Histol Histopath (1994) 9: 339-374

Histology and Histopathology

Invited Review

Human eosinophils in vitro. An ultrastructural morphology primer

A.M. Dvorak[1] and T. Ishizaka[2]

[1]Departments of Pathology, Beth Israel Hospital and Harvard Medical School and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Masachusetts, and [2]Division of Allergy, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA

Summary. An ultrastructural morphological primer of human eosinophils is presented. Mature and immature eosinophils, obtained from peripheral blood and bone marrow, as well as activated tissue eosinophils are all used to illustrate the various morphologies assumed by eosinophils *in vivo*. The various ultrastructural changes expressed by this cell lineage *in vivo* reflect the impact of differentiation, maturation, activation, secretion, and cell injury on morphology. Nearly all of the changes described *in vivo* are also evident in eosinophils arising in *in vitro* systems. We review published studies of these culture systems, which have been supplemented with various conditioned media containing naturally occurring growth factor(s) that are permissive (or not permissive) for eosinophils or with the recombinant growth factors, IL-5 or IL-3. These studies were helpful in the recognition of eosinophil-promoting, -sustaining and -activating properties of human IL-3 and IL-5. Moreover, mature and immature eosinophils were shown to release a granule matrix protein - eosinophil peroxidase (EPO) - by its transport in small cytoplasmic vesicles, a process termed piecemeal degranulation (PMD), accounting for the gradual emptying of granule contents in the absence of granule fusions to the plasma membrane. Also presented are eosinophil morphologies that occur *in vitro* in suspension cultures of human cord blood supplemented with the c-kit ligand from various sources. The wide variety of eosinophil subcellular changes in the c-kit ligand-supplemented cultures, like the changes of which eosinophils are capable *in vivo*, reflects the processes of differentiation, maturation, activation, secretion and cell injury. Presentation of this ultrastructural morphological primer of human eosinophils *in vitro* should enable investigators to recognize eosinophils in all of their diverse morphologic forms in cultures that contain differentiating and functioning members of other lineages, also present in c-kit ligand-supplemented cultures. These lineages include mast cells, basophils, neutrophils, monocytes, macrophages, megakaryocytes, and endothelial cells.

Key words: Human, Eosinophils, *In vitro*, Ultrastructure, Growth factors

Offprint requests to: Ann M. Dvorak, M.D., Department of Pathology, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215, USA

1. Introduction

The rapid identification of hematopoietic growth factors and culture systems, which specifically enable the development of the eosinophil lineage, has provided an opportunity to learn more about the morphologic expressions of these granulocytes during their maturation and function *in vitro* (Dvorak et al., 1993a). The alterations in the standard, well-known ultrastructural morphology of normal human peripheral blood eosinophils (Dvorak et al., 1991a) that are observed in these culture-derived cells are considerable. Additionally, depending on the growth factor(s) present, eosinophils are found among representatives of other cell lineages. These cells are also undergoing morphological changes secondary to their developmental and functional programs. Therefore, the ultrastructural identification of members of the eosinophil lineage is difficult. For this purpose, a primer of ultrastructural eosinophil morphologies *in vitro* may be useful to the research community.

We review here, briefly, the ultrastructural morphology of normal eosinophils in peripheral blood, of immature eosinophils in bone marrow, and of functioning tissue eosinophils *in vivo*. More complete ultrastructural reviews of these *in vivo* morphologies have recently been published (Dvorak, 1988, 1991b, 1993; Dvorak et al., 1991a, 1993a; Weller and Dvorak, 1994). We also review recent reports of *in vitro* systems that reflect some ultrastructural aspects of mature and

immature eosinophils and their functional morphologies (Dvorak et al., 1985, 1989, 1991b, 1992, 1993f; Jabara et al., 1988; Saito et al., 1988). Newer culture systems, supplemented with fibroblast products, also contain eosinophils, generally as a minor population in early cultures (Dvorak et al., 1993b,c,e, 1994a,b; Mitsui et al., 1993). These suspension cultures provide a challenge to the ultrastructural morphologist that should be facilitated by this documentation of the various morphologies expressed by eosinophils *in vitro*. Thus, identification criteria are presented for mature eosinophils that are granule-poor or granule-free, that contain lipid bodies only, that have increases in small granules and primary granules, and that have a variety of alterations in their major bicompartmental granule population. These ultrastructural images should make it possible to recognize members of the eosinophil lineage and to distinguish them from other cellular lineages simultaneously present. These include mast cells, basophils, neutrophils, monocytes, macrophages, megakaryocytes and endothelial cells and their immature and/or functional forms (Dvorak et al., 1993b,c,e, 1994a,b; Mitsui et al., 1993).

2. Mature human eosinophils

Mature eosinophils (Fig. 1) are granulocytes that have multiple lobes to their nuclei. The nuclear chromatin is condensed, and nucleoli are absent. The cell surface contours are comprised of irregular broad cytoplasmic processes. The cytoplasmic organelles (Fig. 2) include glycogen particles, secretory granules, lipid bodies and tubulovesicular structures. Golgi structures and membrane-bound ribosomes are diminished. The large, round lipid bodies are osmiophilic and are not bound by a trilaminar unit membrane. They may be completely or focally encircled by a single, membrane-like dense shell. Focal, internal electron-lucent areas and dense particles can be observed within lipid bodies, and individual lipid bodies may fuse. Lipid bodies are arachidonic acid-rich organelles (Weller and Dvorak, 1985; Weller et al., 1991a) that contain the enzyme prostaglandin endoperoxide synthase, as demonstrated by ultrastructural autoradiography and immunogold stains, respectively (Weller and Dvorak, 1985; Weller et al., 1991a,b; Dvorak et al., 1994c). The majority of granules in mature eosinophils are large, membrane-bound, oval-shaped structures within which a central, dense, crystalline core compartment and an outer, less dense matrix compartment are seen (Fig. 2). These are secondary or specific granules. In their core compartment is found major basic protein (MBP); eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and tumor necrosis factor-alpha (TNF-α) are found in their matrix compartment by ultrastructural immunogold and cytochemical methods (Egesten et al., 1986; Peters et al., 1986; Dvorak et al., 1991a, 1993a; Beil et al., 1993). Approximately 5% of the granules lack central cores; these are residual primary granules (Fig. 2), which

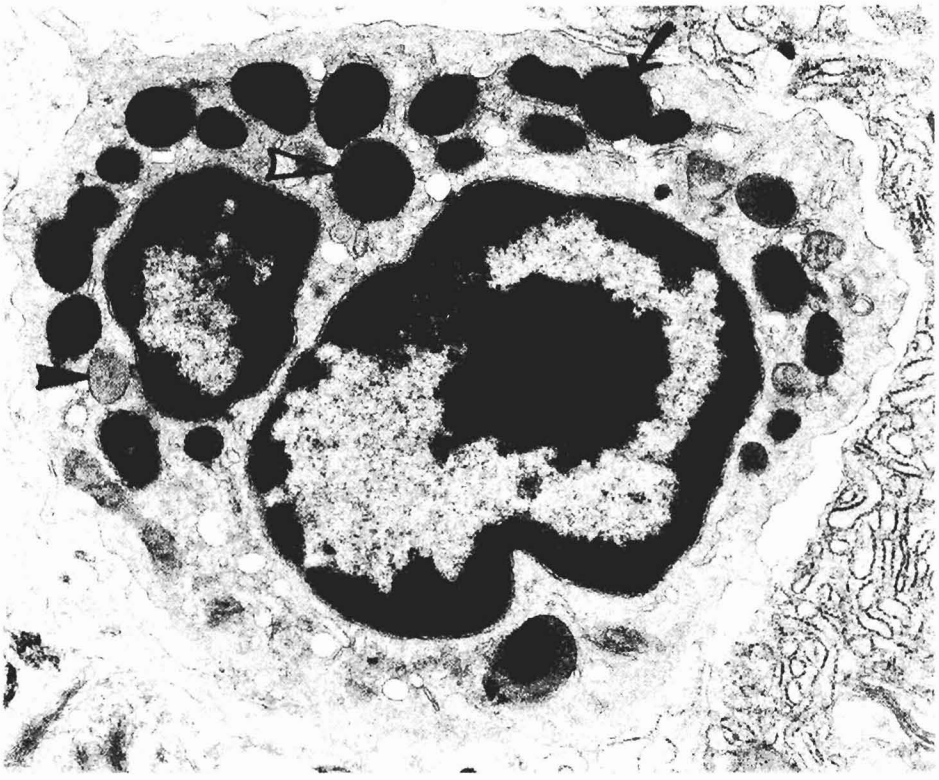


Fig. 1. A mature eosinophil (small intestinal biopsy) shows two nuclear lobes with condensed peripheral chromatin, irregular, broad surface processes and secretory granules. Bicompartmental secondary granules (open arrowhead), a single primary granule (closed arrowhead), an osmiophilic lipid body (arrow), cytoplasmic vesicles and mitochondria are present. x 16,500

have been determined as the granule storage organelle for the Charcot-Leyden crystal protein by ultrastructural immunogold stains (Dvorak et al., 1988). A third granule population, called small granules, contains hydrolytic enzymes (reviewed in Dvorak et al., 1991a); these are small, dense structures of varying shape.

3. Immature human eosinophilic myelocytes

Eosinophilic myelocytes (Fig. 3) are immature eosinophils. They generally are ~3x larger than mature eosinophils and characteristically have an ample cytoplasmic area filled with large, round immature granules, extensive dilated cisterns of rough endoplasmic reticulum and an enlarged Golgi complex. Their surfaces have blunt cytoplasmic processes; the nuclei are large, monolobed, lobular structures with partially condensed chromatin along nuclear membranes. Cytoplasmic glycogen and lipid bodies are generally absent. The large immature granules in eosinophilic myelocytes are precursors to primary and secondary granule populations. Late myelocytes contain mostly the latter. These immature granules are perfectly round and homogeneously dense. Dense, central crystalline cores with irregular shapes are present in small numbers in the maturing secondary granules. Earlier myelocytes have larger numbers of immature primary granules. These structures have loosely arranged, moderately dense, finely granular contents and are at times circumscribed by an outer layer of small vesicles beneath granule membranes. Myelocytes with mixtures of these immature granules do exist. When crystalline cores appear in immature granules, however, they are secondary granule precursors.

