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Similarities in the ultrastructural morphology and developmental and secretory mechanisms of human basophils and eosinophils

Ann M. Dvorak, MD *Boston, Mass.*

Basophils and eosinophils are distinctive granulocytes that can readily be distinguished from one another by ultrastructural analysis. Despite these morphologic distinctions, many morphologic similarities between these two granulocyte lineages exist in their mature, developmental, and functional forms. In this review we address the morphologic similarities of human basophils and eosinophils. The emphasis is on recent work with routine, autoradiographic, cytochemical, immunogold, and enzyme–affinity gold preparations for ultrastructural studies. These new studies have been instrumental in the documentation of subcellular localizations of important materials and of a vesicular transport mechanism for secretion. Older literature regarding ultrastructural morphologic aspects of the cell biology of human basophils and eosinophils can be found in previous reviews.[1-12]

Abbreviations used

BM:	Basophilic myelocyte
CLC:	Charcot-Leyden crystal
ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
EM:	Eosinophilic myelocyte
EPO:	Eosinophil peroxidase
FMLP:	N-formyl-methionyl-leucyl-phenyl-alanine
HES:	Hypereosinophilic syndrome
IL:	Interleukin
MBP:	Major basic protein
PMD:	Piecemeal degranulation
RER:	Rough endoplasmic reticulum
rhIL:	Recombinant human interleukin
TNF-α:	Tumor necrosis factor-α
TPA:	Tetradecanoyl phorbol acetate

MATURE CELLS

Mature human basophils (Fig. 1) and eosinophils (Fig. 2) are cells of similar size that have polylobed nuclei within which the chromatin is condensed. Nucleoli are absent. Their surface contours are similar, consisting of irregularly placed, thick cellular processes. Both cell types contain cytoplasmic secretory granules, glycogen, mitochondria, free ribosomes, and small Golgi structures. Lipid bodies, nonmembrane-bound reservoirs of lipid, have been described in both cells. The secretory granules in human basophils differ both morphologically and biochemically from those in human eosinophils. Human peripheral blood basophils contain, for the most part, a single granule type, which is membrane-bound and contains dense particles or fine granular con-

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.
Supported by United States Public Health Service grant AI-33372.
Reprint requests: Ann M. Dvorak, MD, Department of Pathology, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.
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FIG. 1. This mature human peripheral blood basophil was exposed to cationized ferritin after fixation and prepared with a reduced osmium technique for electron microscopy. With these methods, cationized ferritin binds to the plasma membrane in a uniform, dense layer; cytoplasmic particle-filled secretory granules, enclosed by dense granule membranes, are well-visualized; five nuclear lobes are present, but with this processing technique, condensed nuclear chromatin is not well-delineated. The cytoplasm has a small Golgi structure, cytoplasmic, vesicles, dense glycogen particles, and several mitochondria. Multiple dense concentric membranes within several secretory granules are extensive. (Original magnification ×13,000.)

tents (Fig. 3). These granules are often subcompartmentalized by multiple, dense membranous arrays within them (Fig. 3, *B*). Variable numbers of typically shaped, or amorphously shaped, Charcot-Leyden crystals (CLCs) reside within the particle granules (Fig. 3, *B* and *C*). A second granule population[13] has homogeneous contents in small structures. These granules are infrequent and are located near nuclei. Small numbers of a new granule morphotype emerge during recovery from degranulation of peripheral blood basophils (Dvorak et al. Unpublished data) and during the development and secretion of basophils in c-*kit* ligand-supported suspension cultures of human cord blood cells.[14] These granules are often similar in size to the more numerous particle granules, but their contents are uniformly moderately electron-dense. CLCs, multiple dense membranous arrays, and particles are not present within them. As demonstrated by immunogold staining, they contain CLC protein (Dvorak et al. Unpublished data). The morphologic appearance and biochemical contents of these granules suggest, as in eosinophils,[15] that they are residual primary granules.

Human eosinophil granules are comprised of large numbers of secondary biocompartmental granules (Figs. 2 and 4), smaller numbers of

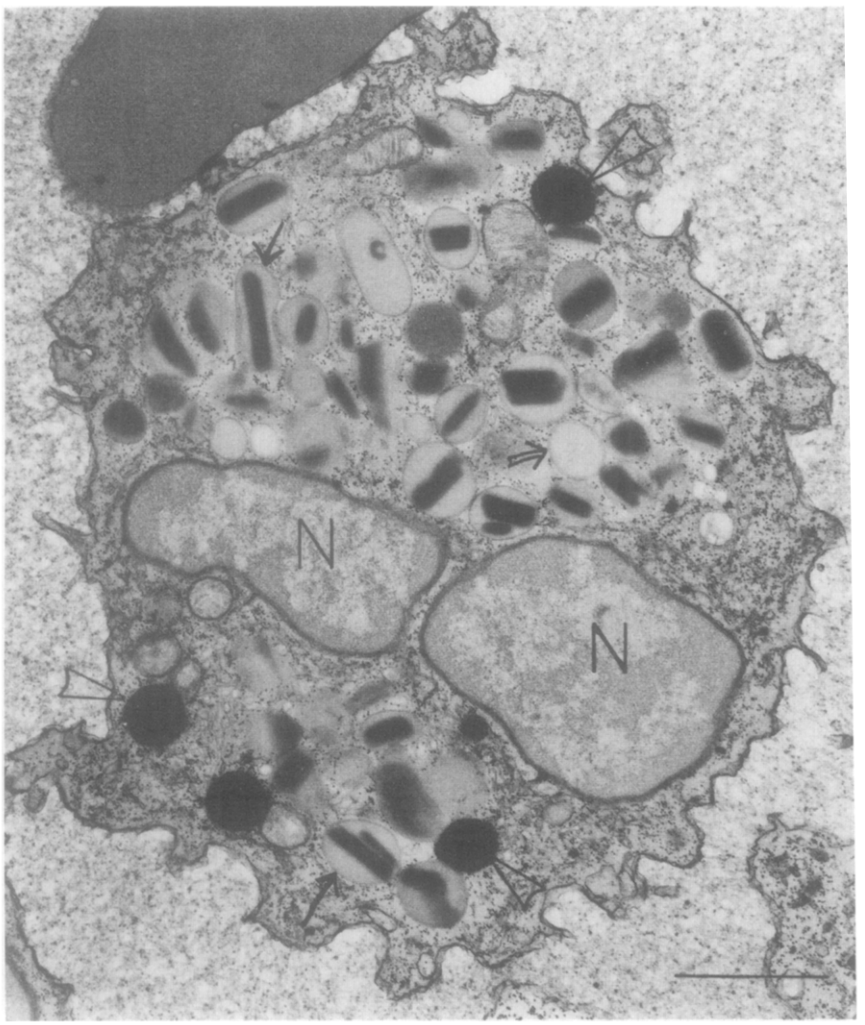


FIG. 2. This mature human peripheral blood eosinophil was prepared with a reduced osmium technique for electron microscopy. Comparison with the similarly prepared basophil in Fig. 1 demonstrates essential similarities and differences in these mature representatives of two granulocyte lineages. They are of similar size and have similar, irregular, short, thick surface processes. Although both cell types display separate nuclear lobes, basophil nuclei generally have multiple lobes, and eosinophil nuclei are generally bilobed *(N)*. The cytoplasmic secondary secretory granules *(closed arrows)* clearly show their bicompartmental nature. The electron-dense central cores are surrounded by a less dense matrix and bounded by a granule membrane. Primary granules *(open arrow)* have a uniform content; dense, central cores are absent. Large, round, osmiophilic lipid bodies *(arrowheads)* are present in this eosinophil, obtained from a patient with the hypereosinophilic syndrome. The cytoplasm also contains dense glycogen particles and mitochondria. (Original magnification ×14,000.) (From Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344.)

primary granules (Fig. 4, *B*), and small granules (Fig. 4, *B*).[3] The numerous secondary granules in mature cells are bound by membranes and consist of a central, dense, crystalloid core compartment and a less dense outer matrix compartment (Fig. 4, *A*). Ultrastructural immunogold and cytochemical preparations have disclosed the presence of major basic protein (MBP) in the core compartment of mature granules,[16, 17] eosinophil peroxidase (EPO) (Fig. 4, *C*),[3, 17] eosinophil-derived neurotoxin (EDN),[16] eosinophil cationic protein (ECP),[16, 17] and tumor necrosis factor-α (TNF-α)

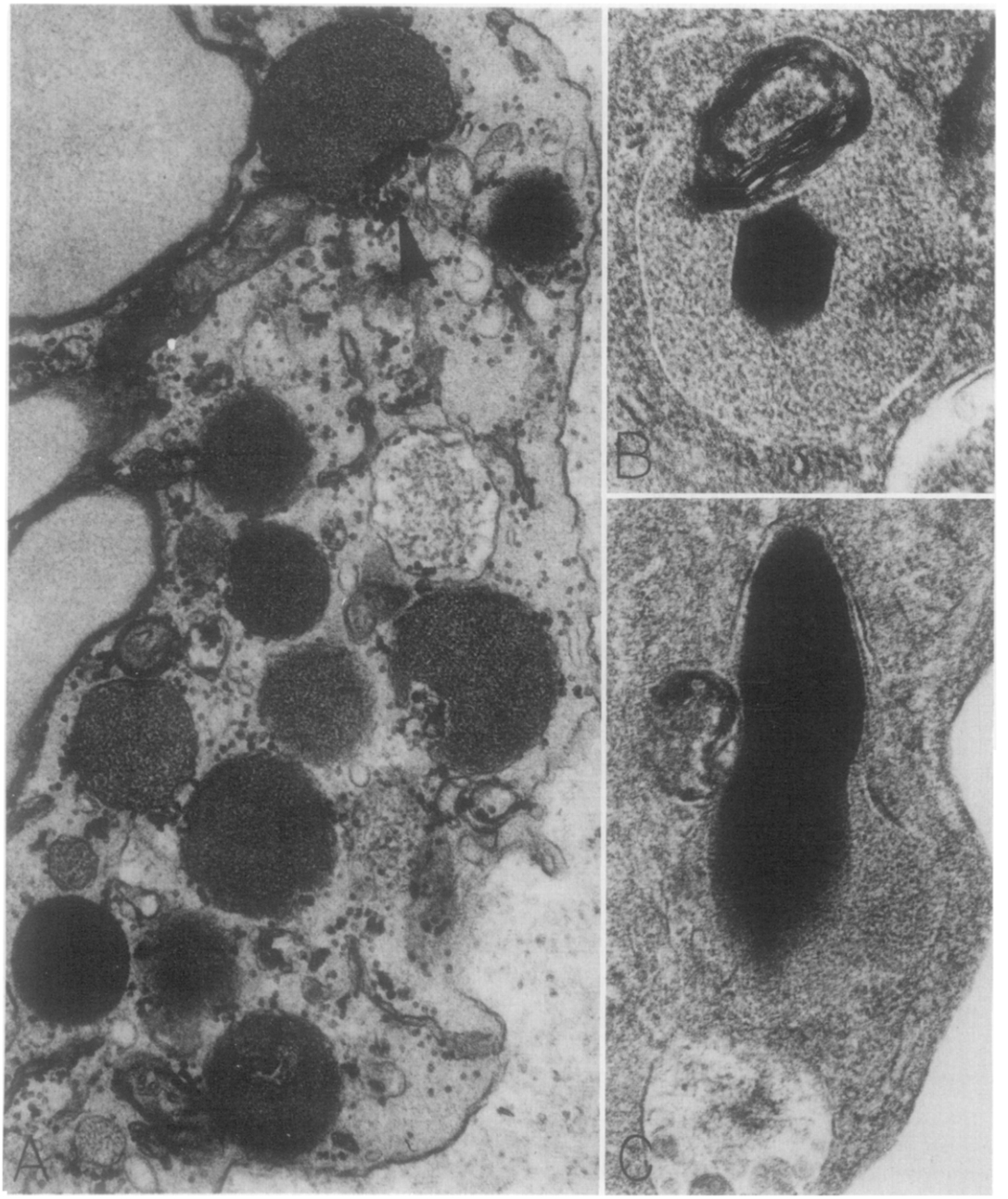


FIG. 3. Mature human basophil granules are shown at higher magnification. The peripheral blood basophil in **A** shows granules that are completely filled with particles. Note the monoparticulate, dense glycogen particles in the cytoplasm. A small cluster of glycogen particles occupies a semicircular indentation of one granule *(arrowhead).* The basophil granules in **B** and **C** (cord blood cell–derived basophils in cultures supplemented with rhIL-5 for 5 weeks) contain particles surrounding hexagonal and elongated CLCs that are homogenously electron-dense. In **B** a collection of dense concentric membranes enclosing granule particles is present. (Original magnifications: **A,** ×37,500; **B,** ×69,000; **C,** ×61,000.) **Panels B** and **C** from Dvorak AM, Saito H, Estrella P, Kissel S, Arai N, Ishizaka T. Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. Lab Invest 1989;61:116-32. Copyright by The United States and Canadian Academy of Pathology, Inc.

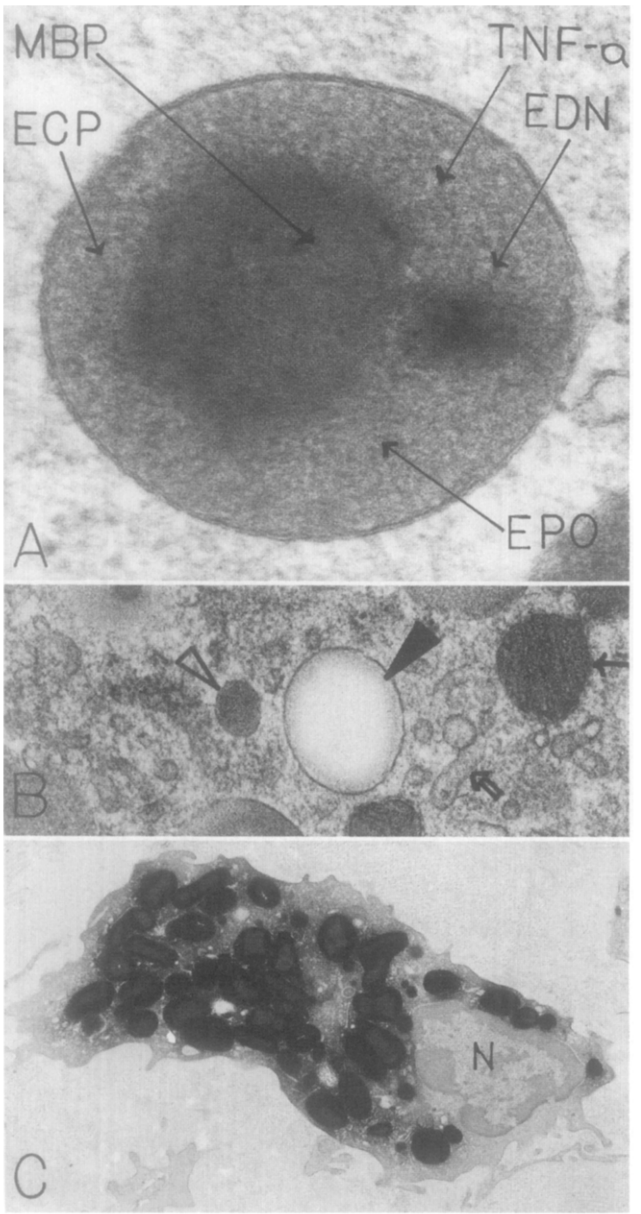


FIG. 4. Mature human eosinophil granules are shown at higher magnification (**A** and **B**) or after a cytochemical procedure to detect peroxidase (**C**). In **A**, a bicompartmental secondary granule (duodenal biopsy) is enclosed by a trilaminar unit membrane. The finely granular matrix compartment (TNF-α, ECP, EDN, EPO) surrounds an electron-dense crystalline core compartment (MBP), which reveals a regular crystalline periodic array. In **B** (skin biopsy specimen from a patient with hypereosinophilic syndrome [HES], control immunogold preparation, in which nonimmune IgG was substituted for specific primary antibody to CLC protein), a primary granule *(closed arrowhead)* has homogenous contents and does not artifactually bind the immunogold reagent. A small granule *(open arrowhead)*, mitochondria *(closed arrow)*, and smooth endoplasmic reticular tubule *(open arrow)* are nearby. In **C** (small intestine biopsy specimen), the eosinophil shows dense EPO in the matrix compartment of secondary granules. *N*, Nucleus. (Original magnifications: **A**, ×152,000; **B**, ×47,500; **C**, ×8000.) **Panel A** from Dvorak AM. Ultrastructural studies on mechanisms of human eosinophil activation and secretion. In: Gleich GJ, Kay AB, eds. Eosinophils in allergy and inflammation. New York: Marcel Dekker, 1993:159-209. Reprinted from Ref. 10 by courtesy of Marcel Dekker, Inc. **Panel B** from Dvorak AM, Weller PF, Monahan-Earley RA, Letourneau L, Ackerman SJ. Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) and peroxidase in macrophages, eosinophils and extracellular matrix of the skin in the hypereosinophilic syndrome. Lab Invest 1990;62:590-607. Copyright by The United States and Canadian Academy of Pathology, Inc.

