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Secretion of Primary Granules from Developing Human Eosinophilic Promyelocytes (40352)

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Mature eosinophils contain two types of membrane-delimited secretory granules. The primary granules are large (0.6–1.2 microns) in diameter, spherical, homogeneously dense, and are produced in the promyelocyte stage of development (1). The secondary granules produced in the myelocyte stage contain a crystalline core and represent the vast majority of the secretory granules of the mature eosinophil. Both granules stain strongly for peroxidase (1, 2). This developmental scheme has been most carefully studied in rats and rabbits (1) but is believed to occur in humans as well (2, 3).

Little is known concerning the functions of the eosinophilic promyelocyte. This is due, in part, to the very small number of these cells (less than 1%) present in normal marrow. In the present electron microscope cytochemical study we examined bone marrows of patients with disease states associated with increased numbers of eosinophilic promyelocytes along with other immature developing cells. Our evidence indicates that the contents of the primary granules of eosinophilic promyelocytes are secreted by exocytosis into the extracellular space of the marrow while the membrane surrounding the granules is retained within the cell. This secretion appears to occur simultaneously with the synthesis and production of new granules.

Materials and methods. Specimens consisted of bone marrow aspirates obtained from patients in the various stages of chronic myelocytic leukemia (initial diagnosis, remission, and blastic transformation) and nonleukemic states including metastatic adenocarcinoma and idiopathic thrombocytopenic purpura (ITP). These patients had increased numbers of promyelocytes and myelocytes as well as blasts in the case of CML with blastic transformation. One of the patients with CML was studied three times; during primary diagnosis, during remission while on busulfan, and in the blast phase. The second CML patient was studied at primary diagnosis, and the third CML patient was examined during chronic phase while under treatment with busulfan. The patient with adenocarcinoma, metastatic to bone, did not have demonstrable metastatic cells on the aspirate or biopsy of the specimen examined in this study. The ITP case was newly diagnosed and on no therapy at the time the bone marrow was obtained.

The bone marrow chips were prepared for cytochemical studies according to the methods of Bainton, *et al.* (4). In brief, the marrow chips were fixed for 10 minutes in cold cacodylate-buffered 1.5% glutaraldehyde with 1% sucrose and then rinsed in cold cacodylate-buffer with 7% sucrose for 24 hr. To demonstrate myeloperoxidase activity, tissue was first soaked in the medium of Graham and Karnovsky (5) (pH 7.6) without substrate (H_2O_2) for 10–15 min at room temperature and then incubated in the full cytochemical medium for 45 min at room temperature. Sucrose (5%) was added to all incubations. For these cytochemical studies controls consisted of H_2O_2-free media. All controls showed no demonstrable reaction product.

Following incubation, the cells were rinsed in cold 7.5% sucrose, post-fixed in cold cacodylate-buffered 1% OsO_4 for 1 hr, rinsed with cold 7.5% sucrose, and soaked *en bloc* with veronal acetate buffered uranyl acetate for 30 min at room temperature. They were then rinsed in cold 7.5% sucrose, dehydrated in a graded series of ethanols and propylene oxide and embedded in Epon.

Silver to grey thin sections were cut on a Porter Blum MT2-B ultramicrotome, lightly stained with lead citrate, and examined on a

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JEOL JEM-100 electron microscope operated at 80 kv. Electron micrographs were taken at initial magnifications of 4000–15,000.

Results. As previously described in the promyelocyte stage of eosinophil development, all the secretory granules are large, homogeneous, and spherical, while the secondary granules with their characteristic crystalline core begin to appear during the myelocyte stage (1, 2). The rough endoplasmic reticulum (RER) of the eosinophilic promyelocyte contains reaction product for myeloperoxidase (MPO) (Figs. 1, 2) and is more saccular and dilated than its PMN promyelocyte counterpart.

We found repeated evidence that developing eosinophilic promyelocytes release the contents of their MPO positive secretory granules into the extracellular space of the bone marrow by exocytosis (Figs. 1–3). The same cells that show this degranulation also contain a MPO positive RER and Golgi apparatus (Figs. 1–3).