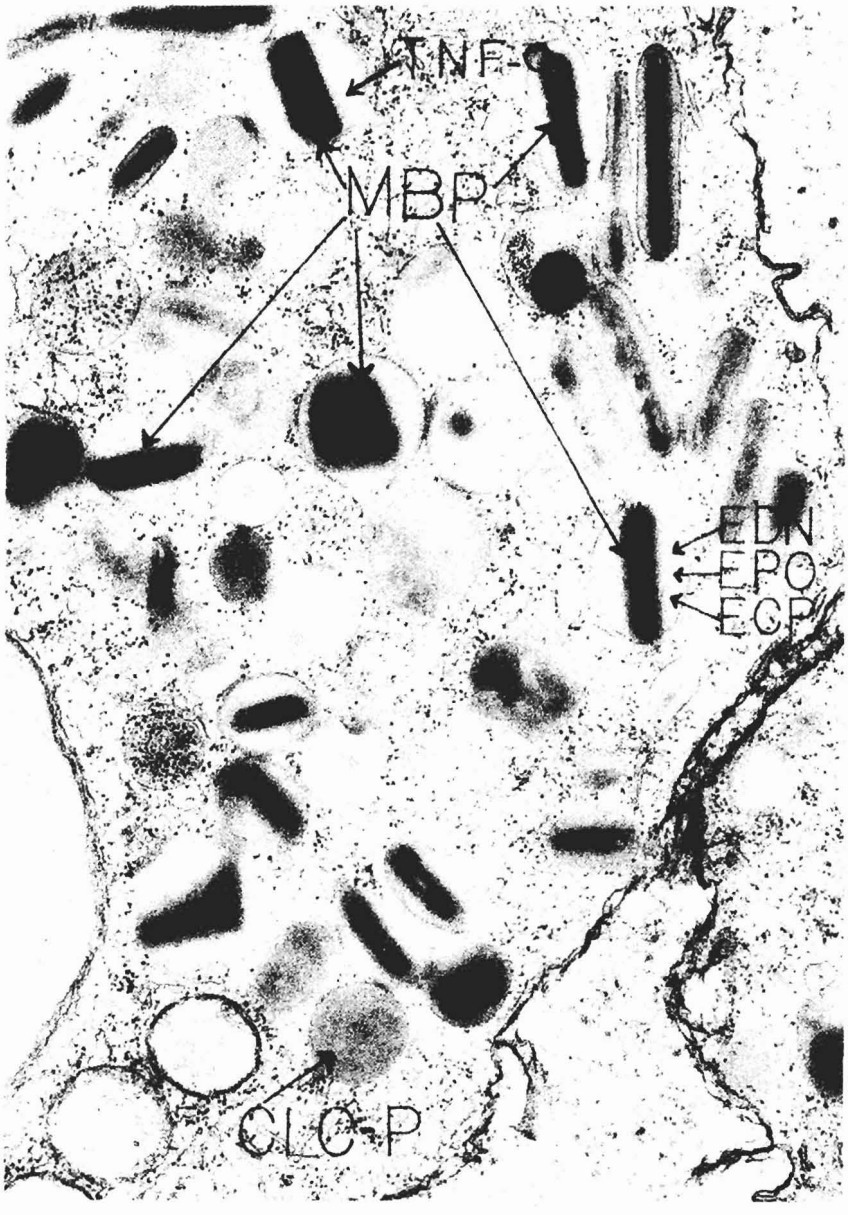


Fig. 2. The cytoplasm of a mature eosinophil (peripheral blood sample) shows secretory granules. Specific (secondary) granules contain dense cores filled with major basic protein (MBP), surrounded by less dense matrix where eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP) (reviewed in Dvorak et al., 1991a), and tumor necrosis factor-α (TNF-α) (Beil et al., 1993) reside. Primary granules, the location of Charcot-Leyden crystal protein (CLC-P), are homogeneous and do not contain central crystalline cores (Dvorak et al., 1988) (with permission, Dvorak, 1991b). x 29,000

4. Tissue eosinophils

Tissue eosinophils (Figs. 4-7) display many ultrastructural morphologic changes in a wide variety of diseases (Dvorak, 1980; Dvorak et al., 1980, 1982, 1990a,b, 1993d; and reviewed in Dvorak, 1988; Dvorak et al., 1991a). Subcellular features include both quantitative and qualitative alterations in granules, tubulovesicles and lipid bodies. The general processes in which tissue eosinophils participate include activation, secretion and cell injury. Ultrastructural changes in cells undergoing secretion include those analogous to regulated exocytosis (Fig. 4) (Dvorak et al., 1993d) and piecemeal degranulation of specific granule contents (Fig. 5) (Dvorak, 1980; Dvorak et al., 1991b, 1992). Morphologically, cell alterations induced by injury to eosinophils can be categorized as apoptosis, necrosis, and mixtures of these processes (Dvorak et al., 1982, 1990b; Weller and Dvorak, 1994; Weller et al., 1994). Cell activation changes include those of increased granule populations (Fig. 5) (other than secondary granules), lipid bodies and tubulovesicles (Dvorak et al., 1991a). Many of the morphologic changes that have been recorded in tissue eosinophils also occur in factor-supplemented cultures of mature human eosinophils (Dvorak et al., 1991b, 1992).

Tissue eosinophil granule changes include decreased numbers of specific granules (Fig. 6), looses from one or both compartments of these bicompartmental granules with retention of granule membranes, a process termed piecemeal degranulation (PMD) (Fig. 5), as well as increased numbers of primary (Fig. 5) and small granules (Fig. 5), tubulovesicles and lipid bodies (Dvorak et al., 1991a). Lipid bodies may also increase considerably in size and display focal lucencies and punctate densities in their interiors (Dvorak et al., 1991a). Tissue eosinophils have been shown to extrude membrane-free secondary granules into large, internal degranulation chambers or into the extracellular milieu through multiple pores in the circumferential cell surface (Fig. 4) (Dvorak et al., 1993d). Cell injury of eosinophils with the characteristic

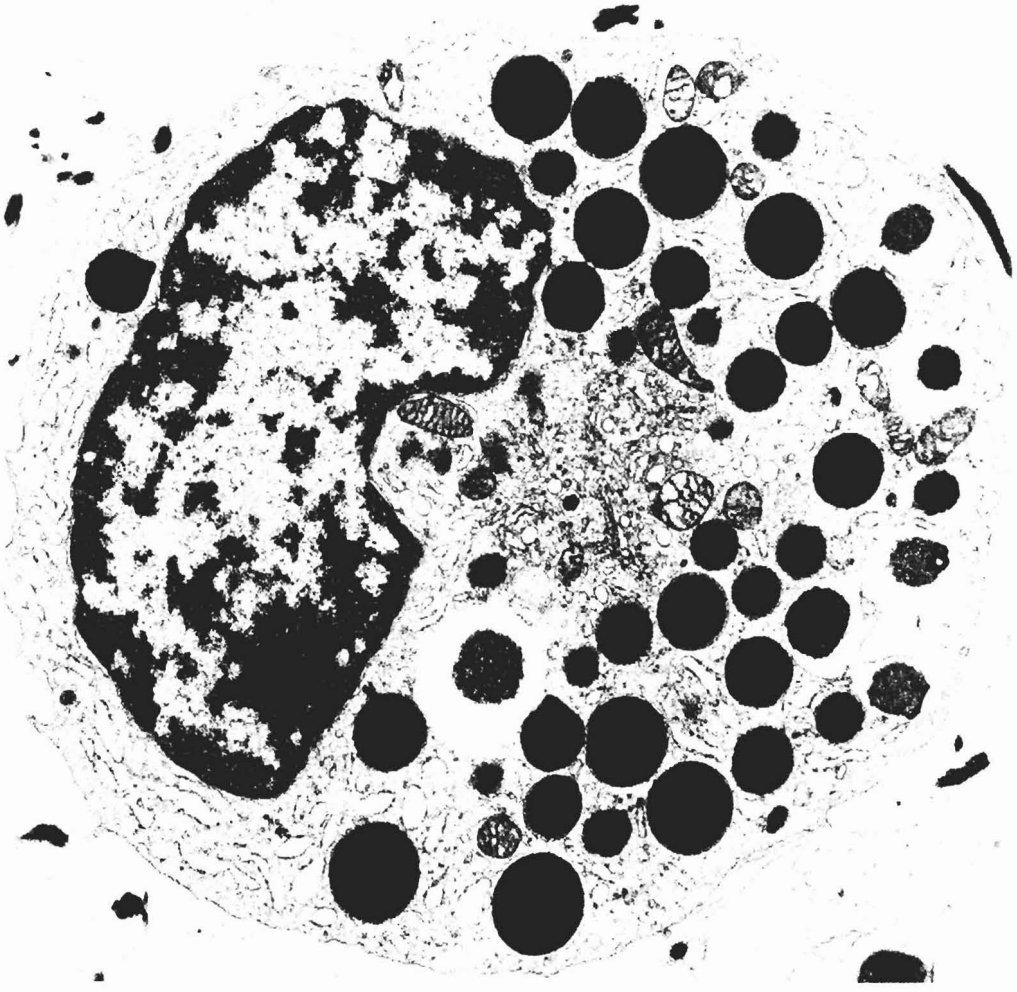


Fig. 3. An eosinophilic myelocyte (bone marrow sample) shows a single, eccentrically located lobular nucleus, extensive dilated cisterns of rough endoplasmic reticulum (RER), a Golgi structure, which contains a small progranule, and numerous large, dense, round immature secondary granules. Several immature granules, with finely granular material and surrounding electron-lucent space, are immature primary granules (with permission, Dvorak, J. Electron Microsc. Tech. 6, 255-301, 1987). x 11,500