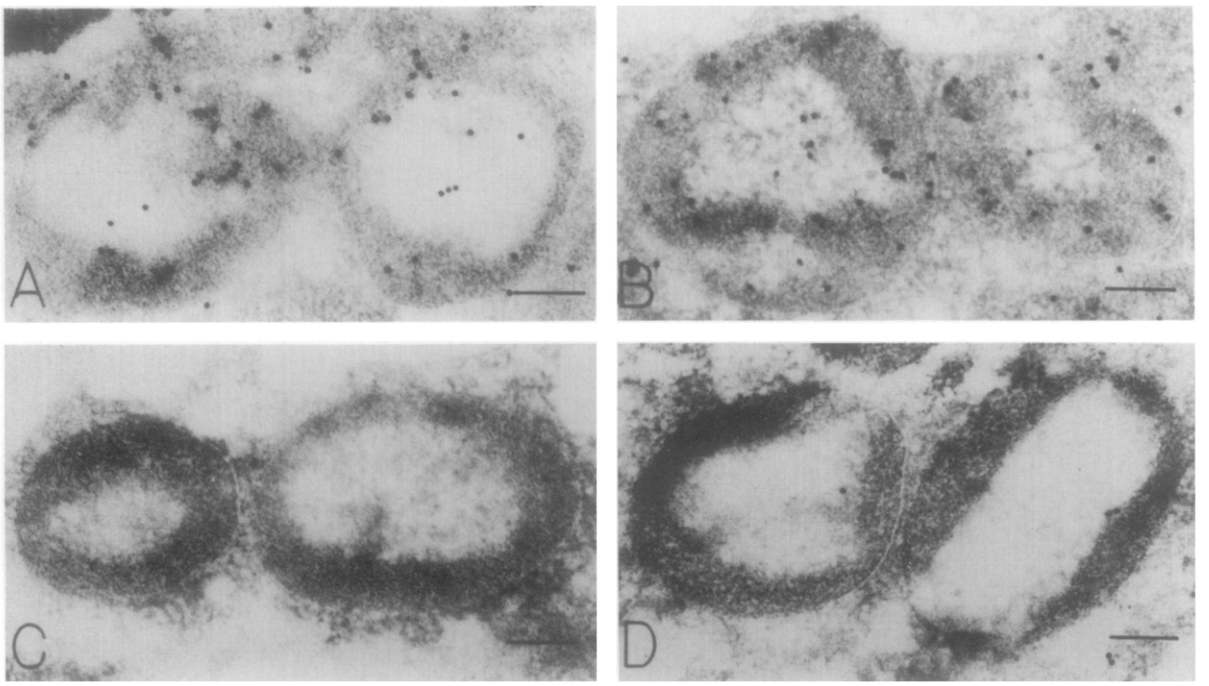


FIG. 5. Mature human eosinophil secondary granules (peripheral blood, patient with HES, immunogold preparation to detect TNF-α [**A** and **B**] and controls for specificity [**C** and **D**]) show gold particles over the matrix compartment, indicating the presence of TNF-α (**A** and **B**), which was absent when normal rabbit serum (**C**) or rhTNF-absorbed primary antibody (**D**) were substituted for the specific antibody to TNF-α in the immunogold sequence. Bars = 0.15 μm. (Reproduced with permission from Beil WJ, Weller PF, Tzizik DM, Galli SJ, Dvorak AM. Ultrastructural immunogold localization of tumor necrosis factor-α to the matrix compartment of eosinophil secondary granules in patients with idiopathic hypereosinophilic syndrome. J Histochem Cytochem 1993;41:1611-5.)

(Fig. 5)[18] in the matrix compartment of mature secondary granules (Fig. 4, *A*). Immunogold ultrastructural studies have determined that the granule storage site for CLC protein is in the homogeneously dense primary granules, which occur in small numbers and do not have crystalline cores[15] (Fig. 6). Small granules in eosinophils contain hydrolytic enzymes.[3] The primary granules in eosinophils that store CLC protein[15] morphologically resemble a granule, which is densely labeled for CLC protein, that appears in recovering and developing basophils[14] (Dvorak et al. Unpublished data).

The distribution of CLC protein in mature, unactivated basophils and eosinophils is different. In unstimulated eosinophils CLC protein was evident by immunogold staining only in primary granules (Fig. 6); on the other hand, in unstimulated basophils, CLC protein was diffusely present in the main particle granule population, in CLCs within these granules, and in small perigranular vesicles[19] (Fig. 7). Activated eosinophils in vivo[20, 21] or in vitro[22, 23] revealed diffuse cytoplasmic, subplasma membrane and nuclear CLC protein (Fig. 8). We also noted the formation of large cytoplasmic CLCs, which contain CLC protein.[3] Activated human basophils also formed large nuclear and cytoplasmic CLCs, which were gold-labeled, indicating the presence of CLC protein[14] (Dvorak et al. Unpublished data). Additionally, and as in eosinophils, there was diffuse cytoplasmic and nuclear labeling for CLC protein in stimulated basophils[14] (Dvorak et al. Unpublished data).

Lipid bodies are found in both mature basophils and eosinophils (Figs. 2 and 9, *A*). However, these organelles are more generally found in eosinophils than in basophils. Consequently, our completed studies focus on the contents of lipid bodies in eosinophils only[24-26] (Dvorak et al. Unpublished data). Using ultrastructural autoradiography, we initially localized tritiated arachidonic acid to lipid bodies (Fig. 9, *B*). Arachidonic acid is important in the generation of prostaglandins and

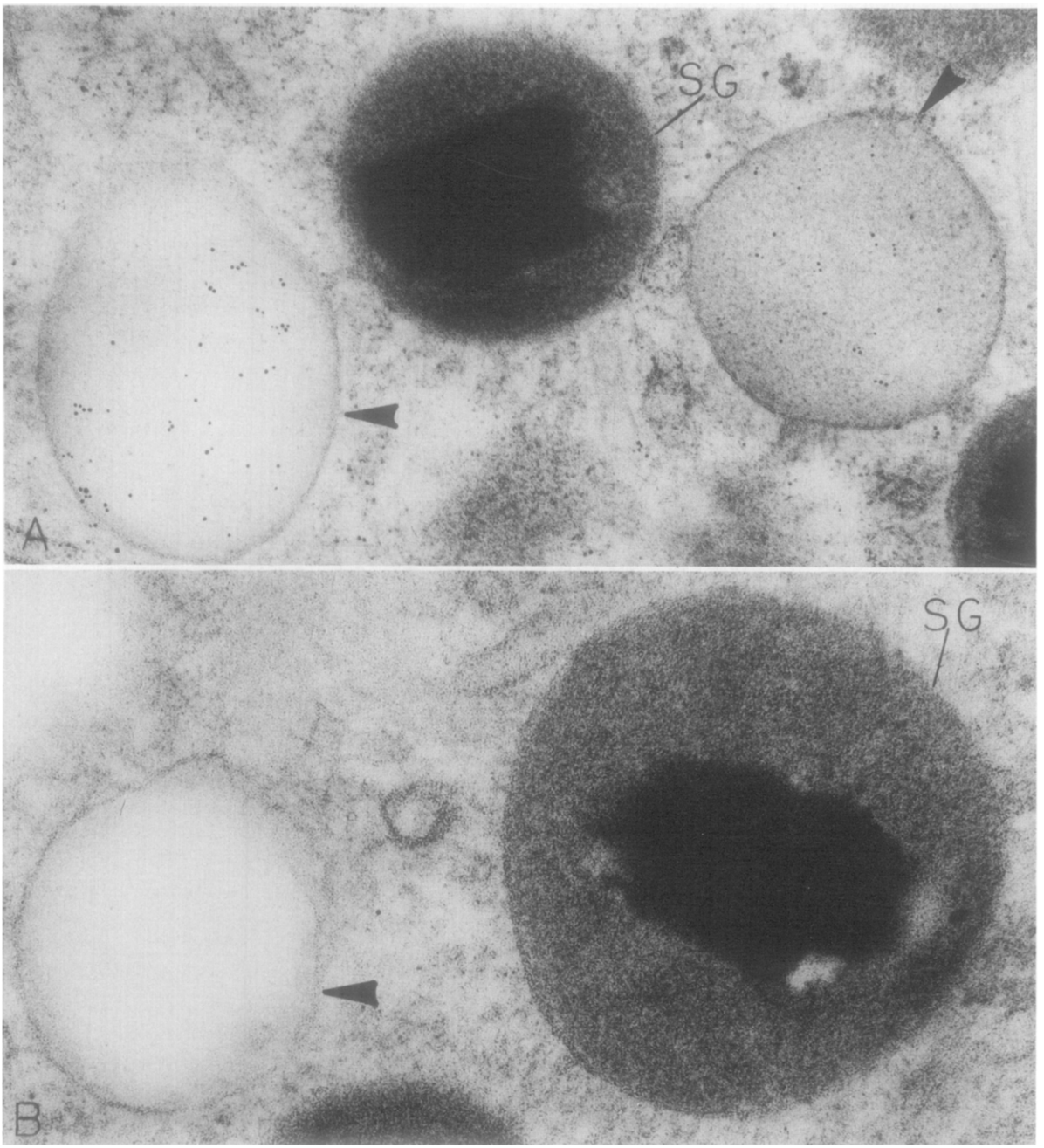


FIG. 6. Mature eosinophil granules (peripheral blood, patient with HES, immunogold preparation to detect CLC protein **(A)** and specificity control, nonimmune IgG **[B]**) show gold-labeled primary granules *(arrowheads)* in **A,** which are not artifactually labeled in the control in **B.** Note that the bicompartmental secondary granules do not contain CLC protein in their matrix or core compartments **(A),** nor do they artifactually bind the gold-labeled secondary antibody **(B).** (Original magnification: **A** and **B,** ×100,000.) **Panel B** from Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344.

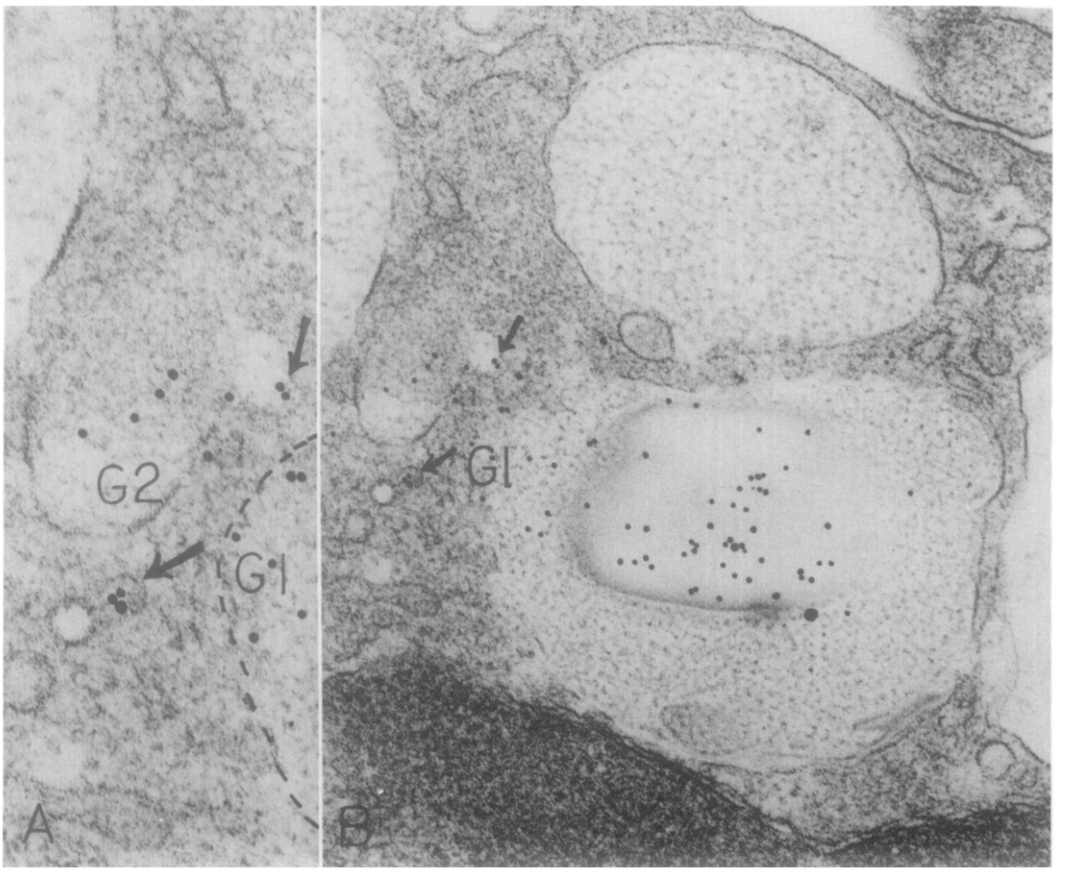


FIG. 7. Mature basophil granules (peripheral blood from a patient with chronic myelogenous leukemia, immunogold preparation to detect CLC protein) shown at two magnifications illustrate a granule *(G1),* which contains a gold-labeled crystal and granule particle-associated gold adjacent to it on one side in **B.** This adjacent area, an oblique section of the far edge of this granule (*G1, dotted line,* shows limits of granule matrix, as shown in **A**), and a second granule *(G2)* are shown at higher magnification in **A.** Gold label, indicating the presence of CLC protein, is seen within several small, smooth perigranular vesicles (*arrows* in **A** and **B**). Other vesicles are empty. (Original magnifications: **A,** ×124,000; **B,** ×68,000.) (From Dvorak AM, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to granules and intragranular crystals in mature human basophils. Lab Invest 1989;60:557-67. Copyright by The United States and Canadian Academy of Pathology, Inc.)

leukotrienes; its identification in lipid bodies[27] stimulated the immunomorphologic studies[28-30] that have disclosed the enzymes (i.e., prostaglandin endoperoxide synthase [cyclooxygenase] [Fig. 9, *C*] and 5-lipoxygenase) necessary for the generation of prostaglandins and leukotrienes in eosinophil lipid bodies.[12, 26, 31]

IMMATURE CELLS

Immature cells, identifiable as basophil or eosinophil precursors on the basis of immature and mature granule mixtures, are termed *myelocytes.* The general ultrastructural features of myelocytes from either lineage are similar and reflect a complement of organelles necessary for protein synthesis. Thus basophilic myelocytes (BMs) (Fig. 10) and eosinophilic myelocytes (EMs) (Fig. 11) are cells that are approximately three times larger than their mature counterparts, have larger lobular nuclei with partially dispersed chromatin, large nucleoli, extensive Golgi structures, and a cytoplasm filled with parallel arrays of distended cisterns of rough endoplasmic reticulum filled with lightly dense material.[32-34] Immature granules and mitochondria fill the remainder of the cytoplasm. In both BMs and EMs, these immature granules are larger than the mature granules of mature cells. In BMs the immature granules are filled with

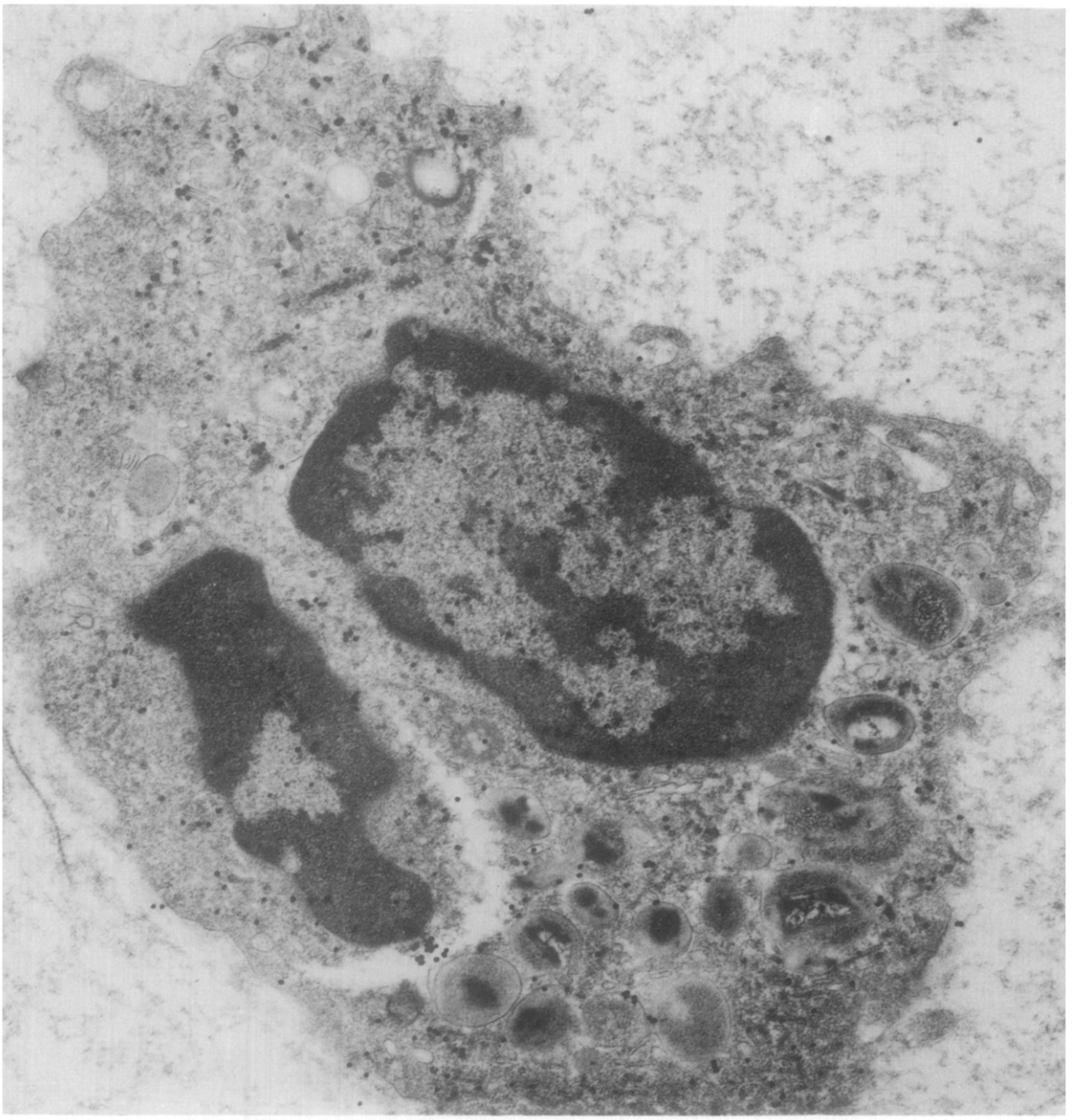


FIG. 8. Mature eosinophil (pancreatic tumor biopsy specimen, immunogold preparation to detect CLC protein) shows numerous 30 nm gold particles in the nucleus and cytoplasm of this activated tissue eosinophil. Secondary granules are not labeled. (Original magnification ×17,500.) (From Dvorak AM, Letourneau L, Weller PF, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to intracytoplasmic crystals in tumor cells of primary solid and papillary neoplasm of the pancreas. Lab Invest 1990;62:608-15. Copyright by The United States and Canadian Academy of Pathology, Inc.)

