neutrophil granule was observed by Bainton and Farquhar\textsuperscript{13}.

Pathologic Granulocytes

In the study of the ultrastructure of granulocytes, it was to be expected these techniques would be applied to leukemic cells in order to detect any unique structures present in them. The present section will deal with ultrastructural elements which have been observed in leukemic cells but which have not been regularly reported in normal cells.

Ackerman et al.\textsuperscript{20} observed changes in the mitochondria of the blast cell of human acute granulocytic leukemia along with other cellular alterations. The mitochondrial changes included swelling, disruption of cristae, infolding of the outer mitochondrial membrane, loss of electron density of cristae and accumulation of a dense material in the matrix between the cristae. An additional feature of these cells was the presence of a cytoplasmic fibrillar formation consisting of parallel membranes and bearing some resemblance to the endoplasmic reticulum although apparently distinct from it.
Anderson\textsuperscript{12} also observed fibrillar formations in the blast cells of acute and chronic granulocytic leukemia. In addition, Ackerman et al.\textsuperscript{20} described vacuoles enclosed by a double membrane which they thought were derived by the reflection of endoplasmic reticulum about areas of cytoplasmic material. Finally, they observed dense inclusions which were in various stages of disruption by membranous structures. These were felt to represent ingested erythrocytes.

In one case of acute granulocytic leukemia out of 23 cases of various types of leukemia, Freeman and Samuels\textsuperscript{37} observed a cylindrical $0.5-2.5$ micron by $0.25-1.0$ micron structure in the progranulocytes. The cylinder was composed of circular fibrils enclosing an inner granular mass. A similar structure was observed in two cases of acute monocytic leukemia, but these cells are not under consideration in the present paper.

Special Ultrastructural Studies

A final topic to be considered is that of the various special ultrastructural studies employed on granulocytes. Electron microscope autoradiography and the ultrastructural localization of acid phosphatase have been previously discussed. Not previously discussed are the studies on the electronmicroscopic appearance of intact ultracentrifuged granulocytes reported by Besis\textsuperscript{38} and Besis and Thiery\textsuperscript{16}. In these studies, a layering of the cellular organelles was described. The specific and nonspecific granules were found
at the outer or centrifugal pole of the cell. Proceeding towards the inner or centripetal pole could be found the nucleus, mitochondria, endoplasmic reticulum and centrosome. Innermost were the cytoplasmic vacuoles. Within the nucleus, a layering out was observed with the nucleolus and chromatin more centrifugally located than the remainder of the nucleoplasm.

Summary

In summary, it can be seen that the ultrastructure of granulocytes has been extensively studied in the relatively limited time that techniques have been available for such studies. Maturational changes, theories of granule formation and specific granule structure have been well worked out. Suggestions for further investigation include additional studies of leukemic cells and studies on the embryonic forms of granulocytes. Of the studies mentioned in this paper, only that of Sorensen²⁴ dealt with embryonic hematopoietic tissue.
REFERENCES