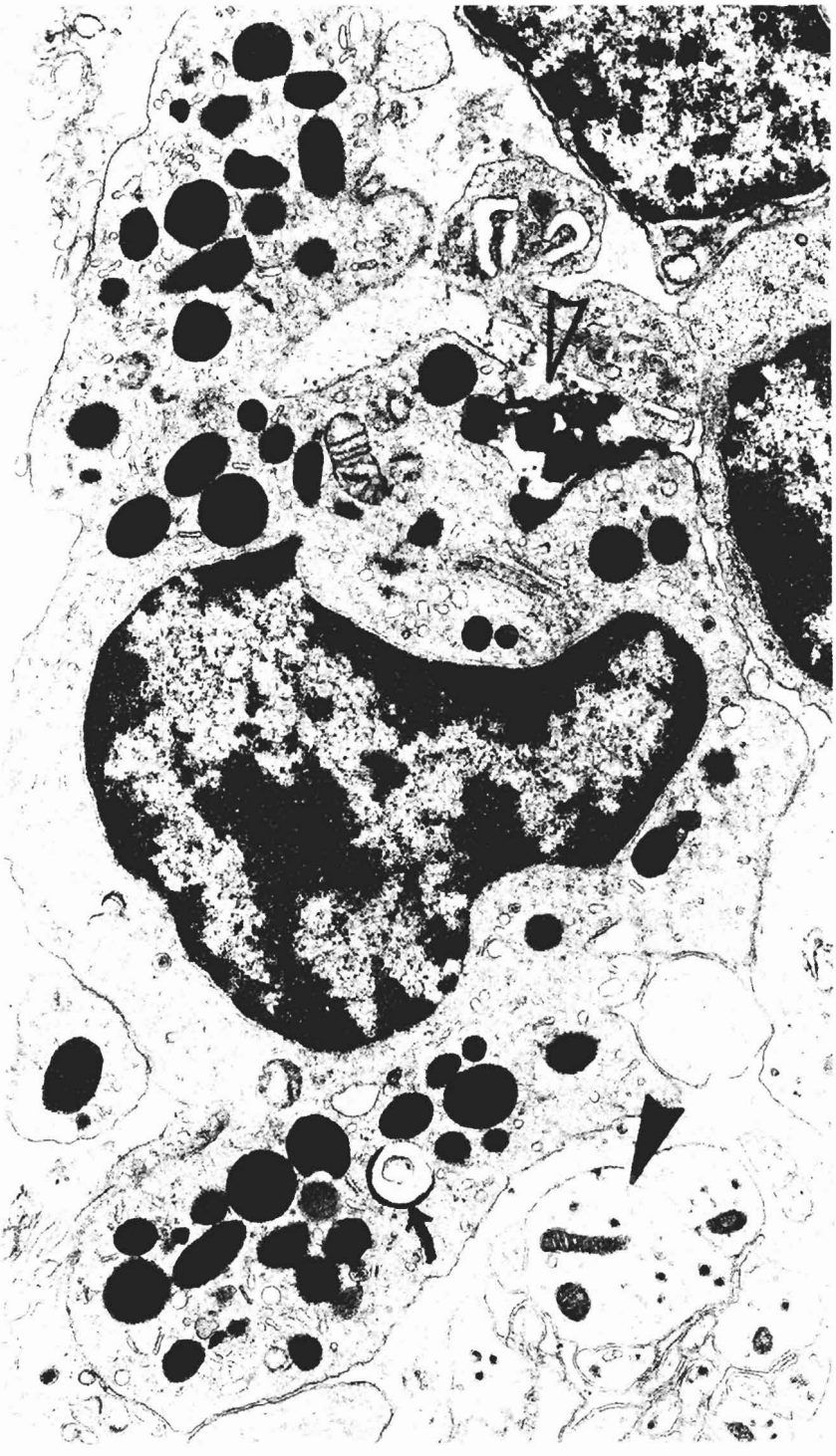


Fig. 4. A tissue eosinophil (Staphylococcal culture-positive biopsy of a continent pouch constructed from small bowel in an ulcerative colitis patient) shows focal extrusion of four secretory granules (open arrowhead). The granule cores retain their shape, and the matrix material has extended into a linear configuration adjacent to the cores. All are contained ajdacent to a single surface pocket of the eosinophil. The remaining cytoplasmic granules show little evidence of morphological change. They are a mixture of secondary, primary and small granules. A single lipid body contains a narrow osmiophilic rim and multiple membranous structures (arrow). Note the collection of swollen enteric nerves with a few dense core granules nearby (closed arrowhead) (with permission, Dvorak et al., 1993d). x 16,000

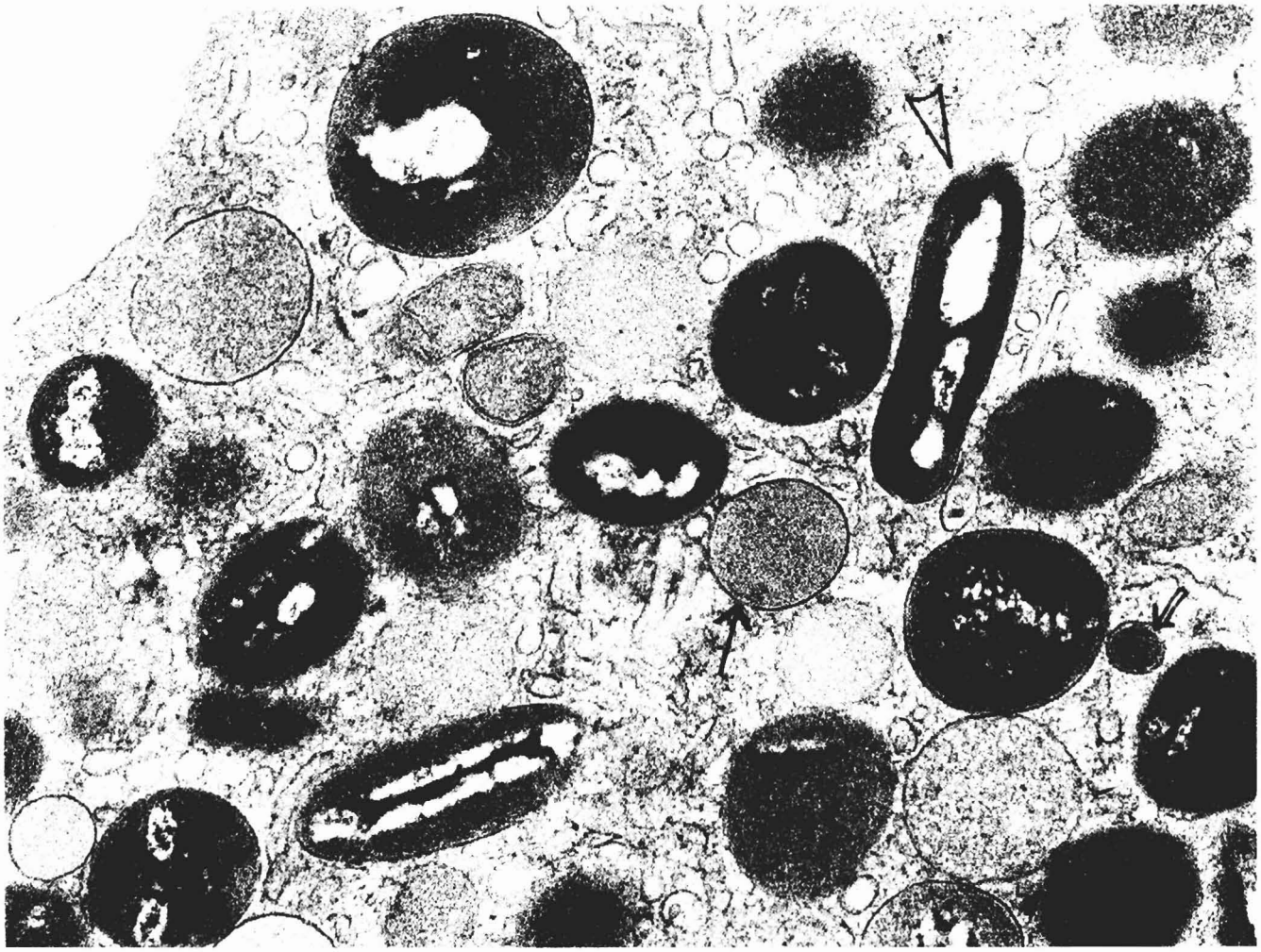


Fig. 5. The cytoplasm of a tissue eosinophil (skin biopsy of a patient with the hypereosinophilic syndrome (HES) and a pruritic skin rash) shows piecemeal degranulation (PMD) characterized by focal losses from the electron-dense cores of secondary granules (arrowhead), increased numbers of primary granules lacking cores (closed arrow), and small granules (open arrow). Also, note the large number of cytoplasmic smooth membrane-bound vesicles and elongated tubules surrounding the cytoplasmic granules. x 41,000

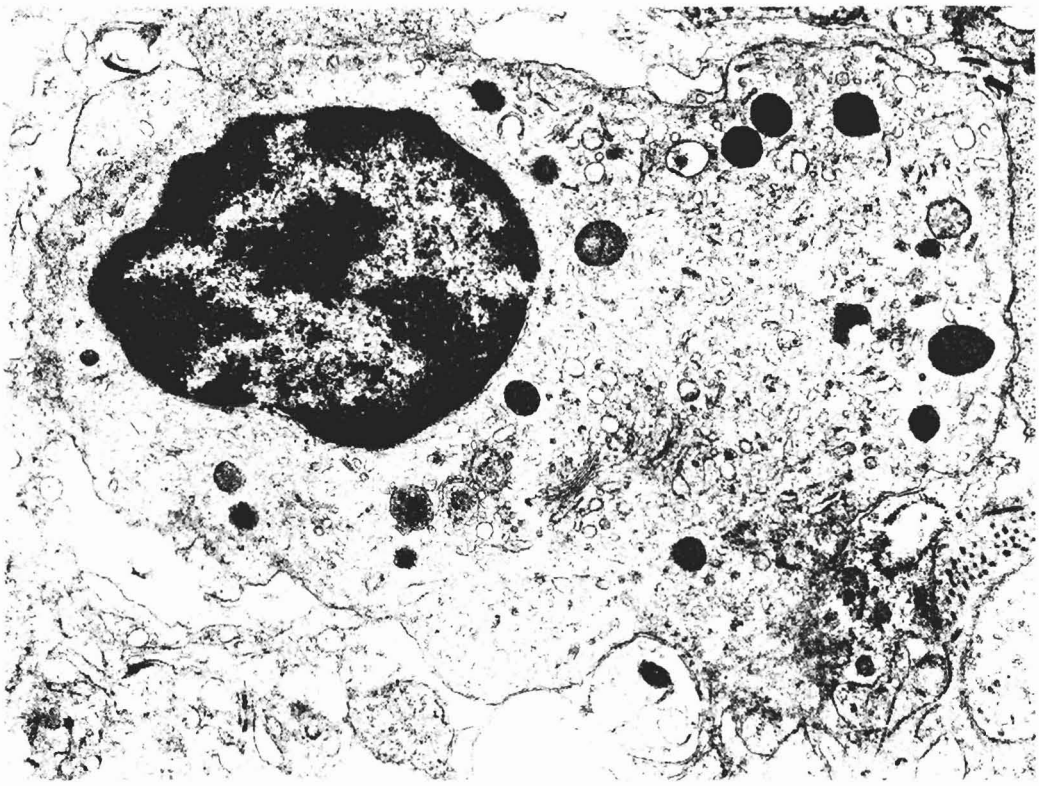


Fig. 6. A tissue eosinophil (Staphylococcal culture-negative biopsy of an ileostomy from a patient with Crohn's disease; damaged axons were present in the same biopsy (Dvorak et al., 1993d)) shows decreased numbers of secretory granules. Several primary granules and one lobe of a typical eosinophil nucleus with condensed chromatin identify this cell as of the eosinophil lineage. x 15,000

features of apoptosis or necrosis ultimately releases membrane-bound specific granules into the tissue microenvironment (Dvorak et al., 1982, 1990b; Weller and Dvorak, 1994, Weller et al., 1994). Necrosis of eosinophils is characterized by cell and organellar swelling, broken cell and organellar membranes, and nuclear chromatolysis (Fig. 7). Apoptosis is characterized by the formation of cytoplasmic apoptotic bodies and nuclear chromatin condensation, pyknosis, and formation of nuclear apoptotic bodies.