lightly dense amorphous material (Fig. 10); in EMs, immature granules contain either moderately dense amorphous material or homogeneously dense material (Fig. 11). In each type of myelocyte, some immature granules contain small, subgranular membrane vesicles. Although the immature granules of BMs and EMs are sufficiently different, allowing correct lineage assignment, the appearance of small, particle-filled granules allows the definitive identification of BMs (Fig. 10, *inset*); the

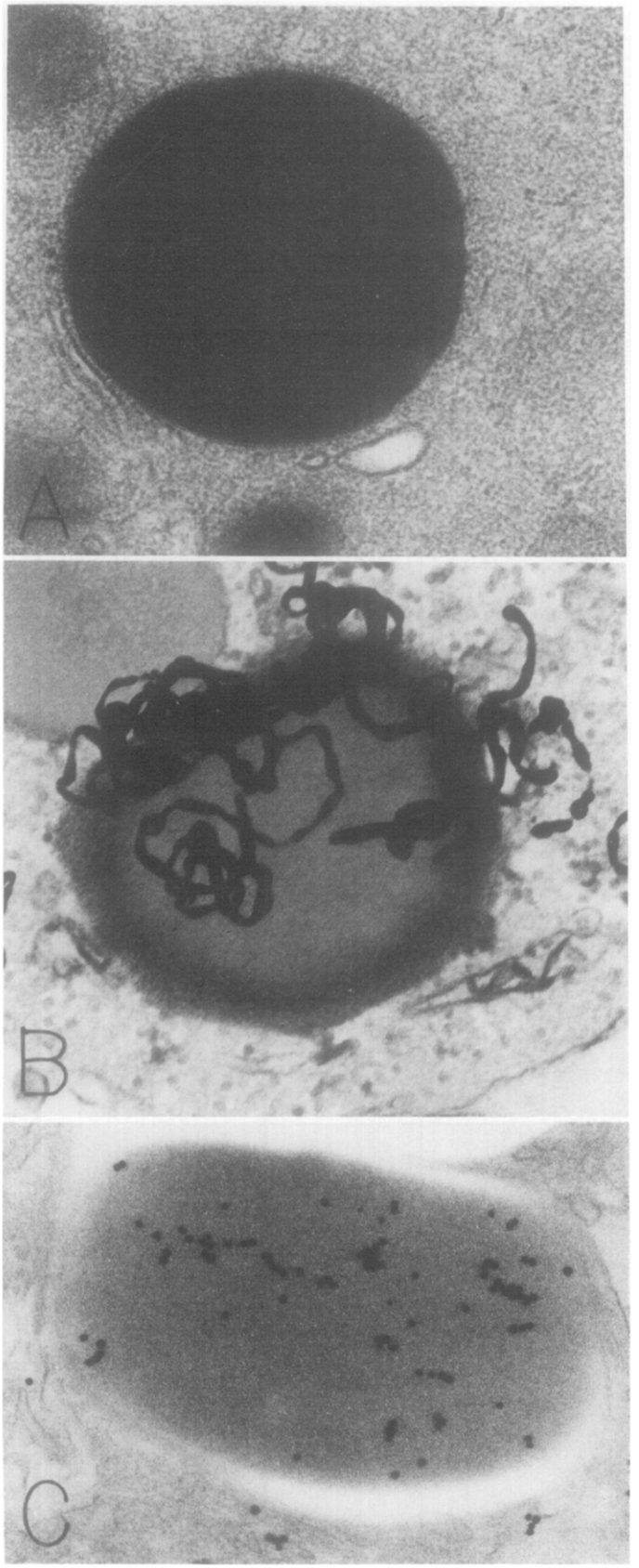


FIG. 9. Mature eosinophil lipid bodies, prepared either with a routine ultrastructural method **(A)**, for autoradiography after a pulse of tritiated arachidonic acid **(B)**, or with an immunogold method to detect prostaglandin endoperoxide synthase (cyclooxygenase) **(C)**. In **A** the large, round, osmiophilic lipid body is homogenously dense; in **B** numerous silver grains label the lipid body, indicating the presence of tritiated arachidonic acid; in **C** the gold-labeled lipid body contains cyclooxygenase. Original magnifications: **A,** ×61,500; **B,** ×57,000; **C,** ×45,500.) **Panel A** from Dvorak AM, Saito H, Estrella P, Kissel S, Arai N, Ishizaka T. Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. Lab Invest 1989;61:116-32. Copyright by The United States and Canadian Academy of Pathology, Inc.

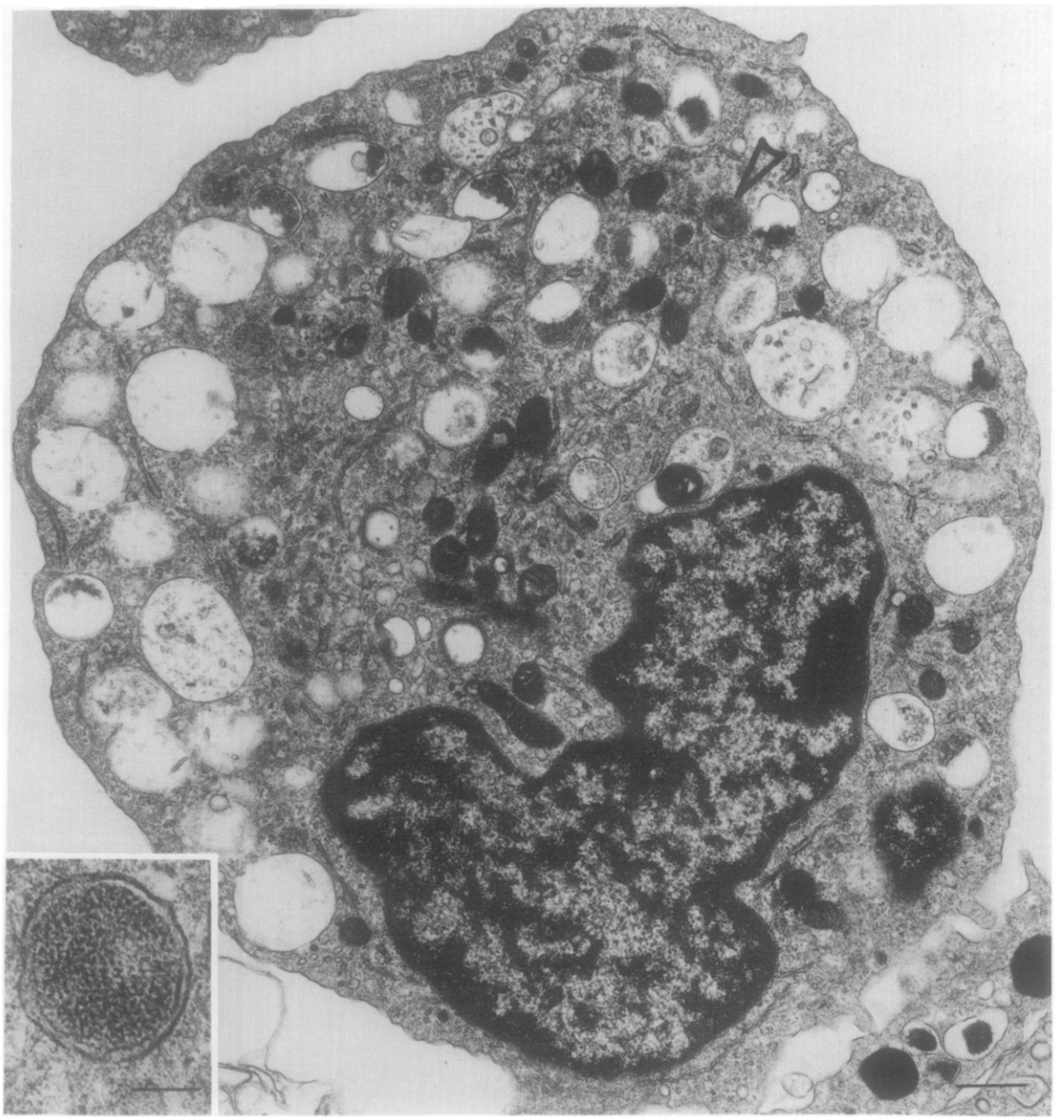


FIG. 10. Basophilic myelocyte (3-week culture of cord blood cells in rhIL-3) shows a single, eccentrically located, lobular nucleus and mitochondria, rough endoplasmic reticulum (RER), and numerous large, immature granules in the cytoplasm. Many of the immature granules contain variable amounts of dense particles and vesicles. Smaller, particle-filled, mature granules *(arrowhead)* are present (shown at higher magnification in the inset). The presence of such a granule in a myelocyte is sufficient to allow assignment of a cell to the basophil lineage. Bars: main panel, 0.7 μm; inset, 0.2 μm. (Original magnifications: main panel, ×15,500; inset, ×60,000.) (From Dvorak AM, Saito H, Estrella P, Kissel S, Arai N, Ishizaka T. Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. Lab Invest 1989;61:116-32. Copyright by The United States and Canadian Academy of Pathology, Inc.)

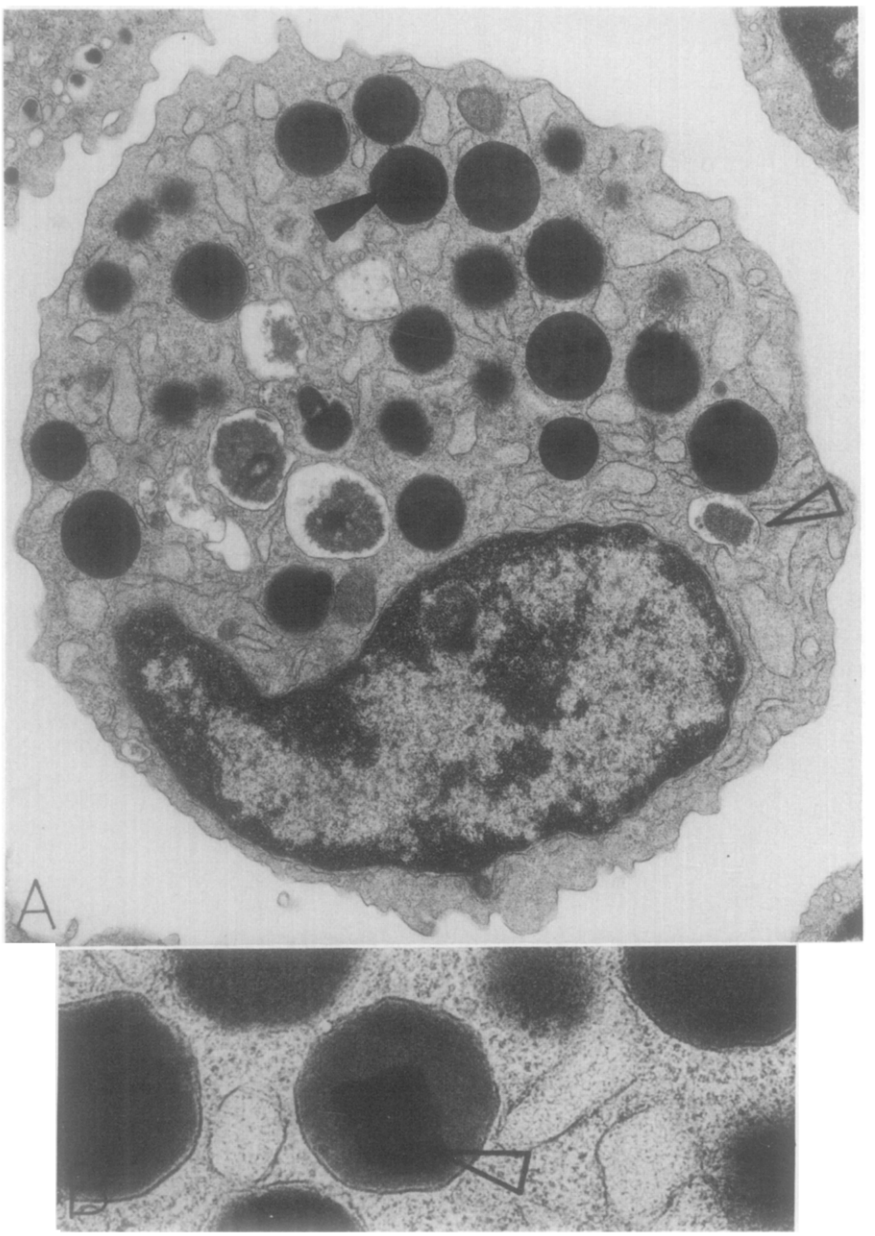


FIG. 11. An eosinophilic myelocyte (3-week culture of cord blood cells in rhIL-3) shows a single, eccentrically located, lobular nucleus; distended cisterns of RER; and a mixture of immature primary *(open arrowhead)* and secondary *(closed arrowhead)* granules in the cytoplasm in **A.** The condensation of a central core *(arrowhead)* in an immature secondary granule from another eosinophilic myelocyte is shown at higher magnification in **B.** The presence of such a granule in the myelocyte is sufficient to allow assignment of a cell to the eosinophil lineage. (Original magnifications: **A,** ×14,000; **B,** 4,000.) (From Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344.)

visualization of crystalline core condensation within immature secondary granules allows the certain identification of EMs (Fig. 11, *B*).

Ultrastructural cytochemical preparations, designed to detect endogenous peroxidases (in this case, EPO) provide additional help in differentiating BMs from EMs.[32, 33] For example, EMs contain EPO in the cisterns (which surround the

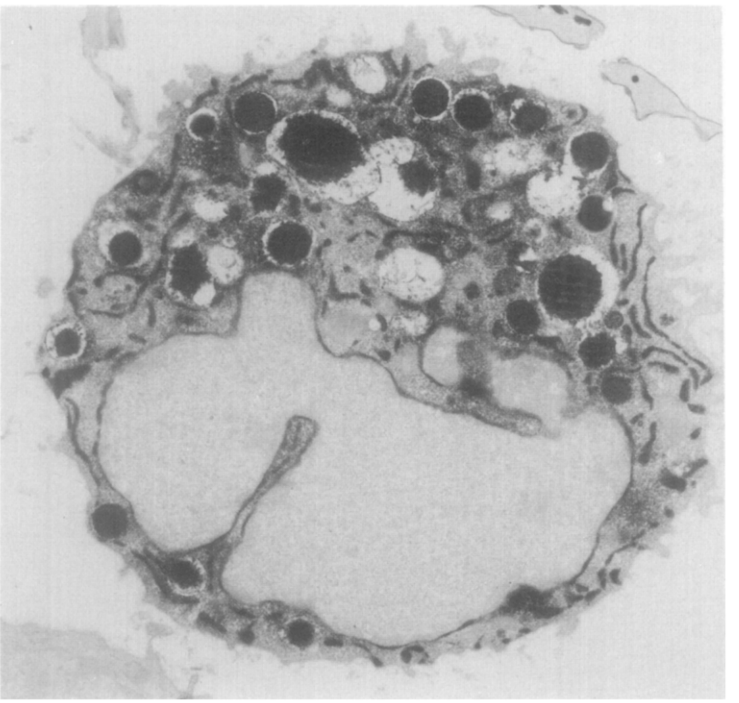


FIG. 12. An eosinophilic myelocyte (3-week culture of cord blood cells in cloned mouse T-cell culture supernatant containing murine IL-3, prepared with a cytochemical method to detect peroxidase) shows EPO in immature granules, cisternae of the RER, and perinuclear cisterna. (Original magnification ×8000.) (From Dvorak AM, Ishizaka T, Galli SJ. Ultrastructure of human basophils developing *in vitro*. Evidence for the acquisition of peroxidase by basophils and for different effects of human and murine growth factors on human basophil and eosinophil maturation. Lab Invest 1985;53:57-71. Copyright by The United States and Canadian Academy of Pathology, Inc.)

nucleus, and those bound by membrane-attached ribosomes), in Golgi structures, and in immature granules (Figs. 12 and 13). The matrix compartment of mature granules is also filled with EPO.[32, 33] In contrast, these synthetic organelles, as well as the immature and mature granules of human basophils, do not contain EPO[32] (Fig. 13).