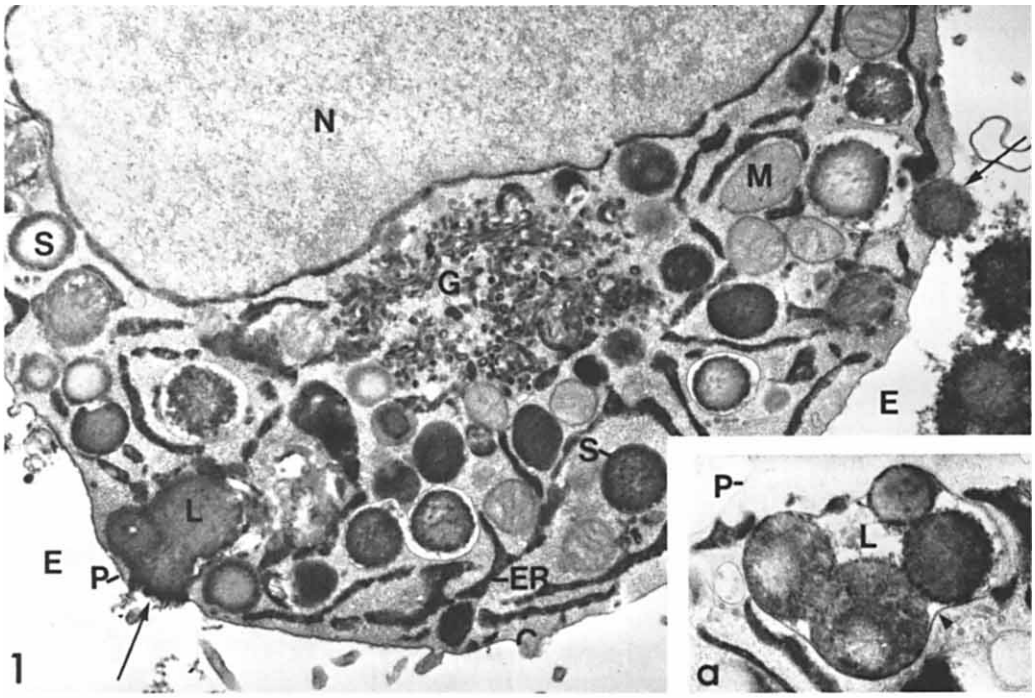
In the eosinophilic promyelocytes undergoing degranulation, there was a coalescence of several individual membrane-bound secretory granules into one or more larger membrane-delimited structures each containing several granules surrounded by a single membrane (Figs. 1, inset a, and 3). Many of these large structures were found to be in a continuity with the extracellular space (Figs. 1–3). Myeloperoxidase was demonstrable within the membrane-delimited granules and was seen to be released into the extracellular space (Figs. 1, 2). The luminal surface of the membrane surrounding these multiple secretory granules often also stain strongly for MPO (Figs. 3, 4). Several of these larger membranous structures, either devoid of any granular content or containing only a single granule, were seen within the cell cytoplasm, appearing as if they were retained in the cell following degranulation (Figs. 3, 4). This degranulation was seen in patients with CML, adenocarcinoma and ITP. We did not observe such degranulation in the numerous PMN promyelocytes nor in developing monocytes, which also contain MPO positive RER and secretory granules. Later stage eosinophilic myelocytes containing characteristic crystalline granules also did not appear to degranulate in this manner.

Discussion. The present study indicates that the homogenous spherical primary granules formed in the eosinophilic promyelocyte can discharge their contents into the extracellular space of the marrow by a process of exocytosis, while the cell is synthesizing MPO and new granules. In a morphological study a similar phenomenon was noted in normal human marrow (6). These observations strongly suggest that secretion from eosinophilic promyelocytes consists of two steps; initial fusion of individual secretory granules to form a compound structure containing several granules surrounded by a single membrane, followed by exocytosis, the fusion of the membrane-delimited compound structure with the plasma membrane permitting access of the granule content into the extracellular space (7). This process resembles the exocytosis described as the secretory mechanism of mast cells (8).