5. Human eosinophils in vitro

Ultrastructural analysis of a large number of cultures of human umbilical cord blood cells, supplemented with a number of growth factor-containing substances, has provided insight into the varied morphology of eosinophils *in vitro*. In a large sense, these studies recapitulate previously documented human eosinophil morphology *in vivo*. The growth factors and sources used for these culture studies include the following:

i. interleukin-2-depleted culture supernatant from

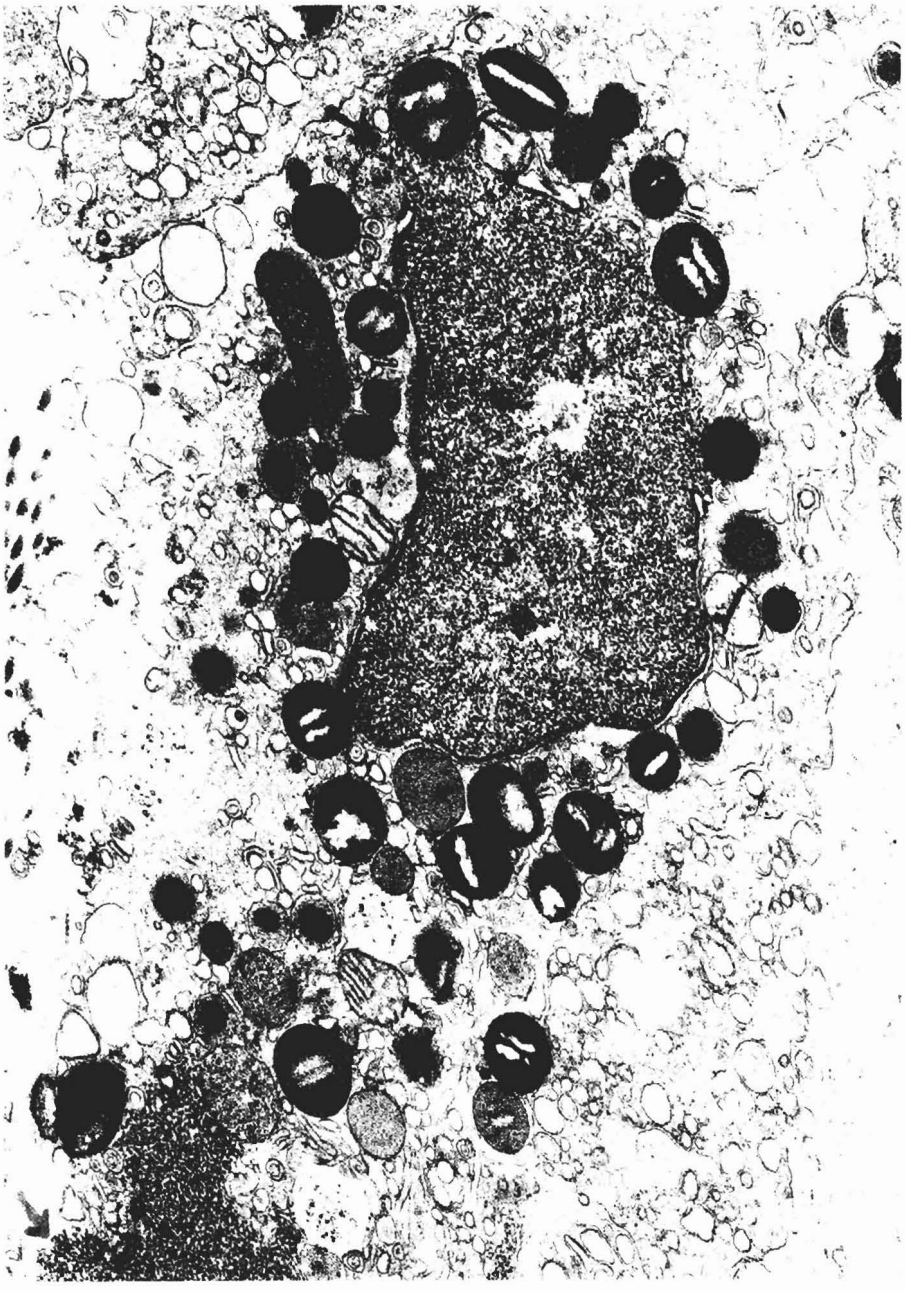


Fig. 7. A tissue eosinophil (skin biopsy of rash in a patient with the hypereosinophilic syndrome) shows necrosis. Note dispersion and leakage of nuclear chromatin into the cytoplasm and extracellular space (arrows) (chromatolysis) as well as the broken nuclear, organellar and plasma membranes. Cytoplasmic contents and organelles are roughly within former cytoplasmic domains. In other areas of this biopsy (data not shown), membrane-bound secondary granules with focal core compartment losses were widely dispersed in the extracellular matrix. x 19,000

phytohemagglutinin (PHA)-stimulated human T lymphocytes, known to contain IL-3 - a human basophilopoietin (Dvorak et al., 1985, 1993f);

ii. murine T lymphocyte conditioned media, known to contain IL-3 - a murine mast cell growth factor (Dvorak et al., 1985, 1993f);

iii. recombinant human interleukin-3 (rhIL-3) (Saito et al., 1988; Dvorak et al., 1989);

iv. recombinant human interleukin-5 (rhIL-5) (Saito et al., 1988; Dvorak et al., 1989);

v. rhIL-5 and the culture supernatant described in (i) above (Dvorak et al., 1991b, 1992);

vi. fibroblast culture supernatants (Mitsui et al., 1993; Dvorak et al., 1994a);

vii. partially purified, naturally occurring mouse c-kit ligand in 3T3 fibroblast culture supernatants (Mitsui

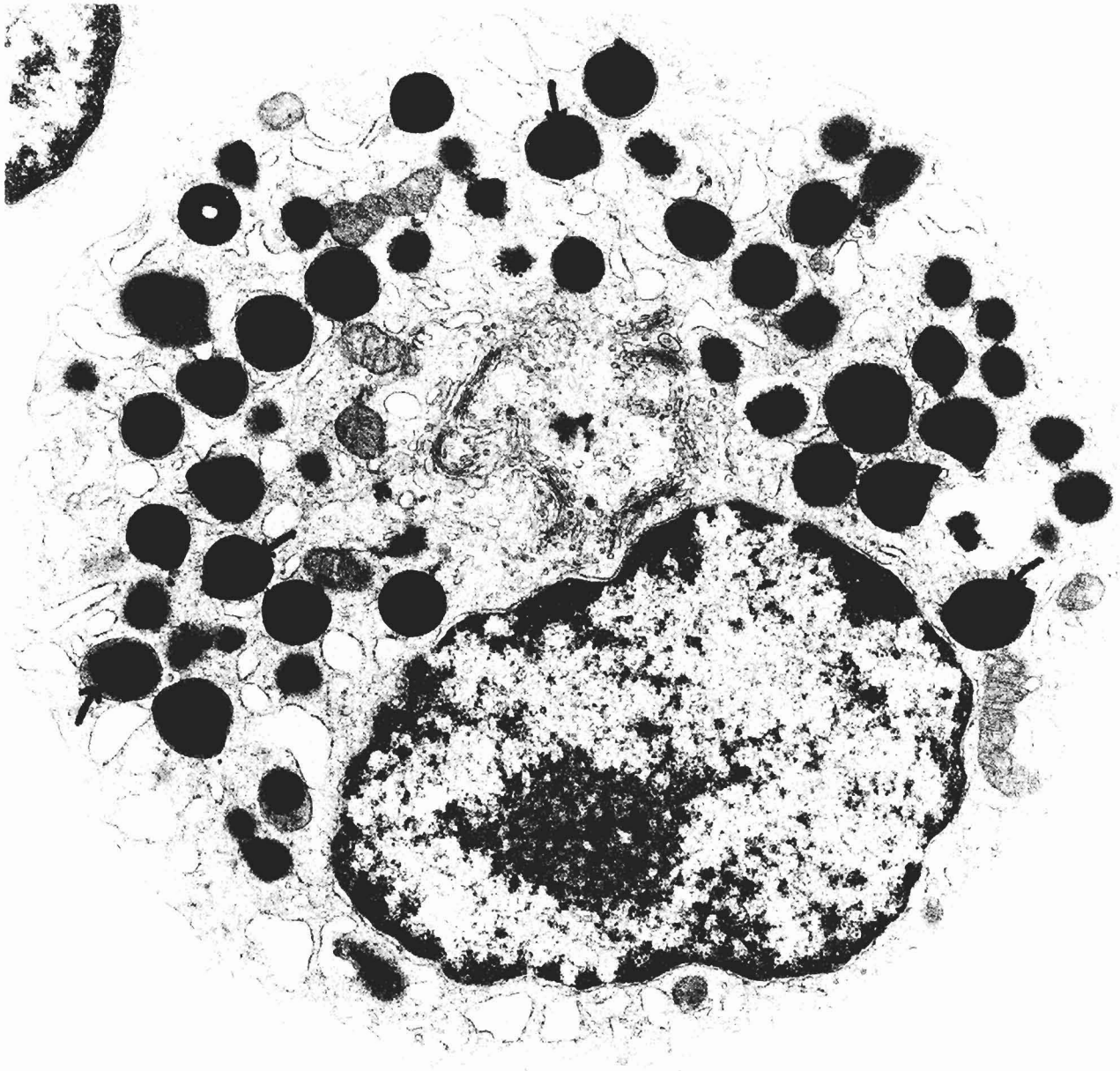


Fig. 8. An eosinophilic myelocyte (umbilical cord blood cell (UCBC) suspension culture with rhIL-3 for three weeks) shows eccentric, monolobed lobular nucleus with dispersed chromatin and large nucleolus, expanded Golgi structures, extensive arrays of dilated cisterns of RER, elongated mitochondria, and round, dense immature secondary granules. Note the formation of irregular condensations of central dense core material (arrows) in the latter. x 16,000

et al., 1993; Dvorak et al., 1993b,c,e, 1994b);
viii. recombinant mouse c-kit ligand (Mitusi et al., 1993; Dvorak et al., 1993b);
ix. recombinant human stem cell factor (rhSCF - also known as recombinant human c-kit ligand and recombinant human mast cell growth factor) (Mitsui et al., 1993; Dvorak et al., 1993b,c,e, 1994b);
x. recombinant human granulocyte-stimulating factor (rhGMCSF) (Weller et al., 1994).