SECRETION BY GRANULE EXTRUSION

Secretion from granulated secretory cells occurs classically as a regulated event in the context of granule-to-granule or granule-to-plasma membrane fusion and extrusion of nonmembrane-bound granule contents.[4] The morphologic end point of such events, in the absence of recovery or preceding recovery, is a granule-poor cell, often having accentuated surface processes, which result from the addition of fused granule membranes to the plasma membrane. Empty granule containers are not present in the cytoplasm. Before the release of granules by extrusion, intracytoplasmic degranulation chambers, constructed from fused granule membranes, may prevail; alternatively, individual granules may fuse with the cell surface and may be ejected through multiple pores formed in this way. In either case this process places nonmembrane-bound granule storage products in the external environment; this process is referred to as *exocytosis.*

Both human basophils and eosinophils undergo regulated secretion by exocytosis.[1, 2, 4, 9, 35-42] Human basophils (Fig. 14) generally extrude granules individually into the microenvironment through multiple plasma membrane pores, resulting in granule-free viable cells. The tempo of these events varies considerably with particular stimulating triggers. In certain cases a granule exocytosis secretion phase is preceded by a piecemeal degranulation (PMD) phase (see "Secretion by PMD"). When a phorbol diester, tetradecanoyl phorbol acetate (TPA), is the trigger, a forme fruste of granule

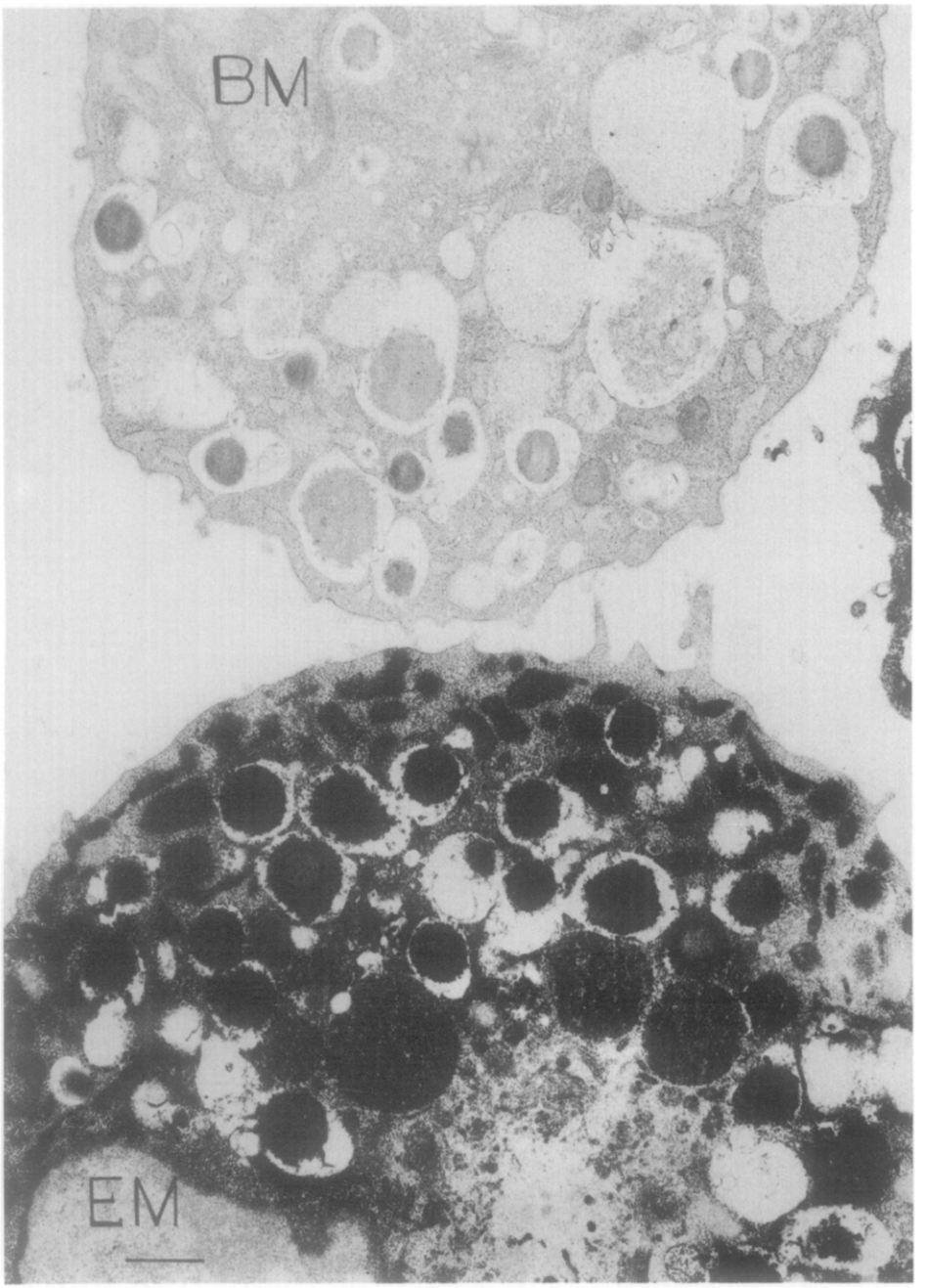


FIG. 13. A basophilic myelocyte (BM) and an eosinophilic myelocyte (EM) (culture of cord blood cells in human T-cell culture supernatant containing human IL-3, prepared with a cytochemical method to detect peroxidase) show EPO-positive immature granules, Golgi vesicles, RER cisterns, and perinuclear cistern in the EM. These synthetic and storage organelles do not display peroxidatic activity in the BM. Bar = 1.2 μm. (Original magnification ×8500.) (From Dvorak AM. Morphologic expressions of maturation and function can affect the ability to identify mast cells and basophils in man, guinea pig, and mouse. In: Befus AD, Bienenstock J, Denburg JA, eds. Mast cell differentiation and heterogeneity. New York: Raven Press, 1986:95-114.)

extrusion (Figs. 15 and 16) takes place, whereby granule fusions with the plasma membrane occur, but exteriorization of granule contents and membranes is frozen in time (Fig. 15). Such cells revealed individual granules bulging beyond the plasma membrane (but still contained by it) and granule-shaped spaces in continuity with the plasma cell membrane (Fig. 16), which often

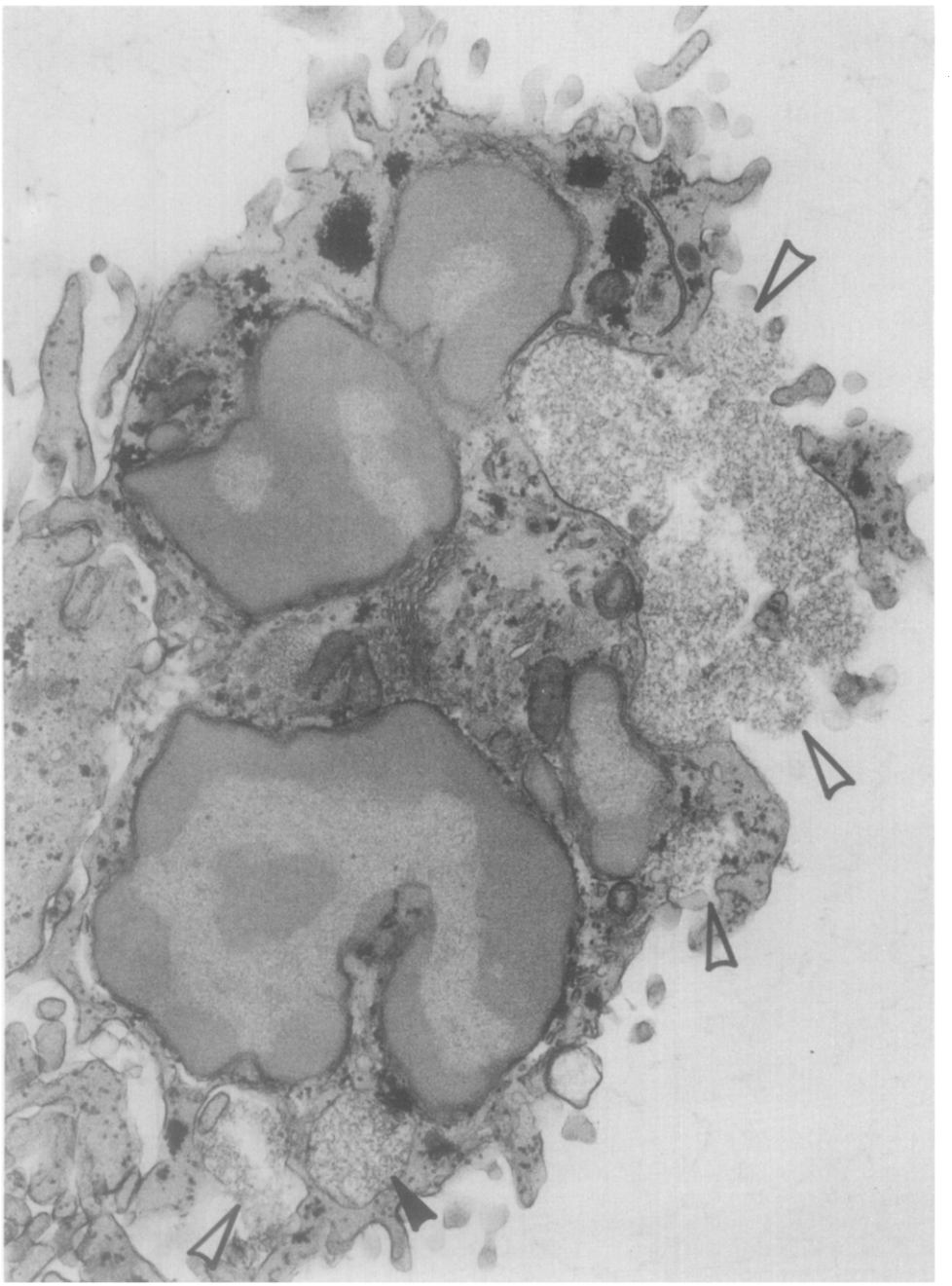


FIG. 14. Basophil (2 minutes after stimulation with FMLP) shows extrusion of membrane-free particle granules at several locations onto the cell surface *(open arrowheads).* One granule remains in the cytoplasm *(closed arrowhead)* of this nearly completely degranulated polymorphonuclear basophil. (Original magnification ×26,000.) (From Dvorak AM, Warner JA, Kissel S, Lichtenstein LM, MacGlashan DW Jr. F-met peptide-induced degranulation of human basophils. Lab Invest 1991;64:234-53. Copyright by The United States and Canadian Academy of Pathology, Inc.)

did not move outward or inward over 45-minute–interval kinetic experiments. Rarely (when stimulated with mannitol), did human basophils form intracytoplasmic degranulation chambers.

Human eosinophils (Figs. 17, 18, and 19) also undergo exocytosis.[3, 7, 10, 12, 37] For example, in diseased, bacteria-invaded human gut tissues examined by electron microscopy, extrusion of membrane-free granules into cytoplasmic degranulation sacs (Figs. 18 and 19) and through multiple

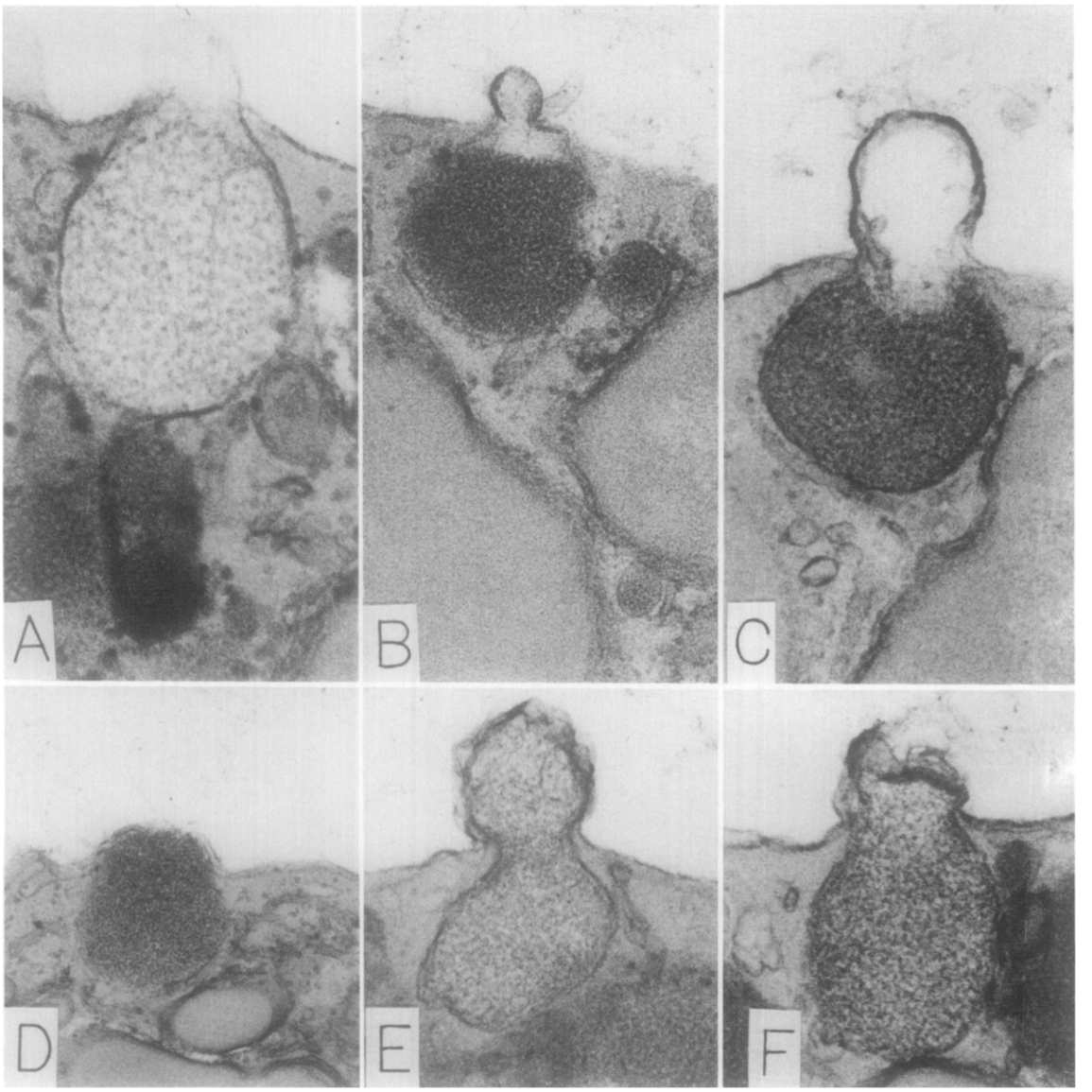


FIG. 15. Basophils show a forme fruste of degranulation characterized by focal blebs in fused granule plasma membranes that protrude outward beyond the perimeter of the cell in stimulated samples at 1 minute **(A)** and 10 minutes (**B** and **C**) after exposure to TPA. The underlying granules show no change in particle packing **(B)**, focal losses in granule particles beneath the blebbed, fused membranes (**B** and **C**) and diminished (altered) packing of granule particles throughout **(A)**. Note that the raised surface bleb is nearly as large as the granule in **C**. This forme fruste of degranulation is further illustrated by granule bulges beyond the cell perimeter **(D-F)**. These granule bulges in TPA-stimulated basophils are small and are of an unaltered, particle-filled granule at 2 minutes in **D** and of an extensively bulged granule in **E**. The particle packing of granules at 2 minutes in **E** and **F** is diminished. All bulged granules are restrained by overlying, dense plasma and granule membranes **(D-F)**. (Original magnifications: **A**, ×90,000; **B**, ×54,500; **C**, ×62,500; **D**, ×40,500; **E**, ×63,000; **F**, ×65,000.) (From Dvorak AM, et al. Am J Pathol 1992;141:1309-22.)

plasma membrane pores (Figs. 17 and 19, *B*) was recorded.[37]

SECRETION BY PMD

PMD is a term we coined to describe the morphology of secretion from human basophils in contact allergy skin biopsy specimens.[43, 44] This process is reviewed in several published studies.[1, 2, 4, 6, 8, 9] In a sequential biopsy study of experimentally elicited lesions, electron microscopy revealed a progressive emptying of human basophil secretory granules. Thus the term *PMD* was used to describe progres-

sive losses of granule particulate contents in the absence of granule-to-granule or granule-to-plasma membrane fusions. The morphologic hallmark of complete release by this mechanism is the retention of empty granule containers in the cell cytoplasm (Fig. 20).

Secretory processes have traditionally been classified as regulated or constitutive processes: regulated secretion refers to exocytosis of storage granules, and constitutive secretion refers to vesicular secretory traffic directly from Golgi structures to the plasma membrane.[4] PMD could be viewed as a diminished regulated secretory event or as an upregulated constitutive secretory event. Ultrastructural morphology does not support an upregulated constitutive secretory event, which bypasses granule storage sites, for at least two reasons: (1) granule containers visibly empty with time, and (2) Golgi structures are diminished in mature human basophils, without evidence of enlargement or increased vesicular components. However, upregulation of constitutive secretion involving vesicular traffic from storage granules is supported by ultrastructural images of particle-filled (or empty) vesicles fused to human basophil granules, as well as the presence of these vesicles in the perigranular and peripheral cytoplasm (Fig. 21).