FIG. 1. Eosinophilic promyelocyte incubated for the localization of peroxidase activity. Note the large, dense, spherical peroxidase-reactive secretory granules (S), reactive saccular endoplasmic reticulum (ER) and Golgi apparatus (G). Inset a shows secretory granules that have fused to form large membrane delimited structure (L). These larger structures appear to release their contents into the marrow extracellular space (E) by exocytosis (arrows). Plasma membrane is at P, mitochondria at M, nucleus at N. × 12,000; Inset a × 13,200.

FIG. 2. Higher magnification view of secretory granule release by exocytosis (arrow) from an eosinophilic promyelocyte. Plasma membrane is at P, and marrow extracellular space at E. Cytoplasm contains secretory granules (S), endoplasmic reticulum (ER) and mitochondria (M). Note the markedly smaller diameter of peroxidase-reactive granules (A) in an adjacent polymorphonuclear leukocyte. × 24,000.

FIG. 3 and 4. Eosinophilic promyelocytes reacted as in Fig. 1. The large vacuolar structures seen at V contain an occasional granule or are entirely devoid of secretory granules (Fig. 4). Note that the inner surface of the membrane of these structures is reactive for peroxidase (I). An example of exocytosis is seen at arrow in Fig. 3. Nuclei are at N, mitochondria at M. Fig. 3 × 10,500; Fig. 4 × 14,000.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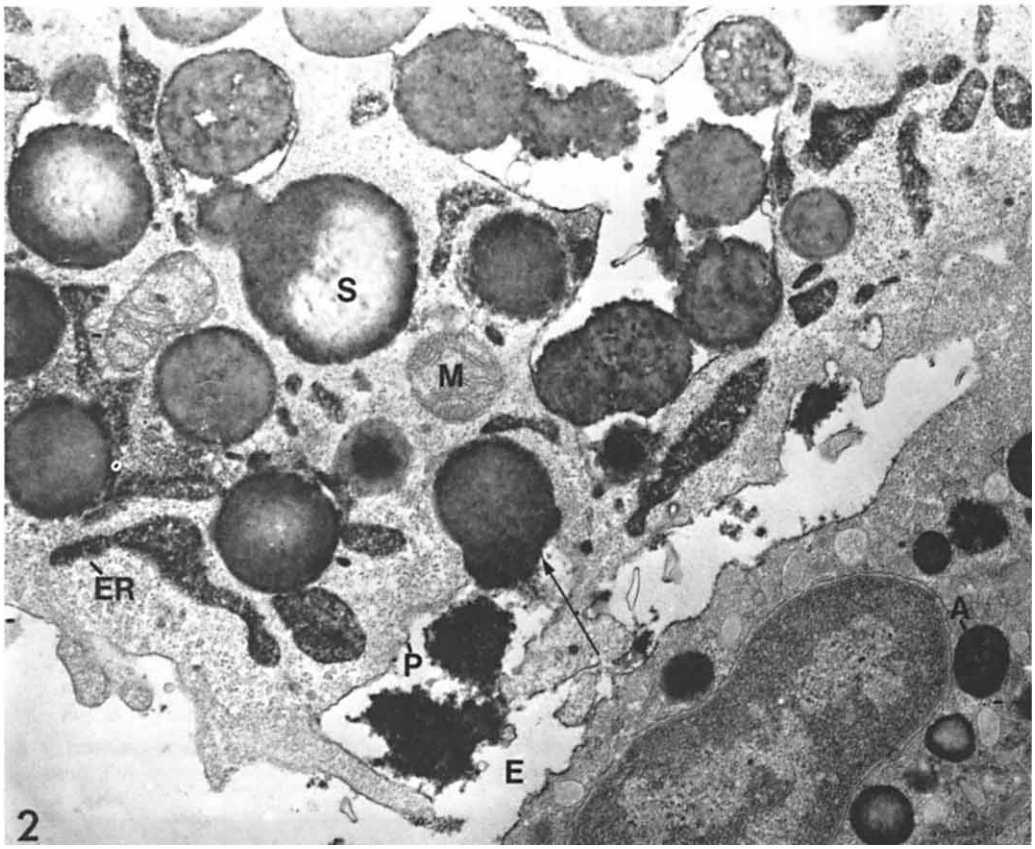
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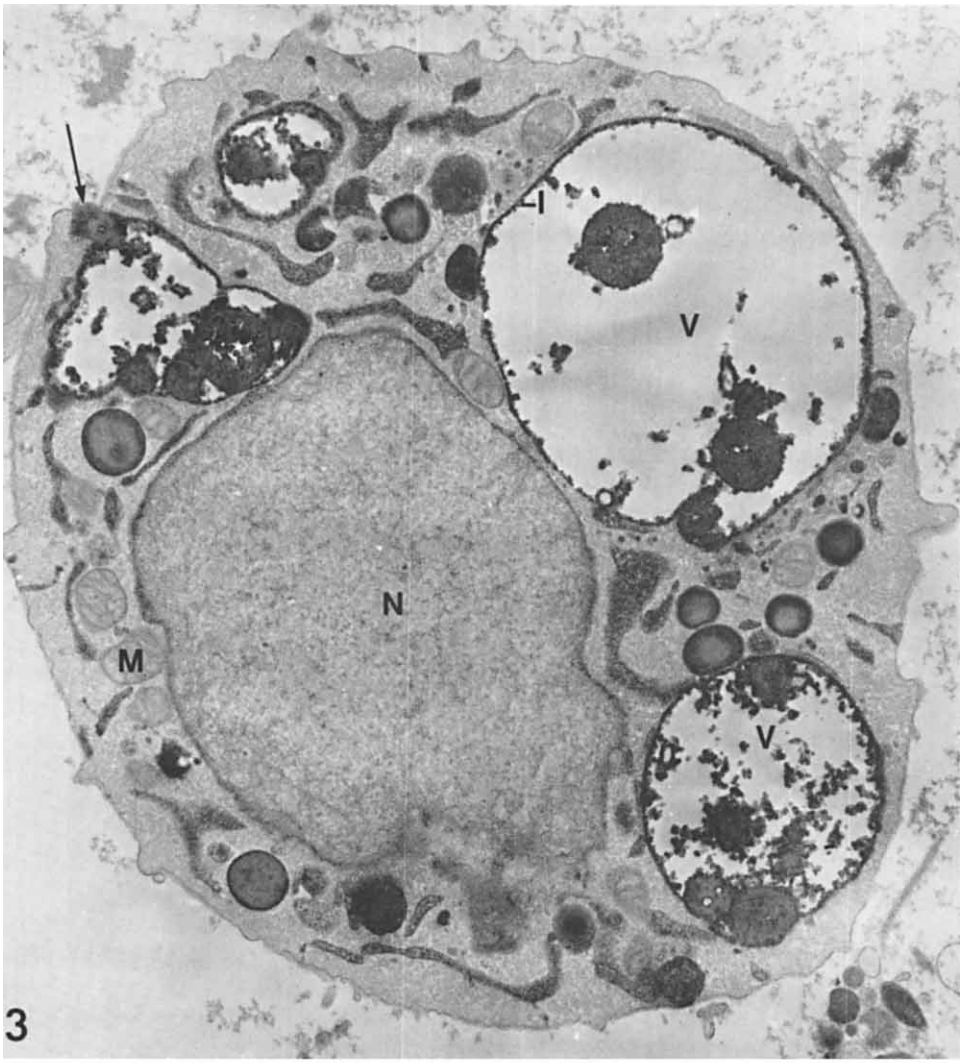