It is now known that additives containing human IL-5 best induce the development of the eosinophil lineage from human umbilical cord cells in suspension cultures (Saito et al., 1988; Dvorak et al., 1989, 1991b, 1992). However, variable numbers of eosinophils are identifiable by electron microscopy in each of the supplemented cultures of human cord blood cells listed

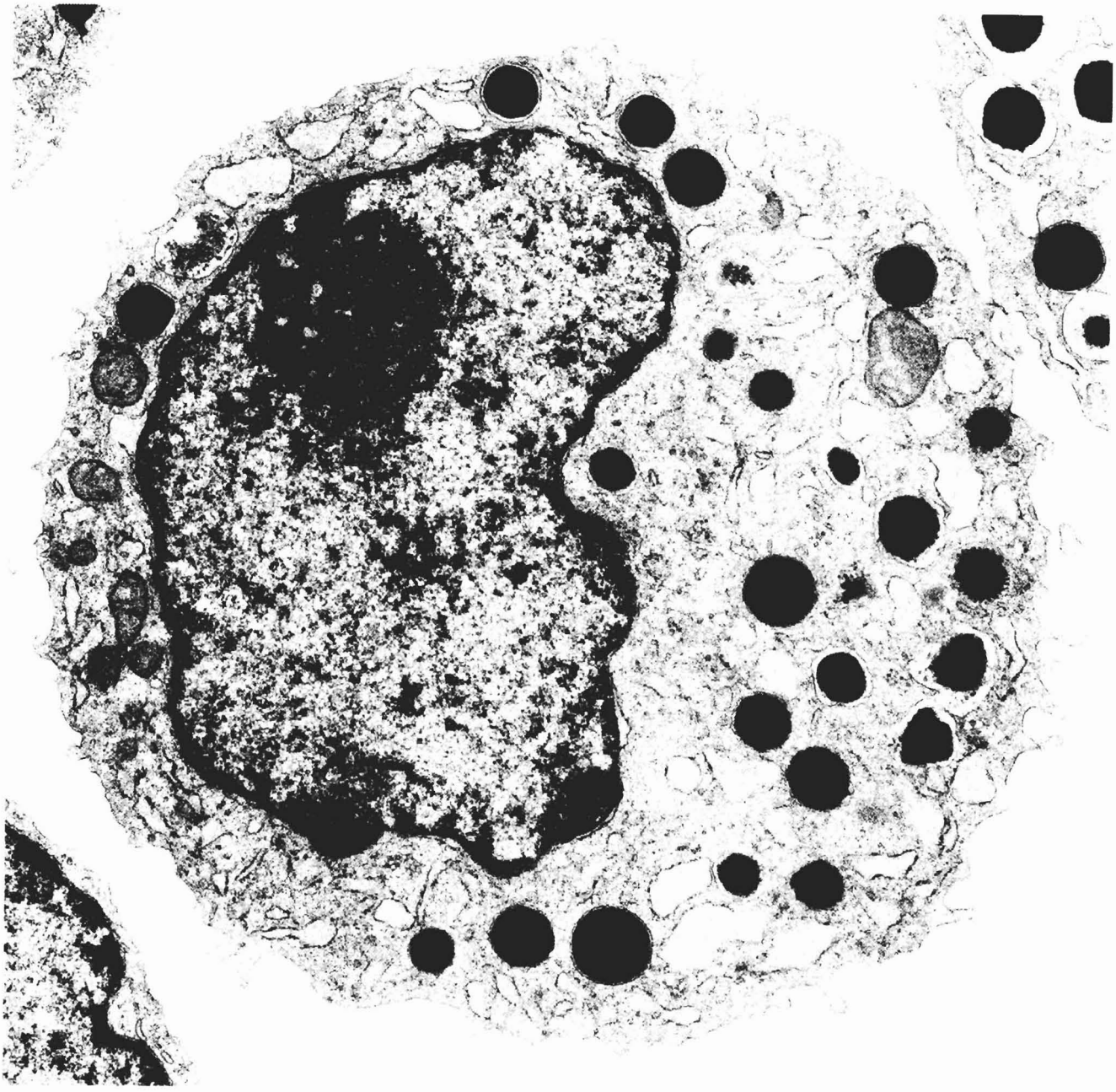


Fig. 9. An eosinophilic myelocyte (UCBC supension culture with rhIL-5 for three weeks) shows a nucleus, cytoplasmic organellar content and immature granules that are similar to those of eosinophilic myelocytes arising in rhIL-3-containing cultures of UCBC (see Fig. 8) (with permission, Dvorak et al., 1989). x 16,000

above. This morphological primer for eosinophils *in vitro* is based on these studies. We present images in a chronological sequence that parallels the course of completion of our studies.

5. 1. Eosinophils develop in cultures supplemented with naturally occurring human IL-3 in stimulated lymphocyte supernatants

Variable numbers of mature eosinophils with poly-lobed nuclei and condensed chromatin developed in these cultures (Dvorak et al., 1985). The secondary granules contained eosinophil peroxidase in the matrix compartment (Dvorak et al., 1985). Mature basophils far exceeded the number of eosinophils in these cultures, however (Dvorak et al., 1985; Ishizaka et al., 1985). Eosinophilic myelocytes were also noted in these cultures. They displayed EPO in the perinuclear and rough endoplasmic reticular cisternae. Golgi structures and immature granules (Dvorak et al.,

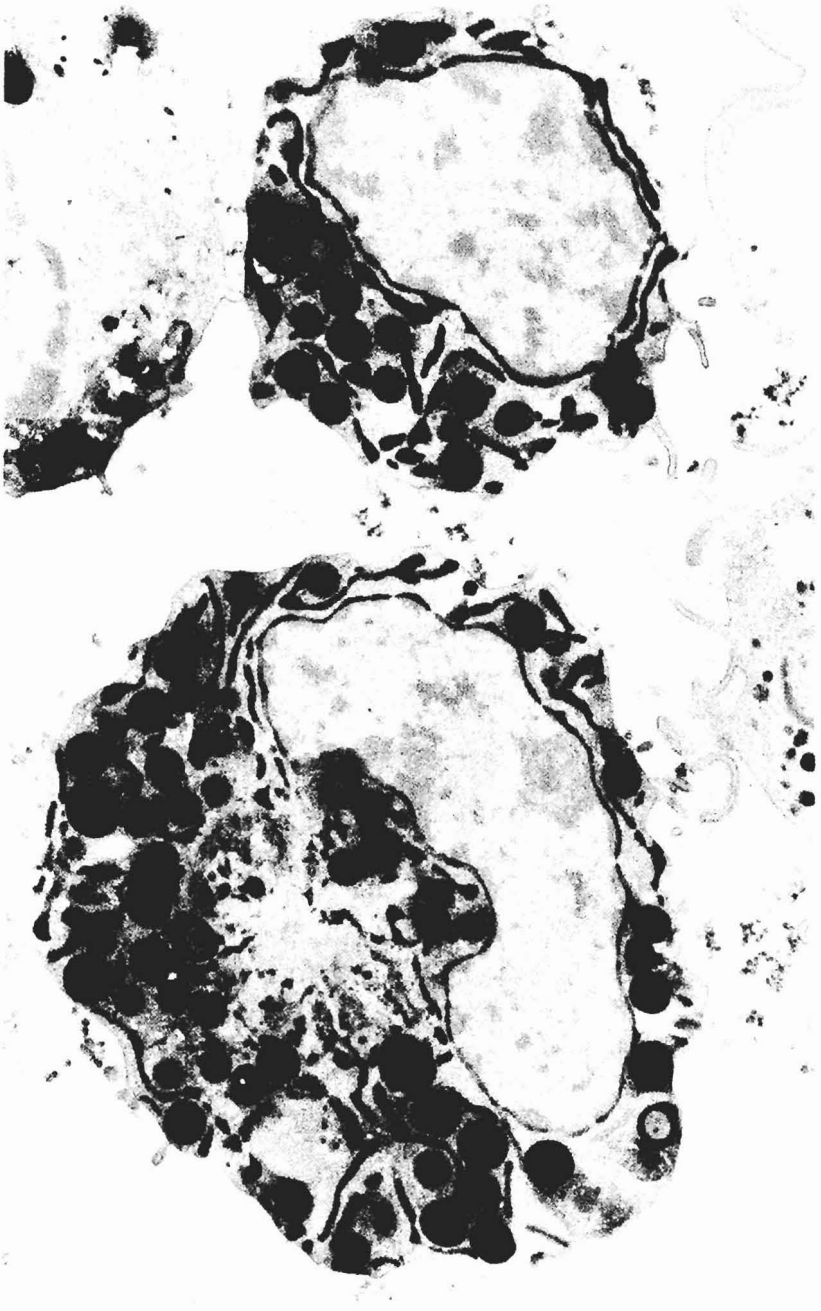


Fig. 10. Eosinophilic myelocytes (UCBC suspension culture with rhIL-5 for three weeks, prepared with a cytochemical method to demonstrate endogenous peroxidase) show EPO in the perinuclear cisterna, RER cisternae, Golgi structures, the matrix compartment of secondary granules, and in immature granules (with permission, Dvorak et al., 1989). x 9,500

1985).