PMD of mature human basophils has been documented in a wide variety of human diseases in vivo[6] and in developing[34] and releasing[35, 36] basophils in vitro. Tissue basophils that migrate into inflammatory and neoplastic microenvironments almost always reveal evidence of PMD in human samples (Fig. 20).[6] Insight into the potential triggering of human basophil PMD was gained when this form of release was found to prevail in human basophils developing in vitro in suspension cultures of human cord blood cells when those cultures were supplemented with either recombinant human interleukin (rhIL)-3[34] rhIL-5[34] (Fig. 22), or the c-*kit* ligand in various forms.[14, 45] It is possible that a wide variety of cytokines initiate PMD in microenvironmental sites that are rich in blood basophils.[6]

Several degranulation models of human basophil-stimulated secretion with peripheral blood basophils have been studied ultrastructurally.[1, 2, 4, 9, 35, 36, 38-42] Included among these are recent kinetic studies of N-formyl-methionyl-leucyl-phenylalanine (FMLP)- or TPA-stimulated human basophils; these studies have revealed PMD as a component of the morphologic response to these triggers.[35, 36] In each instance the morphology of PMD parallels histamine release,

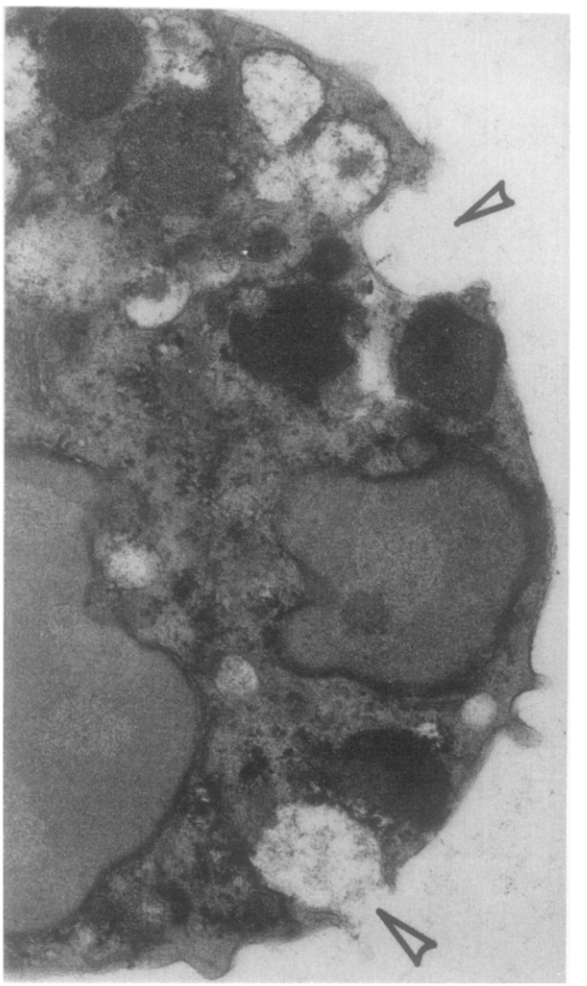


FIG. 16. Forme fruste of degranulation is illustrated in a basophil 1 minute after stimulation with TPA. Two open, empty granules have not externalized their membranes (arrowheads), leaving a granule-shaped, scalloped surface contour in place. (Original magnification ×30,000.) (From Dvorak AM, et al. Am J Pathol 1992;141:1309-22.)

as detected biochemically. In FMLP-stimulated human basophils, PMD appeared early (Fig. 23), was accompanied by large numbers of cytoplasmic vesicles, and merged with typical exocytosis at peak times of histamine release after stimulation.[35] Thus a morphologic continuum for these events, such as the one we proposed in 1975,[46] was documented by the merging of PMD with typical exocytosis in this model.[35] The phorbol diester TPA, in sequential kinetic samples, stimulated extensive PMD (Figs. 24 and 25) that coincided with peak histamine release—a delayed event in this model.[36] For example, approximately 50% of the cytoplasmic granules displayed reduced or empty contents by 45 minutes after TPA stimulation (Fig. 24, *A*). This evidence of PMD was associated with

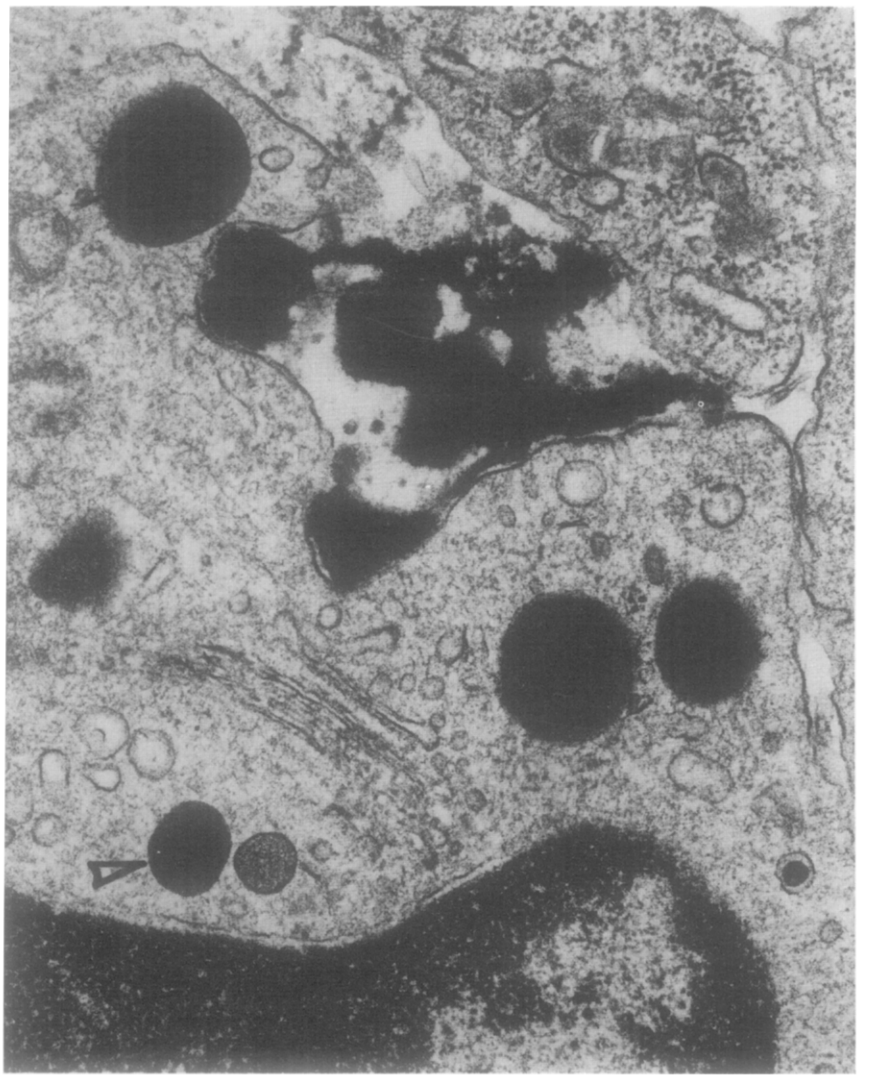


FIG. 17. Eosinophil (staphylococcal culture-positive ileal pouch biopsy specimen from a patient with ulcerative colitis that showed damaged enteric nerves by electron microscopy) shows exocytosis of membrane-free secondary granules. Note that the core material is intact and that the matrix material is spreading into linear arrays along the cell surface adjacent to the degranulation pocket. Primary granules (arrowhead) are present in the Golgi area. (Original magnification ×43,000.) (Reprinted with permission from Dvorak AM. Ultrastructure of human gastrointestinal system. Interactions among mast cells, eosinophils, nerves and muscle in human disease. In: Snape WJ Jr, Collins SM, eds. Effects of immune cells and inflammation on smooth muscle and enteric nerves. Boca Raton, Florida: CRC Press, 1991:139-68. Copyright CRC Press, Inc., Boca Raton, Fla.)

and preceded by a rapid, extensive, and sustained increase in particle-containing cytoplasmic vesicles (Figs. 24, *B* and 25), compared with buffer-incubated controls ($p < 0.001$).[36]

The ultrastructural morphologic evidence supports vesicular transport as the mechanism for effecting PMD from human basophils in vivo and in vitro.[1, 2, 4, 6, 8, 9, 35, 36, 43, 44, 46] Thus visual evidence of vesicles fused to secretory granules, free in the cytoplasm and in peripheral cytoplasmic area, and fused to plasma membranes has been published for human basophils. More recently, new methodology has allowed us to visualize actual secretory granule contents, such as histamine and CLC protein, in cytoplasmic vesicles of human basophils actively undergoing PMD after FMLP stimulation[47] (Dvorak et al. Unpublished data). In the case of histamine, we used a newly developed

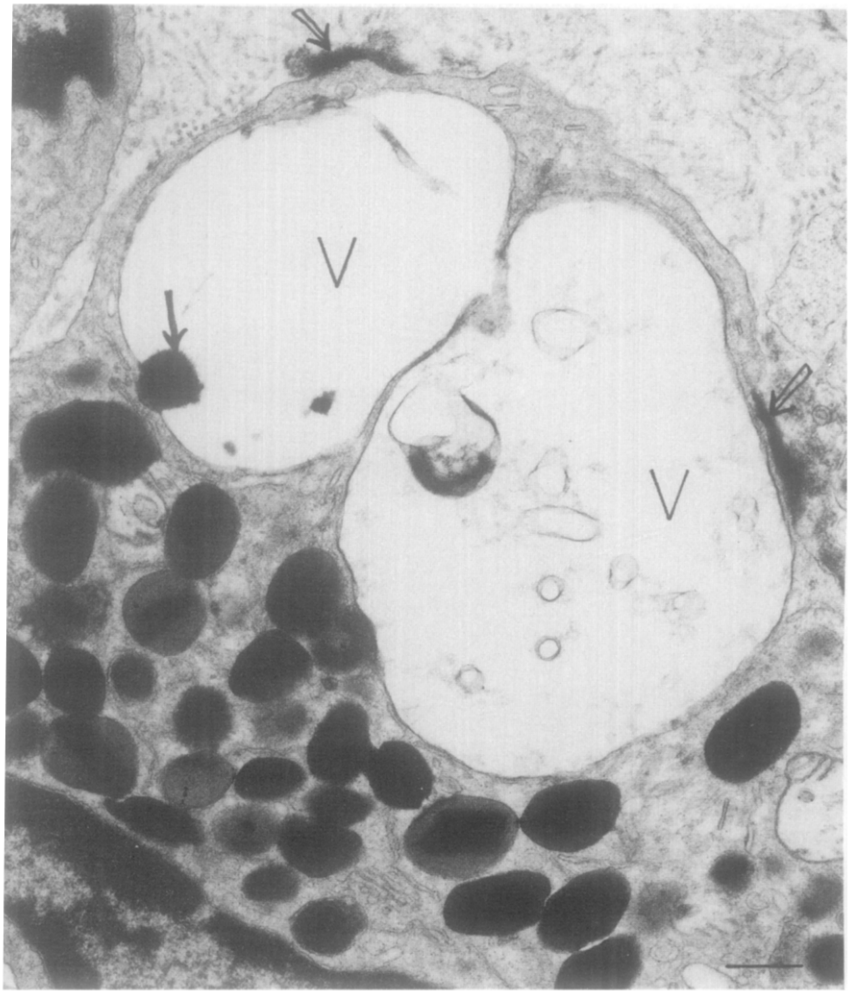


FIG. 18. Eosinophil (bacterial culture-positive ileal biopsy specimen) shows exocytosis of a membrane-free granule *(closed arrow)* into one of two large degranulation vacuoles *(V)* located within the cytoplasm. Two patches of granule matrix are attached to the cell surface overlying the vacuoles *(open arrow).* Numerous unaltered secondary granules with dense crystalline cores are present in the cytoplasm. Bar = 0.5 μm. (From Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344.)

postembedding, ultrastructural enzyme–gold affinity technique (diamine oxidase–gold), which has been shown to bind to histamine in human mast cell granules at high density with excellent specificity and without loss of ultrastructural fine structural details.[48] With this new technique (Fig. 26), histamine was visualized for the first time in cytoplasmic vesicles of FMLP-stimulated, histamine-releasing human basophils.[47] Secretory granules were also labeled (Fig. 26, *A*). In these initial studies cytoplasmic vesicles were significantly labeled with diamine oxidase–gold (Fig. 26, *B*), indicating the presence of histamine ($p < 0.001$).[47] In recent work (Dvorak et al. Unpublished data), using a postembedding immunogold technique to label CLC protein,[15, 19] we have also labeled this human basophil granule protein in cytoplasmic vesicles of FMLP-stimulated cells that were actively secreting histamine. Thus the demonstration of histamine and CLC protein in vesicles of stimulated human basophils undergoing piecemeal granule losses documents vesicular transport as a mechanism for effecting PMD in human basophils.

Human eosinophils, like human basophils, also undergo PMD.[3, 10, 22, 23, 49] Initially, we noted the release from the crystalline core compartment of secondary granules of gut tissue eosinophils in

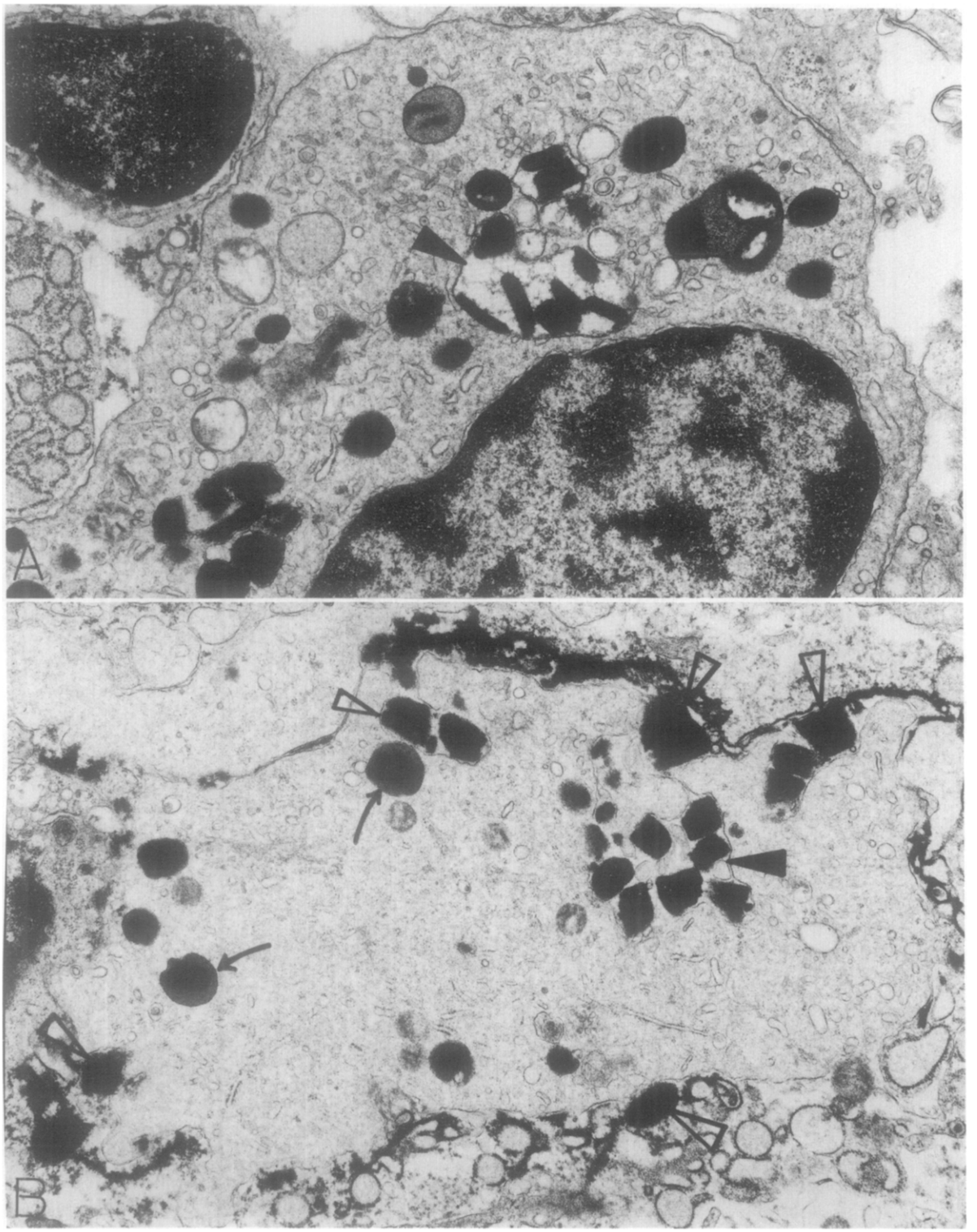


FIG. 19. Eosinophils (bacterial culture-positive ileal pouch biopsy specimens from patients with ulcerative colitis) show extruded secondary granule cores in cytoplasmic degranulation vacuoles *(closed arrowheads)* in **A** and **B** and to the cell surface at multiple openings of the plasma membrane *(open arrowheads)* in **B.** Extruded granule matrix in a linear array is seen along the cell surface in **B.** (Original magnifications: **A,** ×17,000; **B,** ×15,000.) (From Dvorak AM. Ultrastructural studies on mechanisms of human eosinophil activation and secretion. In: Gleich GJ, Kay AB, eds. Eosinophils in allergy and inflammation. New York: Marcel Dekker, 1993:159-209.)

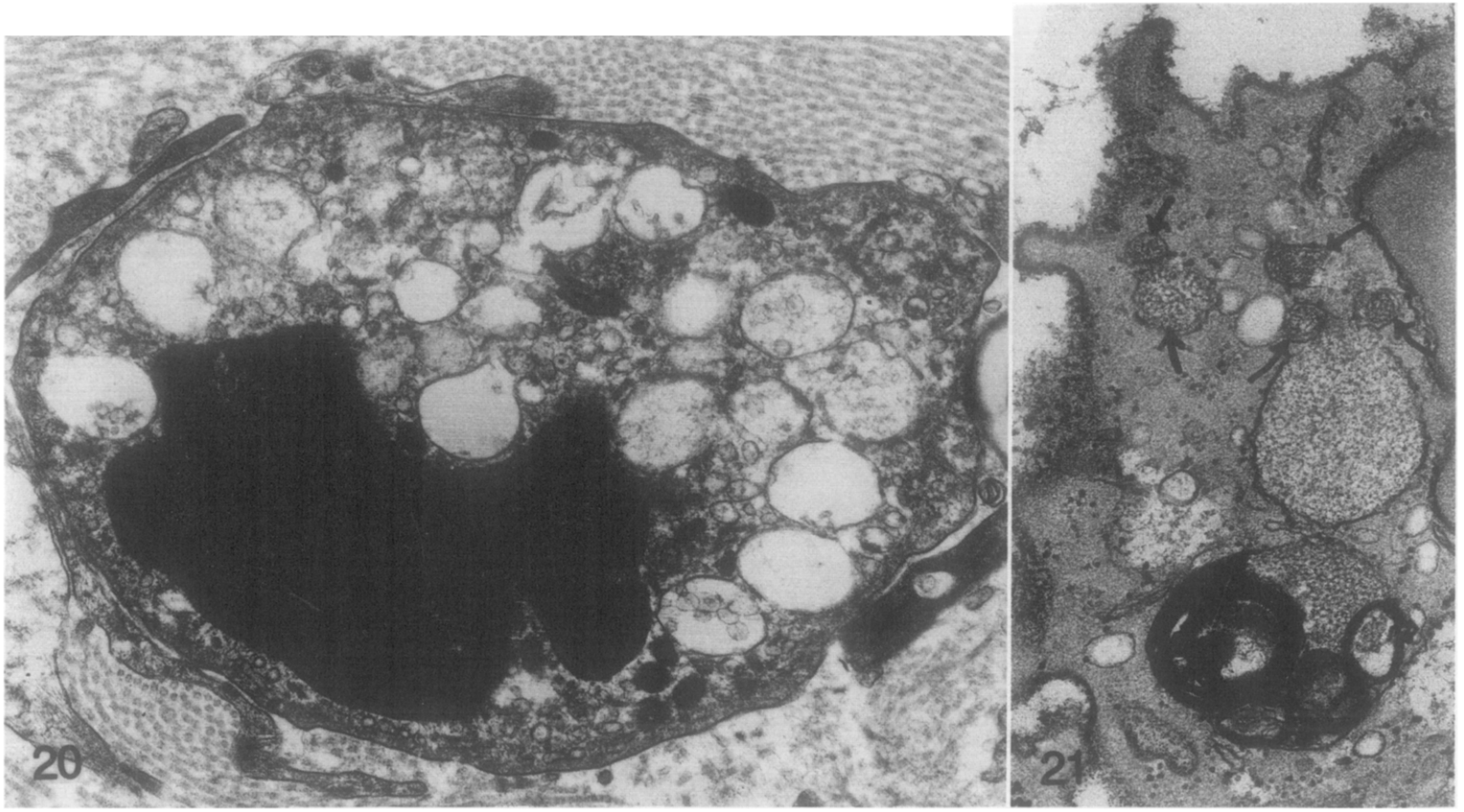


FIG. 20. PMD of a tissue basophil (skin biopsy specimen from a patient with HES) reveals empty, nonfused granule chambers in the cytoplasm. Note one lobe of a granulocyte nucleus with heavily condensed chromatin and large numbers of perigranular cytoplasmic vesicles. Some empty granule chambers also contain vesicles. (Original magnification ×21,000.)