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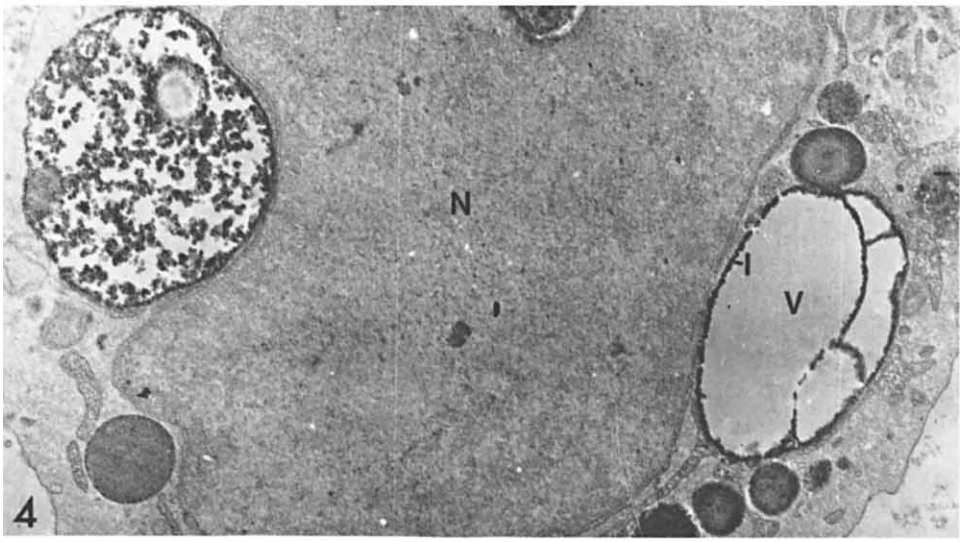

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In cells actively secreting materials by exocytosis, there is a considerable addition of membrane to the cell surface. A compensating endocytotic mechanism appears to retrieve surface membrane back into the cell to maintain a relatively constant surface area (9, 10). The precise nature of such retrieved membrane is not yet understood, particularly its relationship to the original secretory granule membrane. In the present study, the intraluminal surface of the large coalesced secretory granule membrane is labeled with MPO providing a potential membrane marker. It appears that this membrane delimited secretory granule only remains fused with the plasma membrane and patent to the extracellular space for a time sufficient to release the granular contents. We were able to find numerous examples of large MPO labeled cytoplasmic vacuolar structures either devoid of granules or containing very few granules. This evidence suggests that the same fragment of membrane that originally surrounded the secretory granule is retained within the cell. The subsequent fate of this membrane has not been resolved.

Developing promyelocytes of the neutrophil or monocyte series within our preparations, which also contain MPO-positive secretory granules do not show a similar exocytosis of their granules (see also 11). Therefore, we believe the events we observed in eosinophilic promyelocytes are physiological and not merely induced during aspiration of marrow or tissue preparation.

This degranulation of eosinophilic promyelocytes does not appear to be limited to any specific disease state or particular chemotherapeutic regimen. It was observed in several stages of chronic myelocytic leukemia, in adenocarcinoma and in ITP.

The significance of our observations of secretory granule release by eosinophilic promyelocytes is unclear. There is no available biochemical data on the content of these early eosinophil granules. Cytochemical studies have shown that they contain MPO, but it is not clear that these granules are biochemically identical in other respects to the later crystalloid-containing granules that are clearly a part of the lysosomal system (12–14). Previous work has demonstrated that in mature eosinophils phagocytosis is stimulated by antigen–antibody complexes and that granules are released into the phagocytic vacuoles (14–16) but not into the extracellular space. In vitro studies have demonstrated a substance in the eosinophil granule, thought to be associated with myeloperoxidase, which causes the disruption of mast cells (17, 18). These studies hint that we may be viewing a component of an inflammatory response. Further investigation of the chemical content of these granules is clearly indicated.

Summary. This study indicates that the primary large homogenous dense granules of eosinophilic promyelocytes are released into the extracellular space of the marrow by exocytosis while the cell is producing new secretory granules. This process appears to occur in two steps: Initial fusion of several individual granules to form one large myeloperoxidase positive membrane-delimited body, followed by exocytotic release of the granule content. The membrane of this large secretory granule appears to be retained within the cell since empty, myeloperoxidase positive vacuolar structures remain following secretion.

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