5. 2. Murine T lymphocyte-derived conditioned medium (known to contain murine IL-3) stimulates the development of eosinophilic myelocytes but not mature eosinophils. The eosinophilic myelocytes that develop show apoptosis, necrosis and piecemeal degranulation

It was determined, by ultrastructural analysis of the eosinophilic myelocytes derived from these cultures, that several processes were in operation. The morphological criteria of apoptosis, necrosis and mixtures of these injury patterns were evident (Dvorak et al., 1985). Subsequent release of membrane-bound granules, cellular and nuclear debris, and apoptotic bodies resulted in avid phagocytosis by macrophages (Dvorak et al., 1985). Soluble EPO was endocytosed by basophils and was stored in their granules (Dvorak et al., 1985). Morphologically, some eosinophilic myelocytes were not damaged. These cells showed vesicular transport of EPO from specific granule matrix compartments. The surfaces of these actively releasing cells often had released EPO bound to them, but the remaining specific granules and synthetic organelles did not contain residual EPO. Thus, the morphological criteria for PMD were established in eosinophilic myelocytes, which did not complete their maturation to mature cells (Dvorak et al., 1993f).

5. 3. Recombinant human IL-5 (or IL-3) stimulates the development in vitro of human eosinophils

By three weeks in culture with either of these recombinant human interleukins, eosinophilic myelocytes were the predominant cells that developed (Figs. 8, 9) (Saito et al., 1988). These young eosinophils were filled with peroxidase-positive synthetic organelles and immature secretory granules and did not show any morphologic signs of apoptosis or necrosis (Fig. 10). IL-3 also induced the formation of large numbers of viable, peroxidase-negative basophilic leukocytes (Dvorak et al., 1985; Saito et al., 1988). Extending the interval of cultures, which were supplemented with either of these recombinant human interleukins, to five weeks resulted in the maturation of eosinophilic myelocytes to mature

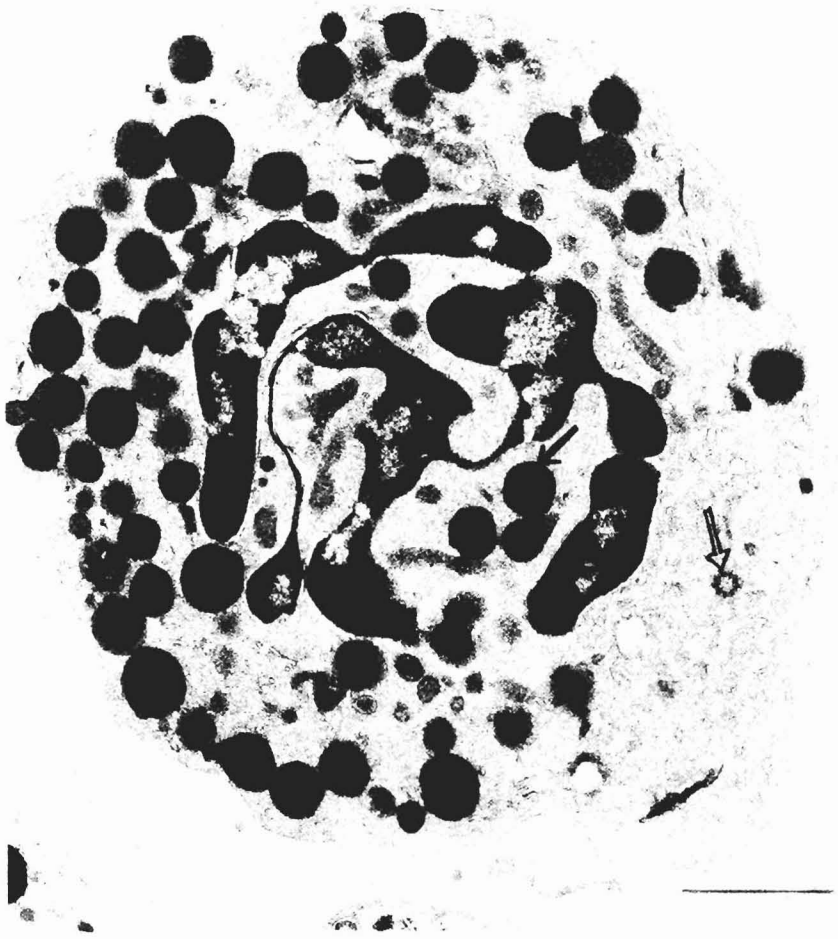


Fig. 11. A mature eosinophil (UCBC suspension culture with rhIL-5 for five weeks) shows a polylobed nucleus with condensed chromatin as well as core-containing secondary granules (closed arrow). The Golgi area surrounds a centriole (open arrow) (with permission, Dvorak et al., 1991a). x 15,000 (Bar = 1 μm).

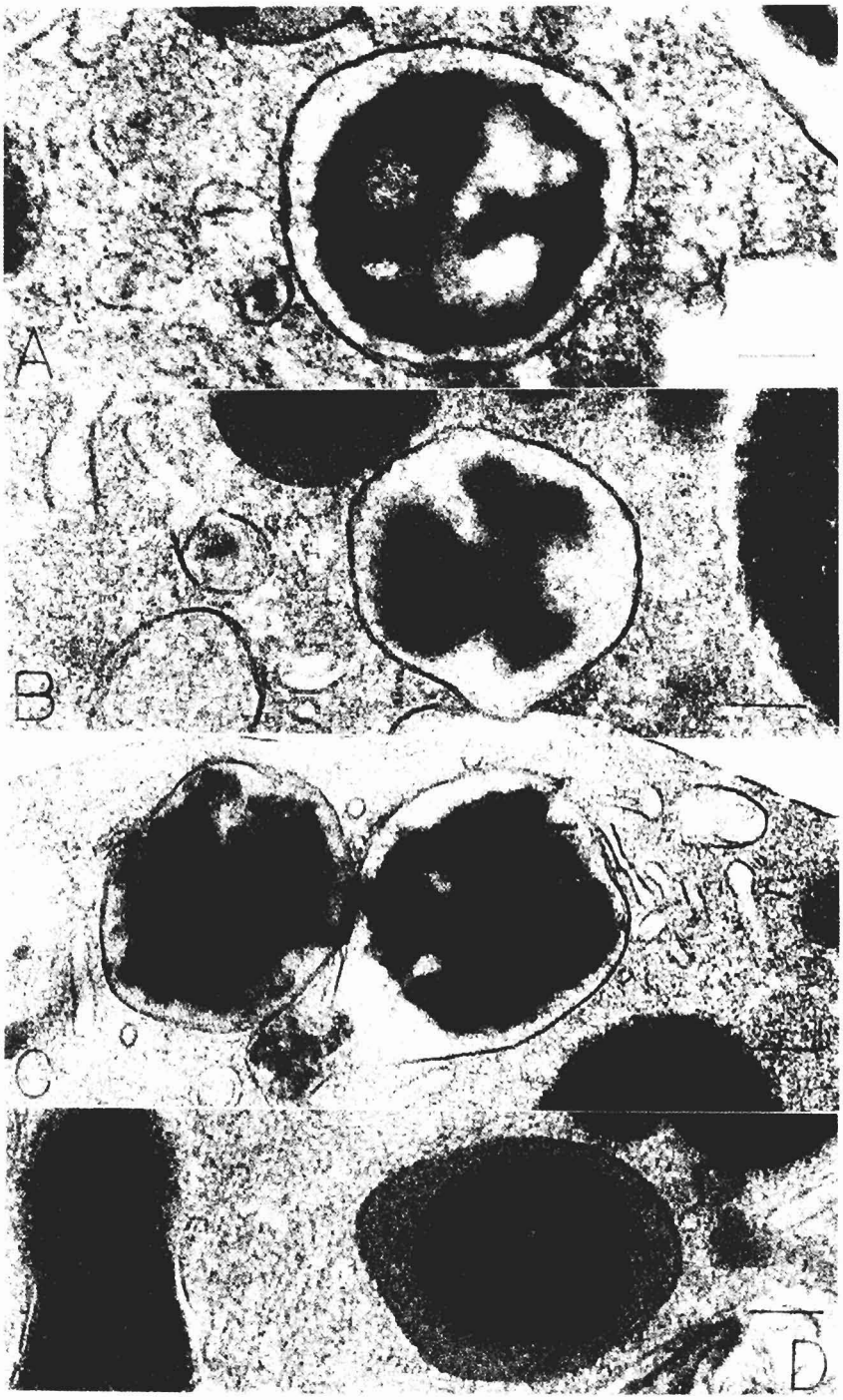


Fig. 12. Portions of the cytoplasm of mature eosinophils (UCBC suspension culture with rhIL-5 for five weeks) show secondary granules at high magnification. Note irregular, thick, dense aggregates of core material **(a-c)** and mature secondary granule with dense core and less dense matrix compartments **(d)** (a, b, d: with permission, Dvorak et al., 1989; c: with permission, Dvorak et al., 1991a). (a) x 75,000; (b) x 81,000; (c) x 79,000; (d) x 51,500 (Bars: a-c = 0.1 μm; d = 0.2 μm).

eosinophils with polylobed nuclei containing condensed chromatin (Fig. 11) (Dvorak et al., 1989). Basophilic myelocytes also underwent maturation (Dvorak et al., 1989).

The maturation of eosinophilic myelocytes was accompanied by size reduction, elimination of synthetic organelles, chromatin condensation, segmentation of nuclei and granulogenesis. Maturation of the crystalloid-containing specific granules was accompanied by the formation of irregular, rope-like material and large irregular blocks of dense core material (Fig. 12). Condensation of these patterns yielded central cores that were often irregularly shaped (Fig. 12). We noted losses from the matrix compartment of these granules, detected by the electron lucency of this compartment. Some mature eosinophils had decreased numbers of specific granules and increased numbers of tubulovesicular structures, lipid bodies, primary and small granules, analogous to the changes seen in tissue eosinophils *in vivo* (Fig. 13).