FIG. 21. Higher magnification of a peripheral blood basophil shows two particle-containing cytoplasmic granules surrounded by particle-filled *(arrows)* and empty vesicles. One granule has multiple compartments delineated by dense concentric membranes. (Original magnification ×38,500.) (From Dvorak AM. Ultrastructural analysis of anaphylactic and piecemeal degranulation of human mast cells and basophils. In: Foreman JC, ed. Immunopharmacology of mast cells and basophils. London: Academic Press, 1993:89-113.)

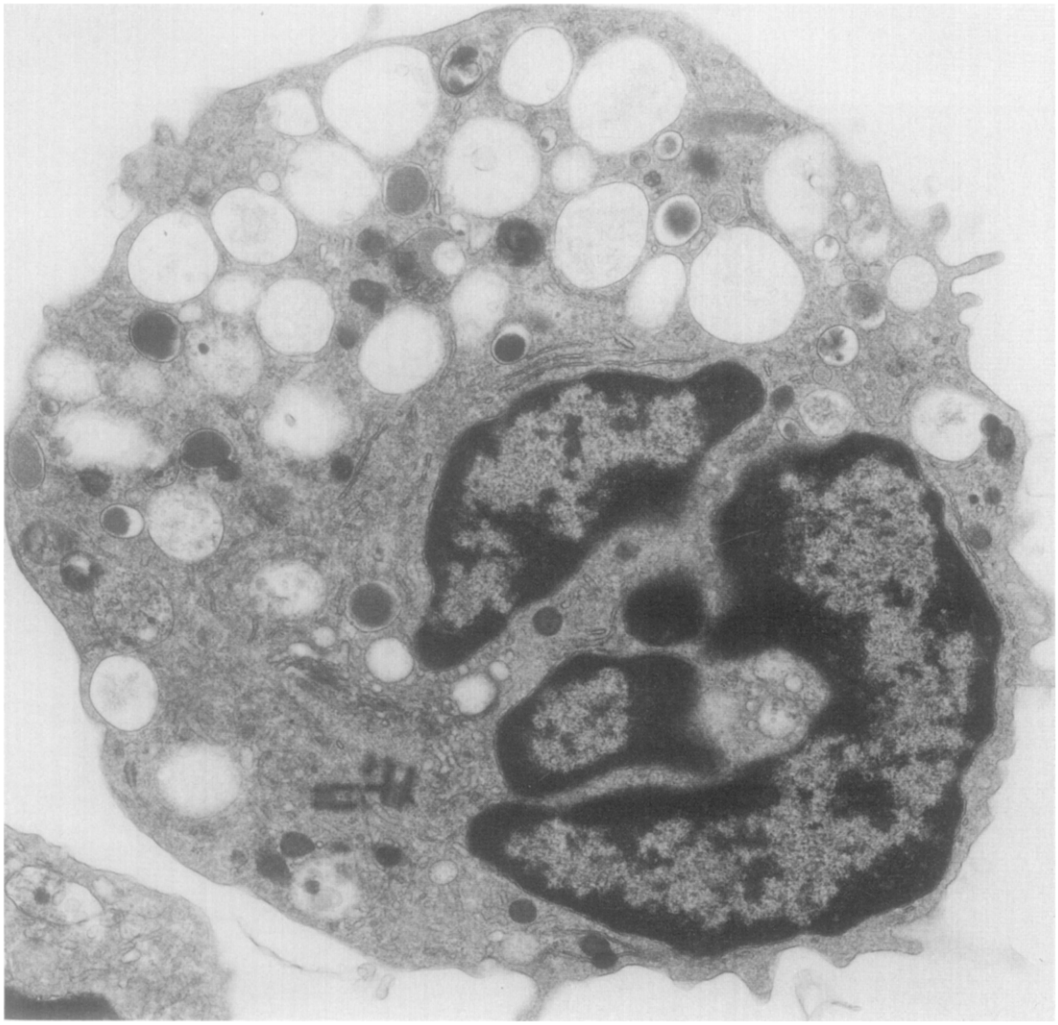


FIG. 22. PMD of a basophil (5-week cord blood cell culture containing rhIL-5 and a fraction of stimulated human T-cell–conditioned media) reveals nonfused, empty, and partially empty granule chambers and numerous cytoplasmic vesicles with granule particles. Note the polylobed nucleus with condensed chromatin. (Original magnification × 16,000.)

vivo in patients with Crohn's disease[50] (Fig. 27). Subsequently, core or matrix losses from the secondary granules (in the absence of fusions and with retention of partially empty and empty granule containers) were documented in vivo in pleural fluid and tissue eosinophils[3, 5] (Fig. 27). PMD of human eosinophils has also been recorded in vitro in cells developing from human cord blood cells supplemented with murine lymphocyte–conditioned media[49] or with rhIL-3, rhIL-5, and a fraction of IL-2–depleted phytohemaglutinen-stimulated human lymphocyte supernatant.[22, 23] In the cultures supplemented with rhIL-5 and the human lymphocyte–conditioned media, we noted mature eosinophils with partial to complete release of matrix or core compartments of secondary granules[22] (Fig. 28, *A*), analogous to those changes in human eosinophils in vivo.[3, 5] The mature eosinophils in these cultures also failed to show granule-to-granule or granule-to-plasma membrane fusions but retained partially empty and empty granules that contained small vesicles and were surrounded by smooth membrane-bound vesicles (Fig. 28, *B*). Some of these vesicles were filled with electron-dense material; others were electron-lucent[22] (Fig. 28, *B*). In contrast to human basophils, immunogold preparations did not demonstrate vesicles loaded with CLC protein

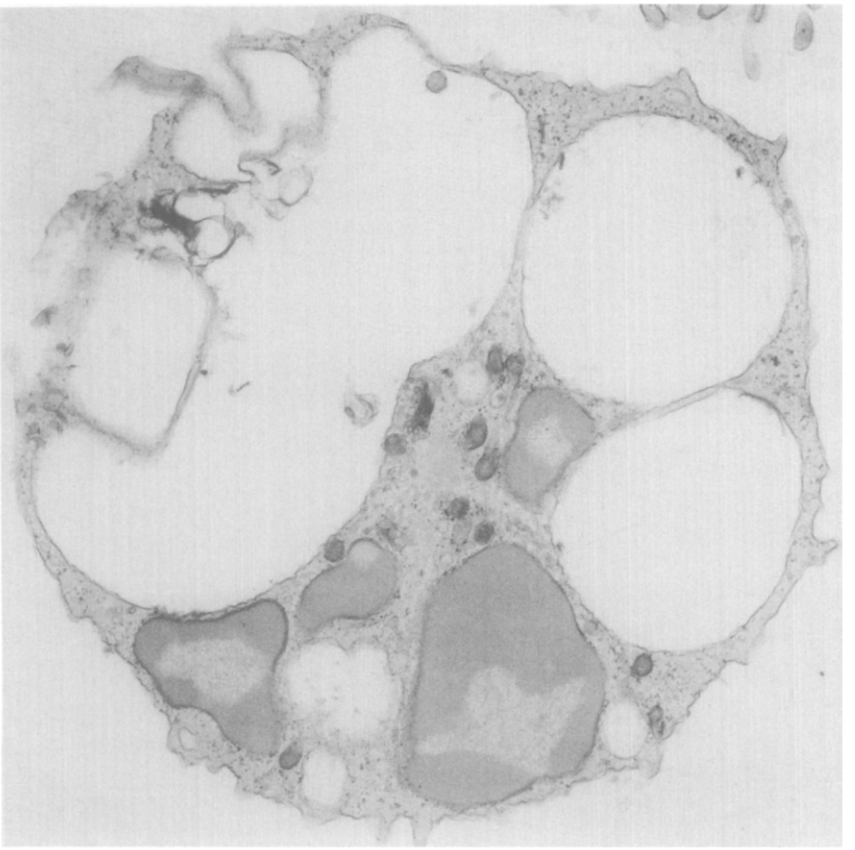


FIG. 23. Basophil (20 seconds after stimulation with FMLP) shows PMD characterized by greatly swollen, empty granule chambers in the cytoplasm. All cytoplasmic granules are involved in PMD in this mature polymorphonuclear basophil. (Original magnification ×15,000.) (From Dvorak AM, Warner JA, Kissel S, Lichtenstein LM, MacGlashan DW Jr. F-met peptide-induced degranulation of human basophils. Lab Invest 1991;64:234-53. Copyright by The United States and Canadian Academy of Pathology, Inc.)

in these perigranular locations of cells undergoing PMD. Rather, there was diffuse and extensive cytoplasmic labeling in these activated secretory eosinophils[22] (Fig. 29). Primary granules contained CLC protein, as previously reported,[15] but secondary granules lacked CLC protein in both matrix and core compartments.[22]

We investigated vesicular transport as a mechanism for effecting PMD in human eosinophils (Figs. 29 to 31), using an ultrastructural cytochemical method to image peroxidase(s)— a secondary granule matrix protein.[23, 49] These studies demonstrated EPO-loaded vesicles (Figs. 29 to 31) attached to secondary granules, adjacent to them in clusters, and in the subplasmalemmal cytoplasm. These vesicles fused with the plasma membrane and released quanta of EPO to the cell surface where it was often seen to be bound in small patches to the outer portion of these trilaminar unit membranes (Fig. 30, *D*).[23] In different culture systems prepared with the cytochemical method to detect EPO, we observed PMD, which occurred either in immature eosinophilic myelocytes (Fig. 31)[49] or in mature eosinophils.[23] In each circumstance, the matrix compartment of many secondary granules was devoid of EPO, retaining only EPO-negative crystalline core material (Fig. 31).[23, 49] As determined with immunogold preparations, the core compartment retained the typical granule protein, MBP, which often completely filled homogeneously dense, EPO-negative granules.[51]

CONCLUDING REMARKS

In this review, we have focused on morphologic studies that highlight similarities between human

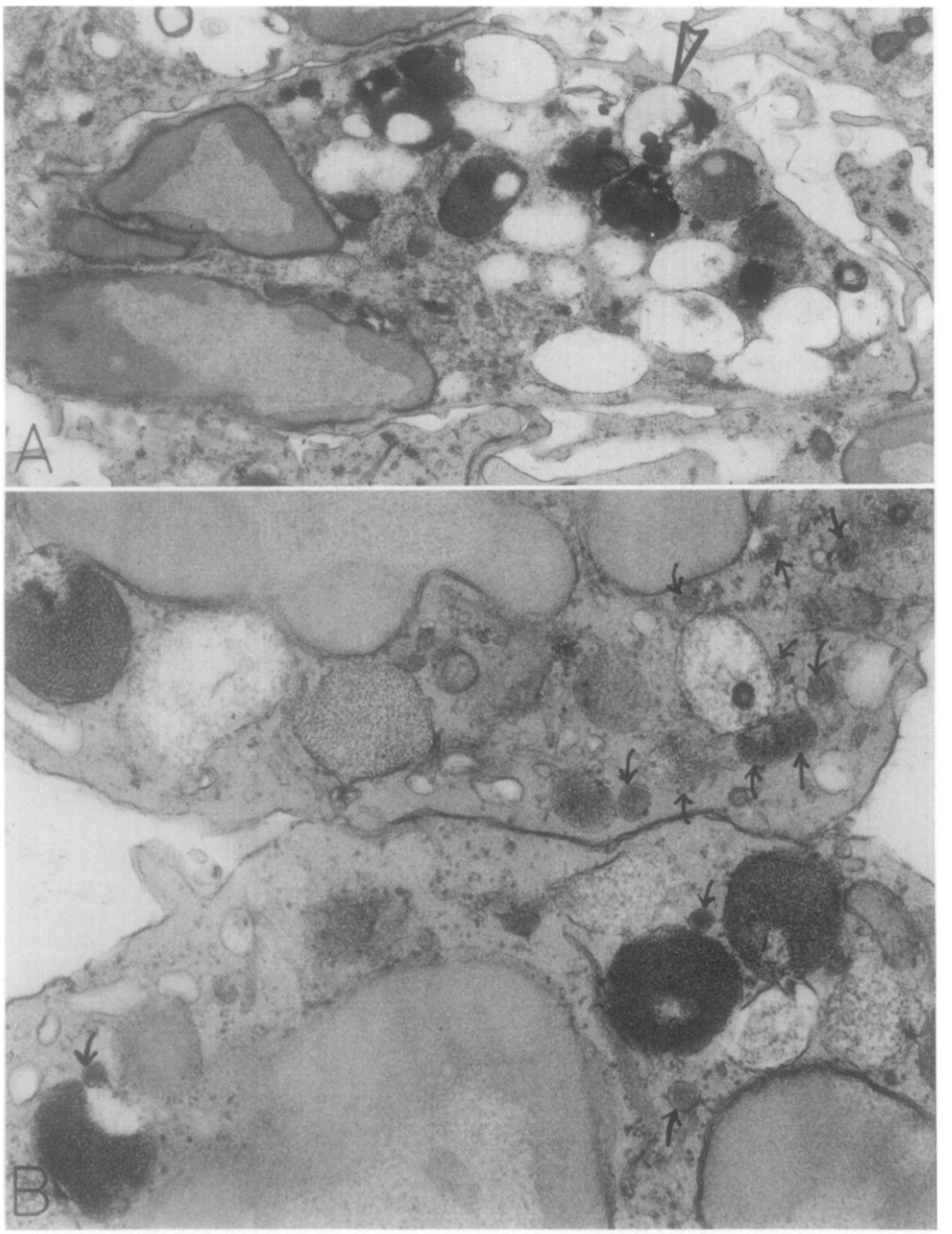


FIG. 24. Basophils at 45 minutes **(A)** or 10 minutes **(B)** after TPA stimulation show PMD of approximately 50% of the cytoplasmic granules in **A.** Focal alterations in granule contents are present in the remaining particle granules. One altered granule *(arrowhead)* contains a CLC and multiple dense concentric membranes. In **B** two basophils are aligned along their surfaces. Both cells show piecemeal losses from particle granules and contain numerous particle-filled vesicles near granules and the cell surfaces *(arrows).* (Original magnifications: **A,** ×14,500; **B,** ×35,500.) **Panel A** from Dvorak AM, et al. Am J Pathol 1992;141:1309-22.

basophils and eosinophils. The similarities between these two granulocyte lineages are demonstrable with routine ultrastructural methods that reveal properties common to mature granulocyte secretory cells and to their developmental and functional programs in vivo and in vitro. Specialized ultrastructural methods have assisted in the documentation of subcellular materials in different granule populations, in different compartments within individual granules, and in lipid bodies. Thus granule localization of histamine, CLC protein, MBP, EPO, EDN, ECP, and TNF-α,

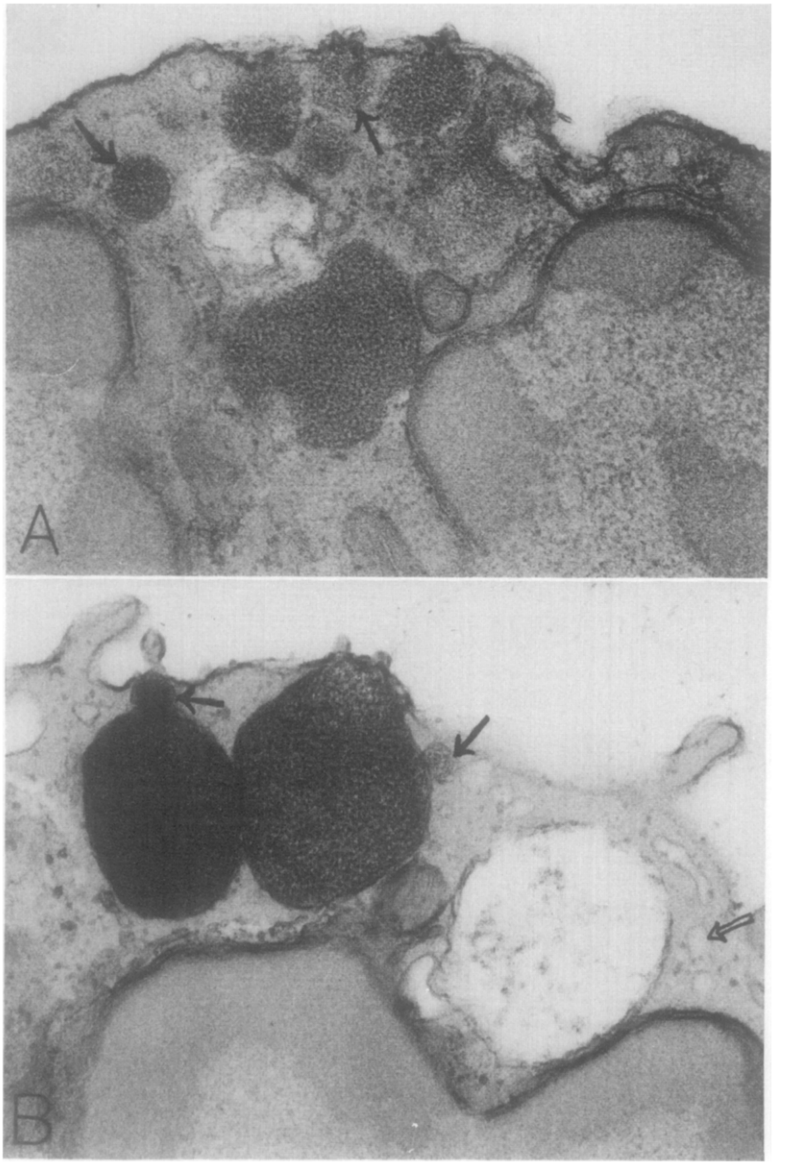


FIG. 25. Basophils (2 minutes after stimulation with TPA) show increased particle-filled vesicles *(closed arrows)* adjacent to granules and plasma membranes. One empty granule in **B** has empty vesicles *(open arrow)* nearby. (Original magnifications: **A,** ×50,500; **B,** ×47,000.) (From Dvorak AM, et al. Am J Pathol 1992;141:1309-22.)

with ultrastructural cytochemical, immunogold, and enzyme affinity–gold techniques, has been achieved. Lipid bodies (in human eosinophils) have been shown to contain arachidonic acid, prostaglandin endoperoxide synthase (cyclooxygenase), and 5-lipoxygenase by ultrastructural autoradiography and immunogold techniques.

Both human basophils and eosinophils undergo regulated secretion by exocytosis and PMD of individual granule contents in the absence of fusions and with retention of granule containers in the cytoplasm. This form of granule content release from human basophils and eosinophils is effected by vesicular transport of granule proteins, a process documented in human basophils by imaging histamine or CLC protein in smooth membrane-bound cytoplasmic vesicles and in human eosinophils by imaging EPO in similar vesicles in cells expressing the ultrastructural morphology of PMD.

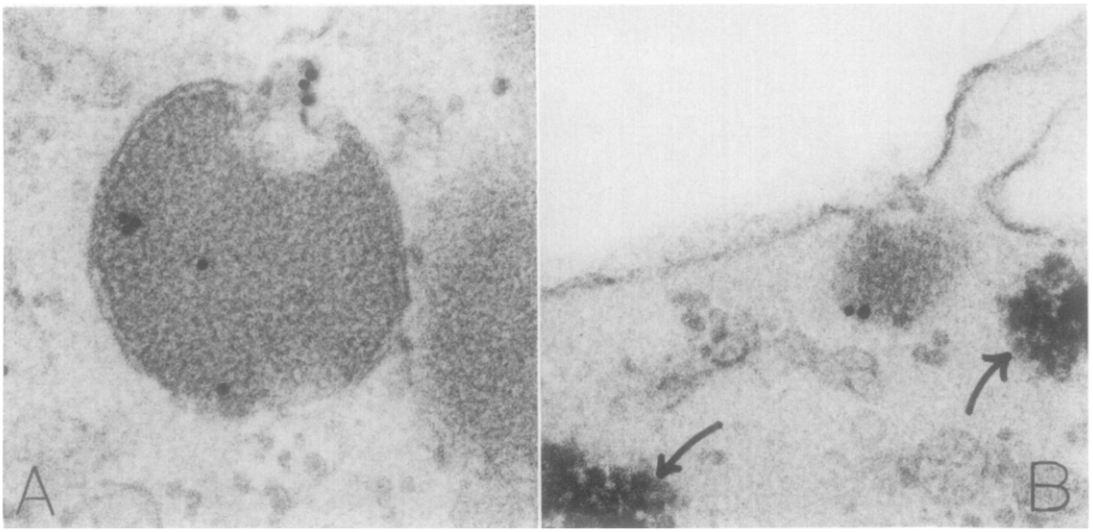


FIG. 26. High magnification views of basophils (20 seconds after stimulation with FMLP, prepared with enzyme affinity–gold to demonstrate histamine) show gold label in a particle granule **(A)** and in vesicles, attached to either a granule **(A)** or the cell surface **(B)**. Electron-dense aggregates of cytoplasmic glycogen are visible in **B** *(arrows)*. (Original magnification: **A** and **B**, ×114,000.) (From Dvorak AM, et al. Activated human basophils contain histamine in cytoplasmic vesicles. Int Arch Allergy Immunol 1994;105:8-11, by permission of S Karger AG, Basel.)

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REFERENCES

1. Dvorak AM. Morphologic and immunologic characterization of human basophils, 1879 to 1985. Riv Immunol Immunofarmacol 1988;8:50-82.
2. Dvorak AM. The fine structure of human basophils and mast cells. In: Holgate ST, vol ed. Mast cells, mediators and disease [Reeves WG, series ed., Immunology and medicine series]. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1988:29-97.
3. Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344.
4. Dvorak AM, ed. Blood cell biochemistry. vol. 4. Basophil and mast cell degranulation and recovery. Harris JR, series ed. New York: Plenum Press, 1991:1-415.
5. Dvorak AM. Ultrastructure of human gastrointestinal system. Interactions among mast cells, eosinophils, nerves and muscle in human disease. In: Snape WJ Jr, Collins SM, eds. Effects of immune cells and inflammation on smooth muscle and enteric nerves. Boca Raton, Florida: CRC Press, 1991:139-68.
6. Dvorak AM. Basophils and mast cells: piecemeal degranulation in situ and ex vivo: a possible mechanism for cytokine-induced function in disease. In: Coffey RG, ed. Granulocyte responses to cytokines. Basic and clinical research. New York: Marcel Dekker, 1992:169-271.
7. Dvorak AM, Ishizaka T, Weller PF, Ackerman SJ. Ultrastructural contributions to the understanding of the cell biology of human eosinophils: mechanisms of growth factor-induced development, secretion, and resolution of released constituents from the microenvironment. In: Makino S, Fukuda T, eds. Eosinophils: biological and clinical aspects. Boca Raton, Florida: CRC Press, 1993:13-22.
8. Dvorak AM, Dvorak HF. Cutaneous basophil hypersensitivity—a 20-year perspective, 1970-1990. In: Foreman JC, ed. Immunopharmacology of mast cells and basophils [Page C, series ed., The handbook of immunopharmacology]. London: Academic Press, 1993:153-80.
9. Dvorak AM. Ultrastructural analysis of anaphylactic and piecemeal degranulation of human mast cells and basophils. In: Foreman JC, ed. Immunopharmacology of mast cells and basophils [Page C, series ed., The handbook of immunopharmacology]. London: Academic Press, 1993: 89-113.
10. Dvorak AM. Ultrastructural studies on mechanisms of human eosinophil activation and secretion. In: Gleich GJ, Kay AB, eds. Eosinophils in allergy and inflammation. New York: Marcel Dekker, 1993:159-209.
11. Dvorak AM. Recent ultrastructural contributions to the understanding of the cell biology of mast cells and basophils: identification, homogeneities, heterogeneities, development, secretion, and recovery. In: Pozzi E, ed. Pathophysiology of pulmonary cells. Milan: Masson, 1994.
12. Weller PF, Dvorak AM. Human eosinophils: development, maturation and functional morphology. In: Busse WW,

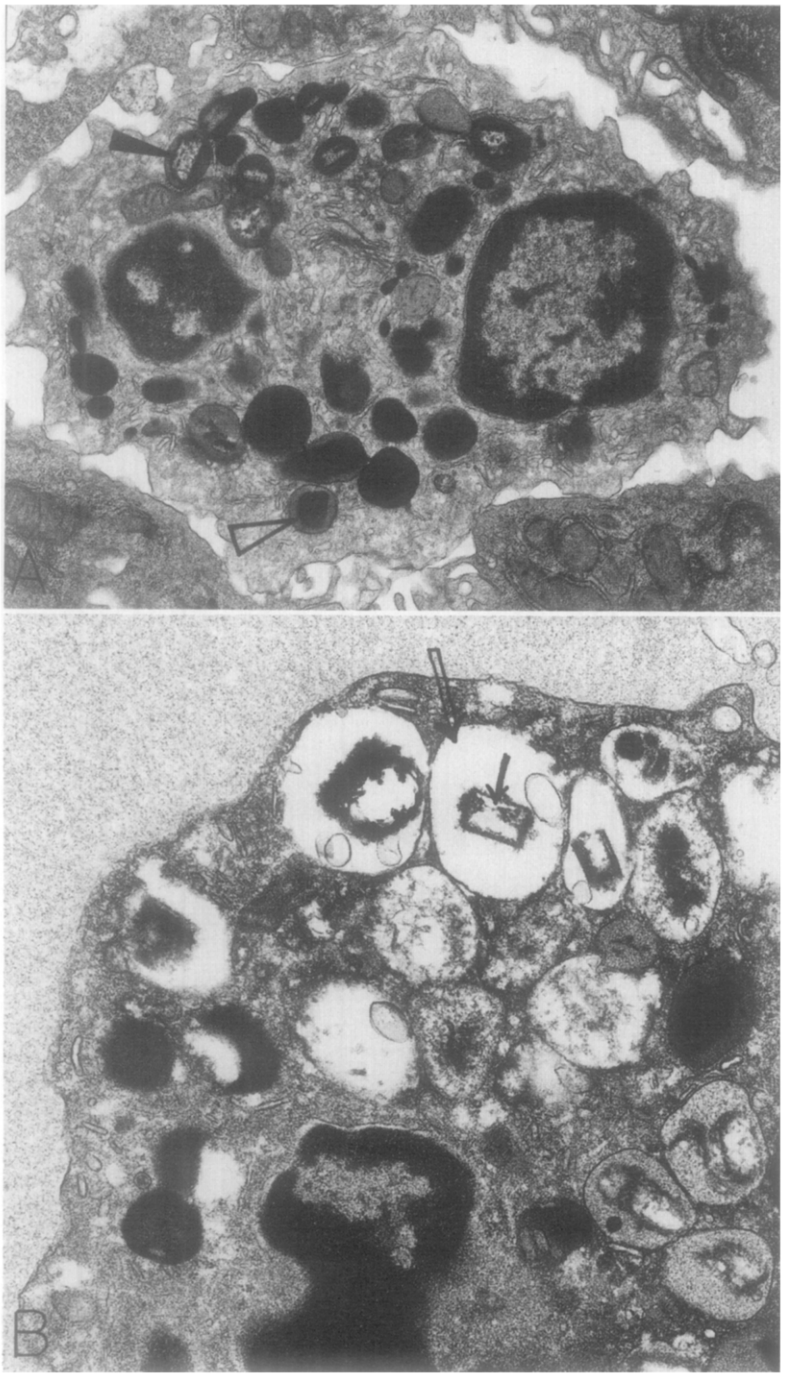


FIG. 27. In **A** PMD of an eosinophil (bacterial cultural-positive biopsy specimen of ileum) reveals focal loss of MBP crystalline core of specific granules *(closed arrowhead).* Many cores are intact *(open arrowhead).* In **B** PMD of an eosinophil (biopsy specimen of ileum from a patient with Crohn's disease) reveals release from individual specific granule cores *(closed arrow),* as well as from the specific granule matrix compartment *(open arrow),* leaving partially emptied, membrane-bound granule containers in the cytoplasm. Note one unaltered granule and structural integrity of the plasma membrane and nucleus in this undamaged eosinophil. (Original magnifications: **A,** ×13,000; **B,** ×19,500.) (Reprinted with permission from Dvorak AM. Ultrastructure of human gastrointestinal system. Interactions among mast cells, eosinophils, nerves and muscle in human disease. In: Snape WJ Jr, Collins SM, eds. Effects of immune cells and inflammation on smooth muscle and enteric nerves. Boca Raton, Florida: CRC Press, 1991:139-68. Copyright CRC Press, Inc., Boca Raton, Fla.)

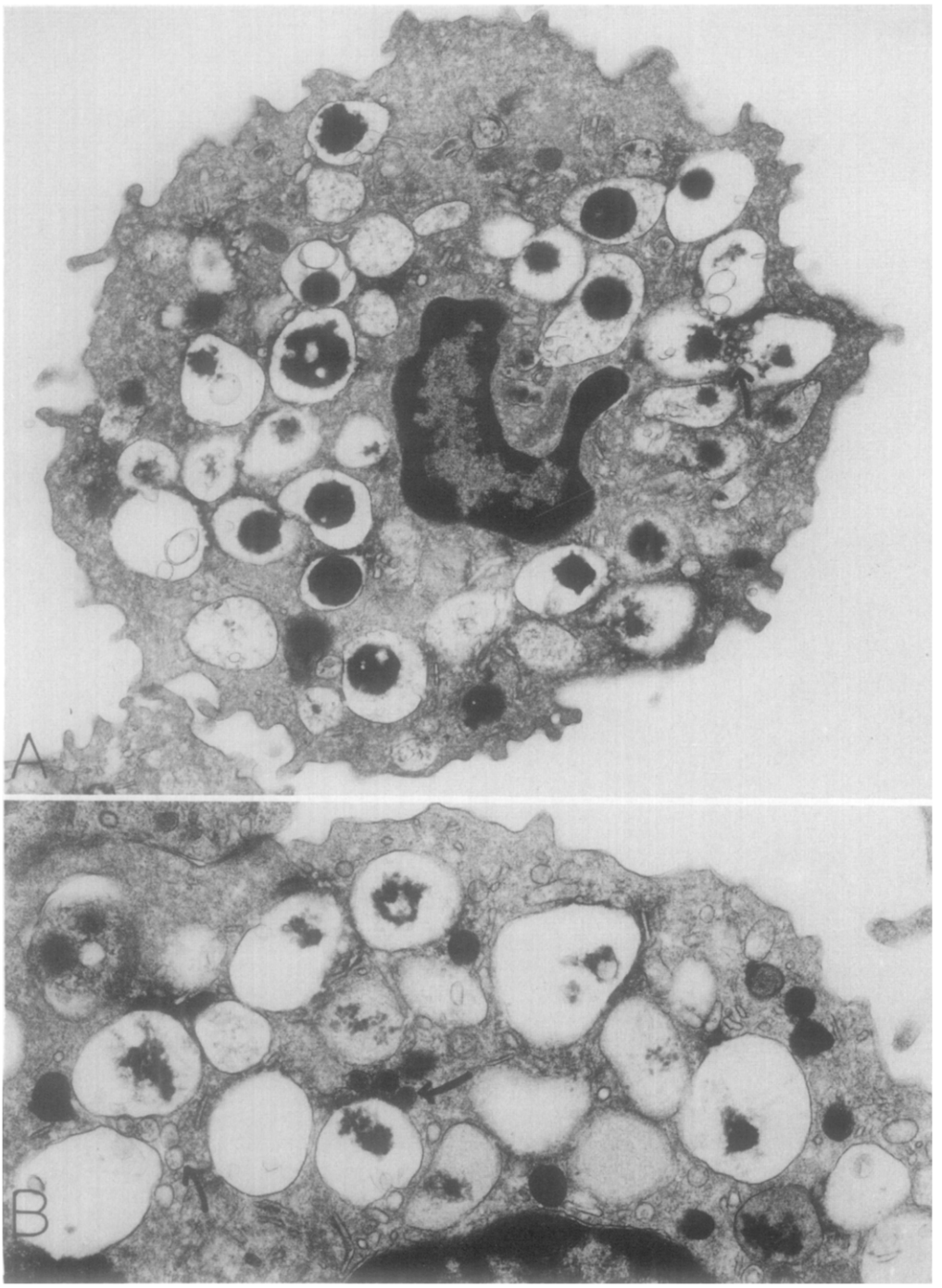


FIG. 28. PMD of eosinophils (5-week cultures of cord blood cells containing rhIL-5 and a fraction of stimulated human T-cell supernatant) reveals emptied secondary granule matrix compartments, residual granule cores that are often enlarged, and empty granule chambers. Clusters of empty and full vesicles *(arrows)* adjacent to altered granules are evident. (Original magnifications: **A,** ×15,000; **B,** ×21,000.) **Panel A** from Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. London: Plenum Publishing Corp, 1991:237-344. **Panel B** from Dvorak AM, et al. Am J Pathol 1991;138:69-82.

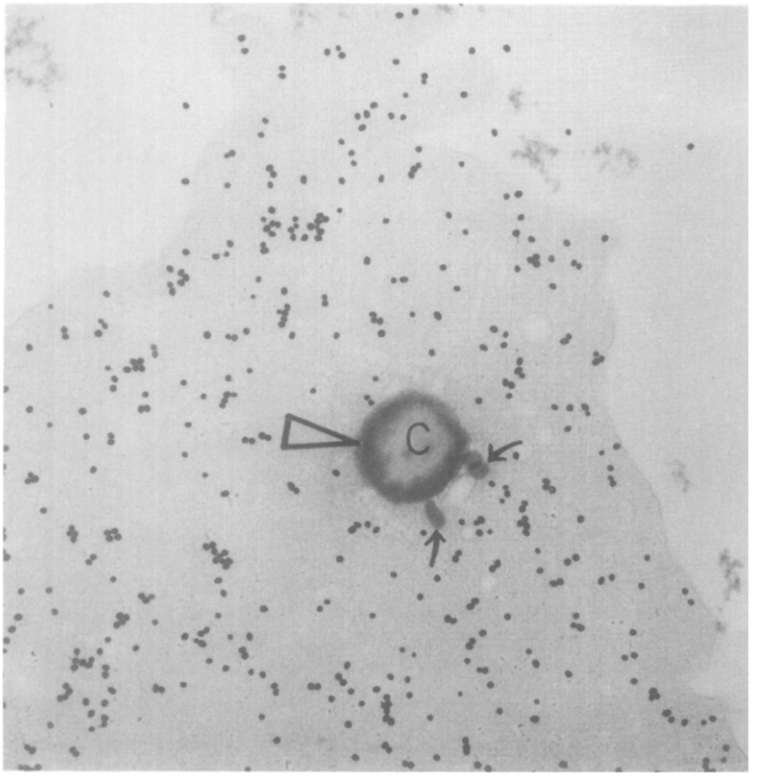


FIG. 29. Eosinophil cytoplasm (5-week culture of cord blood cells containing rhIL-5 and a fraction of stimulated human T-cell supernatant, prepared with a cytochemical method to detect peroxidase and an immunogold method to detect CLC protein) shows 30 nm gold particles diffusely distributed in the cytoplasm. Neither the plasma membrane nor the secondary granule *(arrowhead)* contains CLC protein. The secondary granule has EPO in the matrix; the central core **(C)** does not contain EPO. Several vesicles adjacent to the granule are loaded with EPO *(arrows).* (Original magnification ×28,000.)