5. 4. Recombinant human IL-5- and IL-2-depleted, PHA-stimulated human lymphocyte culture supernatant stimulates the development of, and PMD from, mature human eosinophils

PMD of the specific granules in mature eosinophils was evident in these cultures (Figs. 14, 15) (Dvorak et al., 1991b, 1992). This process was associated with prominent collections of peri- and intragranular vesicles; losses were sustained from both matrix and core compartments, giving rise to empty granule containers in the cytoplasm (Fig. 14) (Dvorak et al., 1991b). Other morphological aspects associated with activation, analogous to those seen in tissue eosinophils, were often also present. The vesicular transport of specific granule matrix peroxidase was demonstrated in cytochemical preparations (Fig. 15). Thus, vesicular transport of this secondary granule matrix protein was demonstrated to be an effector mechanism for PMD of mature eosinophils *in vitro* (Dvorak et al., 1992). The cytochemical preparations showed EPO in small vesicles that were attached to specific granules, adjacent to granules, in the peripheral cytoplasm and closely docked and fused to the plasma membrane. Secreted EPO was attached to the cell surface in focal patches. The peroxidase content of the matrix compartment of specific granules was either unaltered or partially or completely absent (Dvorak et al., 1992). In contrast to the transport and secretion of peroxidase-loaded vesicles from the specific granule matrix compartment, Charcot-Leyden crystal protein (generally stored in primary granules in peripheral blood eosinophils *in vivo*) filled the cytoplasm up to, but not beyond, the subplasma

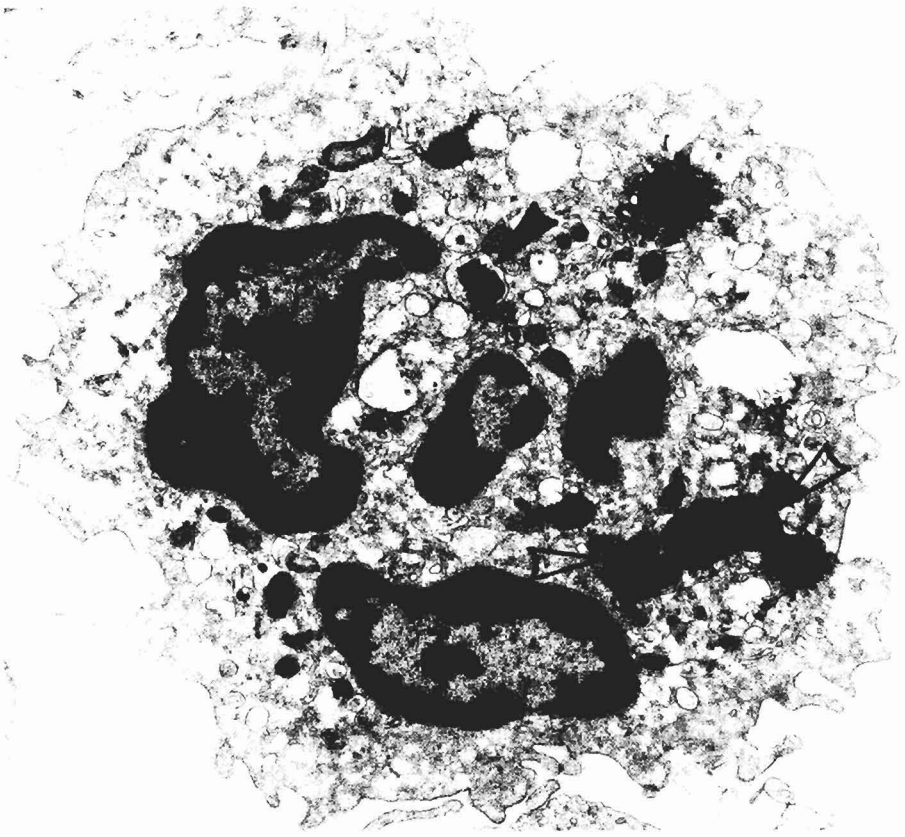


Fig. 13. A mature eosinophil (UCBC suspension culture with rhIL-5 for five weeks) shows the virtual absence of all secondary granules. One core-containing secondary granule with matrix depletion remains (closed arrowhead). Lipid bodies (open arrowheads) and cytoplasmic tubulovesicles are increased. Large, irregular cytoplasmic electron-lucent spaces are glycogen aggregates that are not imaged with the method used here (with permission, Dvorak et al., 1991a). x 13,000

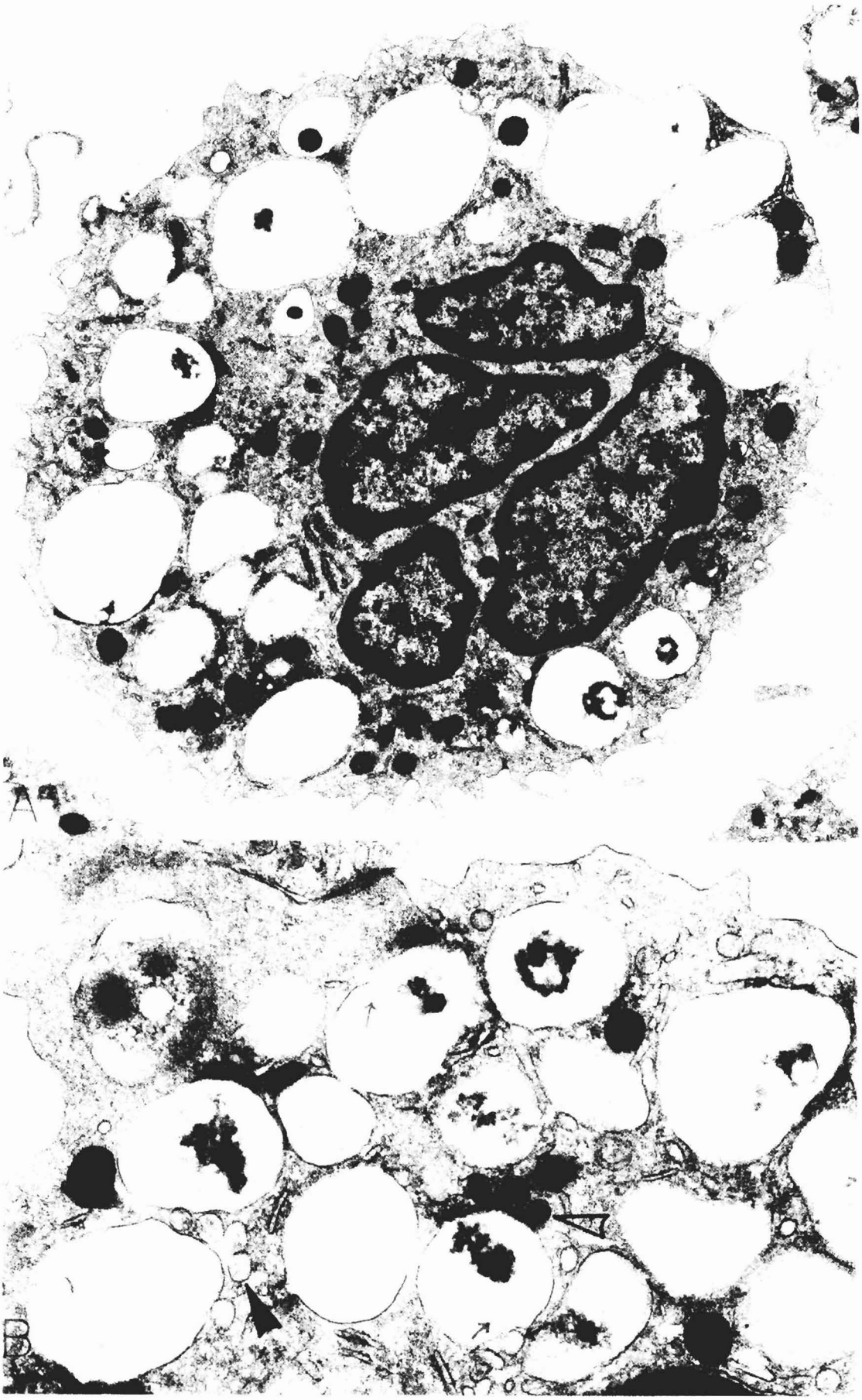


Fig. 14. Mature eosinophils (UCBC suspension cultures with rhIL-5 and IL-2-depleted, PHA-stimulated human lymphocyte culture supernatant for five weeks) show PMD. In **(a)**, note the polylobed, mature nucleus and numerous enlarged, nonfused, empty and partially empty secondary granule chambers. Partially empty granule containers retain some dense granule material and vesicles. The remainder of the cytoplasm contains perigranular vesicles, mitochondria, small granules and a few strands of RER. In **(b)**, at higher magnification the cytoplasm shows clusters of perigranular and intraganular smooth vesicles, some of which are electron-lucent (arrows) or contain lightly dense (closed arrowhead) or very dense contents (open arrowhead). Residual dense granule material is present in some of the granule chambers (with permission, Dvorak et al., 1991b). (a) x 15,500; (b) x 31,500