Holgate ST, eds. Asthma and rhinitis. Boston: Blackwell Scientific Publishers, 1995;255-74.

13. Hastie R. A study of the ultrastructure of human basophil leukocytes. Lab Invest 1974;31:223-31.
14. Dvorak AM, Ishizaka T, Letourneau L, Albee EA, Mitsui H, Ackerman SJ. Charcot-Leyden crystal protein distribution in basophils and its absence in mast cells that differentiate from human umbilical cord blood precursor cells cultured in murine fibroblast culture supernatants or in recombinant human c-*kit* ligand. J Histochem Cytochem 1994;42:251-63.
15. Dvorak AM, Letourneau L, Login GR, Weller PF, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. Blood 1988;72:150-8.
16. Peters MS, Rodriguez M, Gleich GJ. Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. Lab Invest 1986;54:656-62.
17. Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. J Histochem Cytochem 1986;34:1399-403.
18. Beil WJ, Weller PF, Tzizik DM, Galli SJ, Dvorak AM. Ultrastructural immunogold localization of tumor necrosis factor-α to the matrix compartment of eosinophil secondary granules in patients with idiopathic hypereosinophilic syndrome. J Histochem Cytochem 1993;41:1611-5.
19. Dvorak AM, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to granules and intragranular crystals in mature human basophils. Lab Invest 1989;60:557-67.
20. Dvorak AM, Weller PF, Monahan-Earley RA, Letourneau L, Ackerman SJ. Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) and peroxidase in macrophages, eosinophils and extracellular matrix of the skin in the hypereosinophilic syndrome. Lab Invest 1990;62:590-607.
21. Dvorak AM, Letourneau L, Weller PF, Ackerman SJ. Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) to intracytoplasmic crystals in tumor cells of primary solid and papillary neoplasm of the pancreas. Lab Invest 1990;62:608-15.

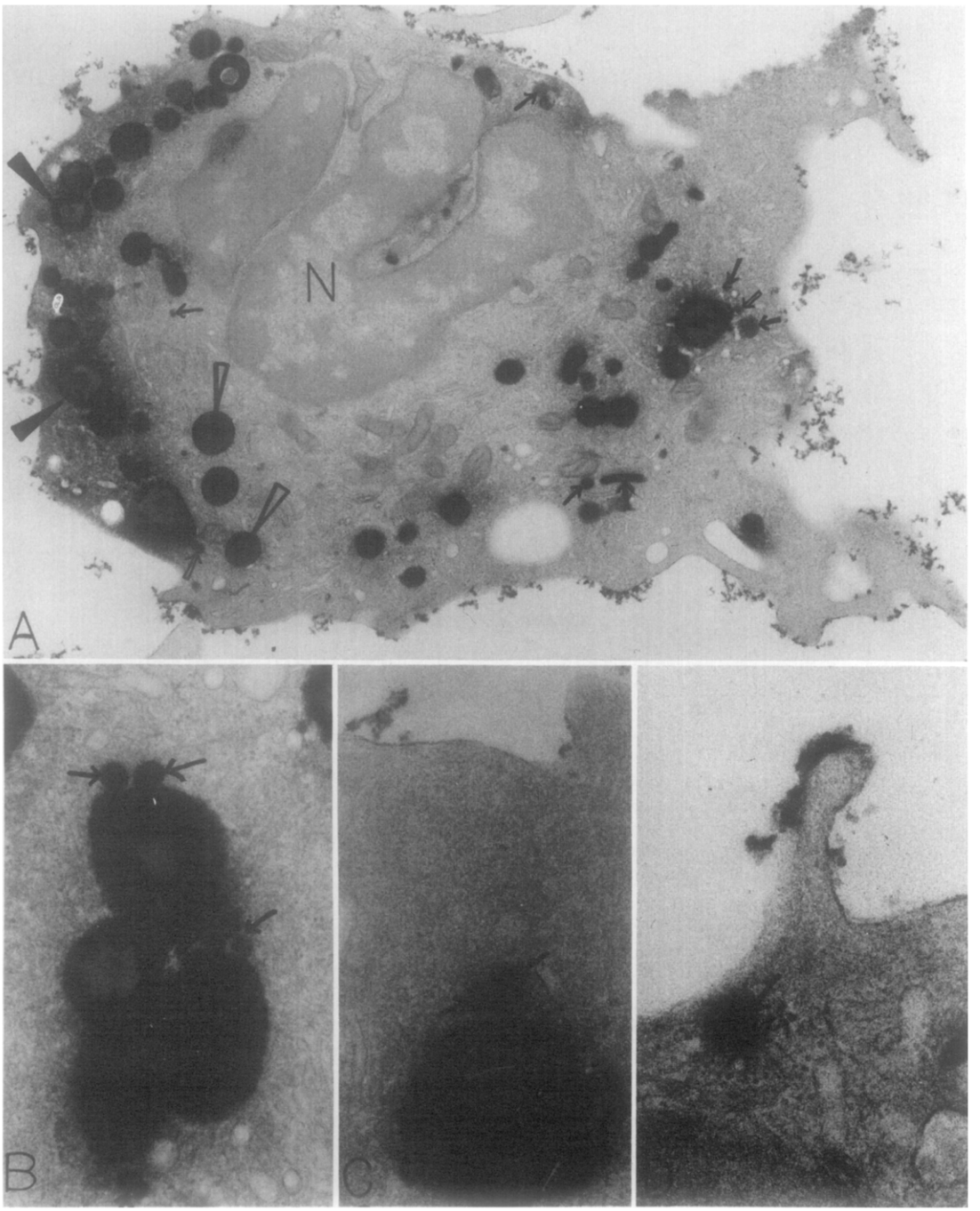


FIG. 30. Eosinophils (5-week culture of cord blood cells containing rhIL-5 and a fraction of stimulated human T cell supernatant, prepared with a cytochemical method to detect peroxidase) show: (in **panel A**) EPO (1) in secondary granule matrix compartments *(closed arrowheads),* (2) in cytoplasmic lipid bodies *(open arrowheads),* and (3) bound to the cell surface; the core compartments of secondary granules do not contain EPO; EPO-loaded vesicles, attached to granules *(open arrows)* and in vesicular and tubular structures in the cytoplasm *(closed arrows),* are evident (*N* = nucleus); (in **panel B**) clusters of EPO-loaded perigranular vesicles *(arrows)* beside the secondary granules that have EPO in their matrix compartments but not in their crystalline cores; (in **panel C**) two EPO-loaded vesicles *(arrows)* adjacent to the secondary granule pole facing the cell surface; (in **panel D**) an EPO-loaded vesicle docked beneath the plasma membrane of the cell; note the dense, secreted EPO attached to the external portion of a surface process. (Original magnification: **A,** ×15,500; **B,** ×41,500; **C,** ×49,000; **D,** ×73,000. (From Dvorak AM, et al. Am J Pathol 1992;140:795-807.)

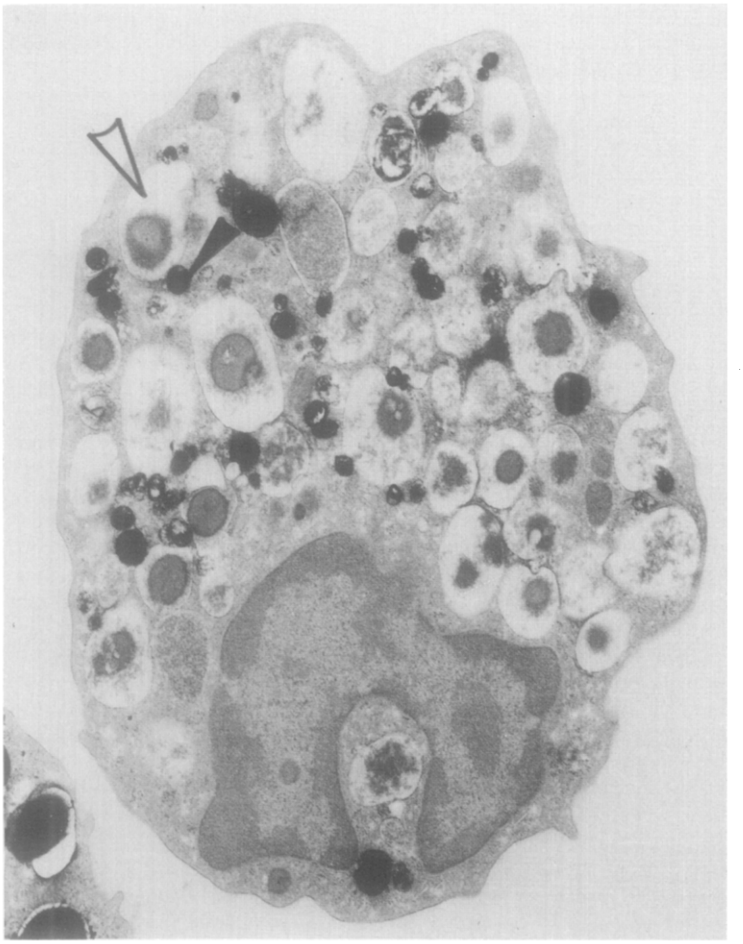


FIG. 31. An eosinophilic myelocyte (3-week culture of cord blood cells in cloned mouse T-cell culture supernatant containing murine IL-3, prepared with a cytochemical method to detect peroxidase) illustrates PMD. Virtually all secondary granule matrix compartments *(open arrowhead)* are devoid of EPO; many retain irregularly shaped, EPO-negative cores. Numerous EPO-loaded perigranular vesicles are evident *(closed arrowhead)* (Original magnification: ×14,000.) (From Dvorak AM, et al. Clin Exp Allergy 1994;24:10-8, by permission of Blackwell Scientific Publications.)

22. Dvorak AM, Furitsu T, Letourneau L, Ishizaka T, Ackerman SJ. Mature eosinophils stimulated to develop in human cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. I. Piecemeal degranulation of specific granules and distribution of Charcot-Leyden crystal protein. Am J Pathol 1991;138:69-82.
23. Dvorak AM, Ackerman SJ, Furitsu T, Estrella P, Letourneau L, Ishizaka T. Mature eosinophils stimulated to develop in human cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. II. Vesicular transport of specific granule matrix peroxidase, a mechanism for effecting piecemeal degranulation. Am J Pathol 1992;140:795-807.
24. Weller PF, Dvorak AM. Arachidonic acid incorporation by cytoplasmic lipid bodies of human eosinophils. Blood 1985;65:1269-74.
25. Weller PF, Monahan-Earley RA, Dvorak HF, Dvorak AM. Cytoplasmic lipid bodies of human eosinophils. Subcellular isolation and analysis of arachidonate incorporation. Am J Pathol 1991;138:141-8.
26. Weller PF, Ryeom SW, Dvorak AM. Lipid bodies: structurally distinct, nonmembranous intracellular sites of eicosanoid formation. In: Bailey JM, ed. Prostaglandins, leukotrienes, lipoxins and PAF. New York: Plenum Press, 1991:353-62.
27. Dvorak AM, Dvorak HF, Peters SP, et al. Lipid bodies: cytoplasmic organelles important to arachidonate metabolism in macrophages and mast cells. J Immunol 1983;131:2965-76 (republished, J Immunol 1984;132:1586-97).
28. Dvorak AM, Morgan E, Schleimer RP, Ryeom SW, Lichtenstein LM, Weller PF. Ultrastructural immunogold localization of prostaglandin endoperoxide synthase (cyclooxygenase) to non-membrane-bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages, type II pneumocytes, and neutrophils. J Histochem Cytochem 1992;40:759-69.
29. Dvorak AM, Schleimer RP, Dvorak HF, Lichtenstein LM,

Weller PF. Human lung mast cell and alveolar macrophage cytoplasmic lipid bodies contain arachidonic acid and prostaglandin endoperoxide synthase (cyclooxygenase), the substrate and enzyme necessary for prostaglandin production. Int Arch Allergy Immunol 1992;99:208-17.
30. Dvorak AM, Weller PF, Harvey VS, Morgan ES, Dvorak HF. Ultrastructural localization of prostaglandin endoperoxide synthase (cyclooxygenase) to isolated, purified fractions of guinea pig peritoneal macrophage and line 10 hepatocarcinoma cell lipid bodies. Int Arch Allergy Immunol 1993;101:136-42.
31. Weller PF, Tzizik DM, Morgan ES, Dvorak AM. Lipid bodies in eosinophils and neutrophils are sites of eicosanoid forming enzymes [Abstract]. J ALLERGY CLIN IMMUNOL 1994;94;93:269.
32. Dvorak AM, Ishizaka T, Galli SJ. Ultrastructure of human basophils developing *in vitro*. Evidence for the acquisition of peroxidase by basophils and for different effects of human and murine growth factors on human basophil and eosinophil maturation. Lab Invest 1985;53:57-71.
33. Saito H, Hatake K, Dvorak AM, et al. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc Natl Acad Sci U S A 1988;85:2288-92.
34. Dvorak AM, Saito H, Estrella P, Kissell S, Arai N, Ishizaka T. Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. Lab Invest 1989;61:116-32.
35. Dvorak AM, Warner JA, Kissell S, Lichtenstein LM, MacGlashan DW Jr. F-met peptide-induced degranulation of human basophils. Lab Invest 1991;64:234-53.
36. Dvorak AM, Warner JA, Morgan E, Kissell-Rainville S, Lichtenstein LM, MacGlashan DW Jr. An ultrastructural analysis of tumor-promoting phorbol diester-induced degranulation of human basophils. Am J Pathol 1992;141: 1309-22.
37. Dvorak AM, Onderdonk AB, McLeod RS, et al. Ultrastructural identification of exocytosis of granules from human gut eosinophils *in vivo*. Int Arch Allergy Immunol 1993;102:33-45.
38. Ishizaka T, Dvorak AM, Conrad DH, Niebyl JR, Marquette JP, Ishizaka K. Morphologic and immunologic characterization of human basophils developed in cultures of cord blood mononuclear cells. J Immunol 1985;134:532-40.
39. Dvorak AM, Newball HH, Dvorak HF, Lichtenstein LM. Antigen-induced IgE-mediated degranulation of human basophils. Lab Invest 1980;43:126-39.
40. Dvorak AM, Lett-Brown M, Thueson D, Grant JA. Complement-induced degranulation of human basophils. J Immunol 1981;126:523-8.
41. Findlay SR, Dvorak AM, Kagey-Sobotka A, Lichtenstein LM. Hyperosmolar triggering of histamine release from human basophils. J Clin Invest 1981;67:1604-13.
42. Dvorak AM, Lett-Brown MA, Thueson DO, et al. Histamine-releasing activity (HRA). III. HRA induces human basophil histamine release by provoking noncytotoxic granule exocytosis. Clin Immunol Immunopathol 1984;32: 142-50.
43. Dvorak HF, Mihm MC Jr, Dvorak AM, et al. Morphology of delayed type hypersensitivity reactions in man. I. Quantitative description of the inflammatory response. Lab Invest 1974;31:111-30.
44. Dvorak AM, Mihm MC Jr, Dvorak HF. Degranulation of basophilic leukocytes in allergic contact dermatitis reactions in man. J Immunol 1976;116:687-95.
45. Dvorak AM, Mitsui H, Ishizaka T. Ultrastructural morphology of immature mast cells in sequential suspension cultures of human cord blood cells supplemented with c-*kit* ligand: distinction from mature basophilic leukocytes undergoing secretion in the same cultures. J Leukoc Biol 1993;54:465-85.
46. Dvorak HF, Dvorak AM. Basophilic leucocytes: structure, function and role in disease. In: Lichtman MA, ed. Clinics in haematology. Vol. 4. Granulocyte and monocyte abnormalities. London: WB Saunders Company, Ltd., 1975:651-83.
47. Dvorak AM, Morgan ES, Lichtenstein LM, MacGlashan DW Jr. Activated human basophils contain histamine in cytoplasmic vesicles. Int Arch Allergy Immunol 1994;105: 8-11.
48. Dvorak AM, Morgan ES, Schleimer RP, Lichtenstein LM. Diamine oxidase-gold labels histamine in human mast cell granules: a new enzyme-affinity ultrastructural method. J Histochem Cytochem 1993;41:787-800.
49. Dvorak AM, Estrella P, Ishizaka T. Vesicular transport of peroxidase in human eosinophilic myelocytes. Clin Exp Allergy 1994;24:10-8.
50. Dvorak AM. Ultrastructural evidence for release of major basic protein-containing crystalline cores of eosinophil granules in vivo: cytotoxic potential in Crohn's disease. J Immunol 1980;125:460-2.
51. Dvorak AM, Furitsu T, Estrella P, Letourneau L, Ishizaka T, Ackerman SJ. Ultrastructural localization of major basic protein in the human eosinophil lineage in vitro. Histochem Cytochem 1994 (in press).