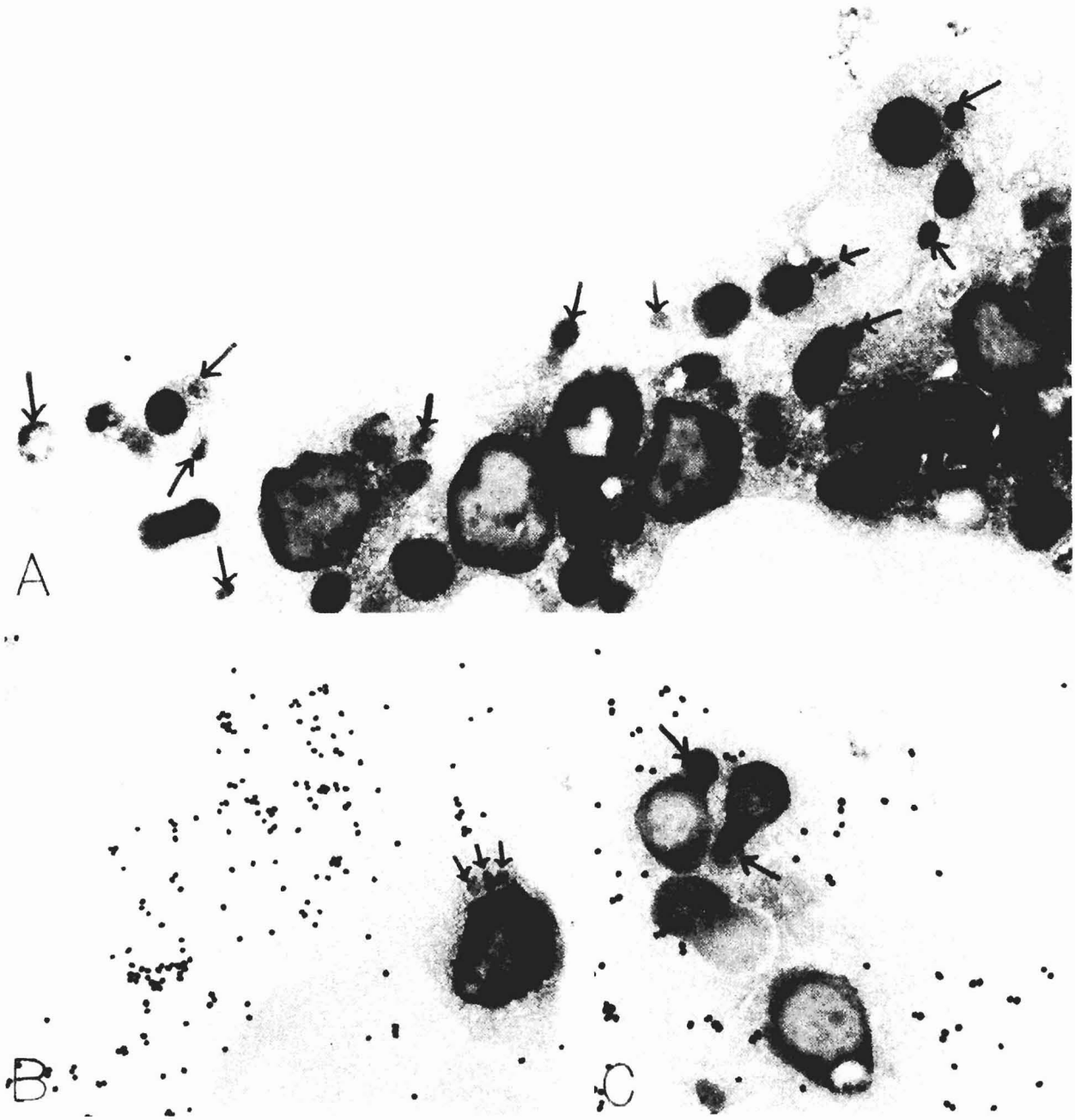
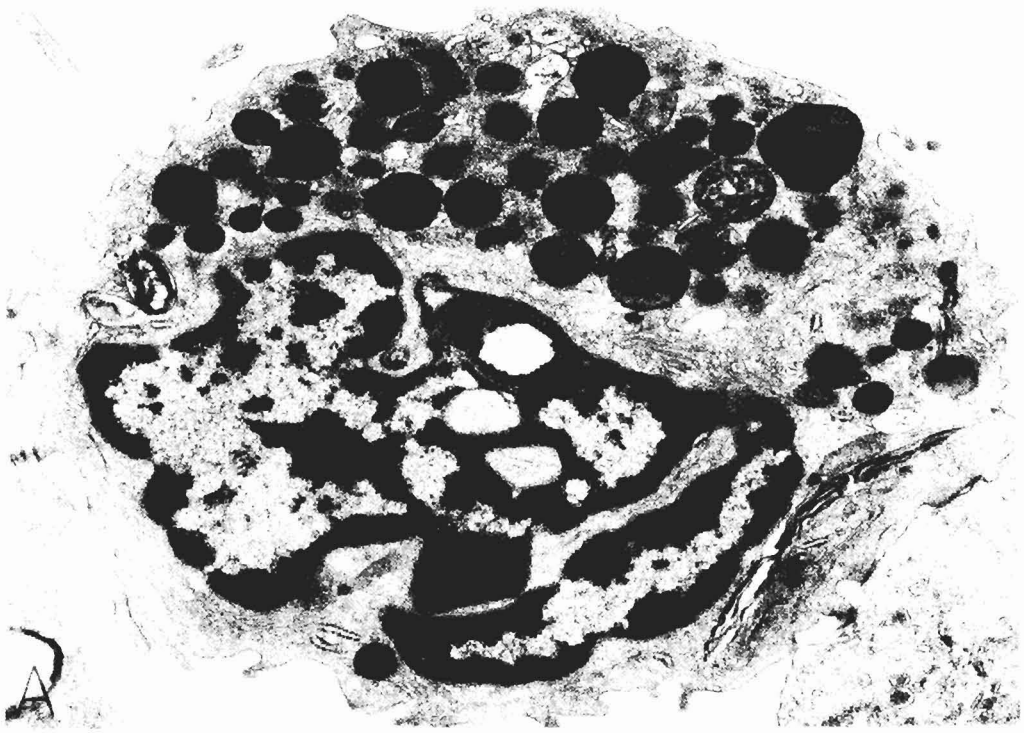


Fig. 15. Mature eosinophils (UCBC suspension cultures with rhIL-5 and IL-2-depleted, PHA-stimulated human lymphocyte culture supernatant for five weeks, prepared with a combined cytochemical technique to demonstrate endogenous peroxidase (a-c) and immunogold method to detect CLC-P (b,c)) show vesicular transport of EPO (a-c) but not CLC-P (b,c) in PMD. In **(a)**, a control non-immune immunoglobulin was substituted for the specific acti-CLC-P primary antibody in the immunogold method. Note a single gold particle in the cytoplasm with none in the nucleus or over the Epon background, indicating exquisite specificity of the cytoplasmic gold-labeled CLC-P in (b,c). Numerous cytoplasmic vesicles contain EPO (arrows), which is of similar density to the EPO in the matrix of secondary granules. Focal areas in several peroxidase-negative secondary granule cores are permeated by EPO (compare morphology to that of Fig. 12a). EPO released from the cells is attached to the cell surface. In **(b,c)**, secondary granules with EPO in their matrix compartments as well as EPO-replete vesicles, either adjacent (b) or attached (c) to EPO-containing granules (arrows), are present. Diffuse gold particles in the cytoplasm represent CLC-P (with permission, Dvorak et al., 1992). (a) x 34,000; (b) x 26,000; (c) x 33,500



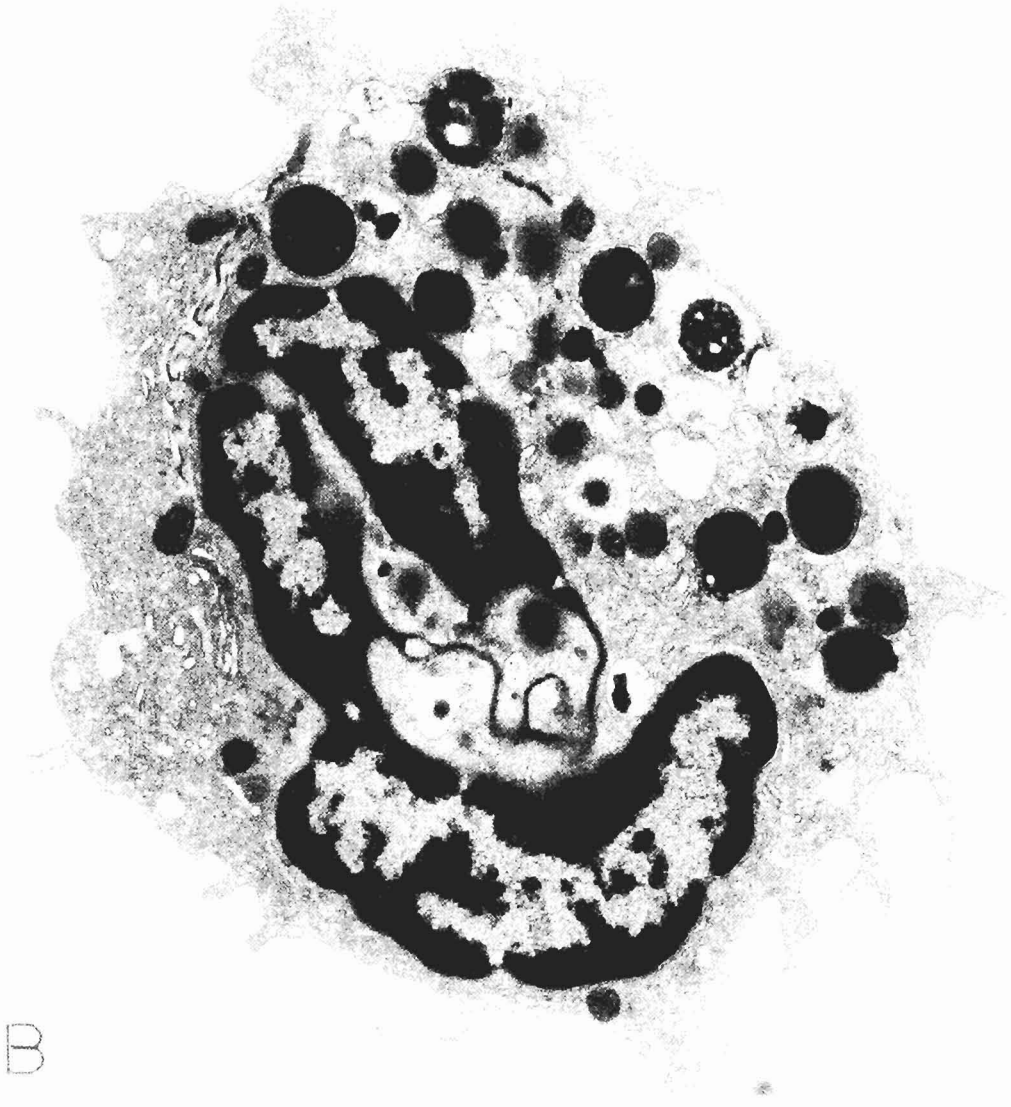


Fig. 16. Mature eosinophils (UCBC suspension culture with rhSCF at six weeks (compare with Fig. 1, small intestine biopsy, and Fig. 11, rhIL-5 containing culture of UCBC)) show polylobed nuclei with condensed chromatin patterns and many secondary granules. Their cell surfaces have irregular, broad processes. The central core compartment of the secondary granules contains irregularly shaped dense core material which is eccentrically located in some granules. In many secondary granules, a well-defined core compartment is not delineated. (a) x 16,500; (b) x 19,000

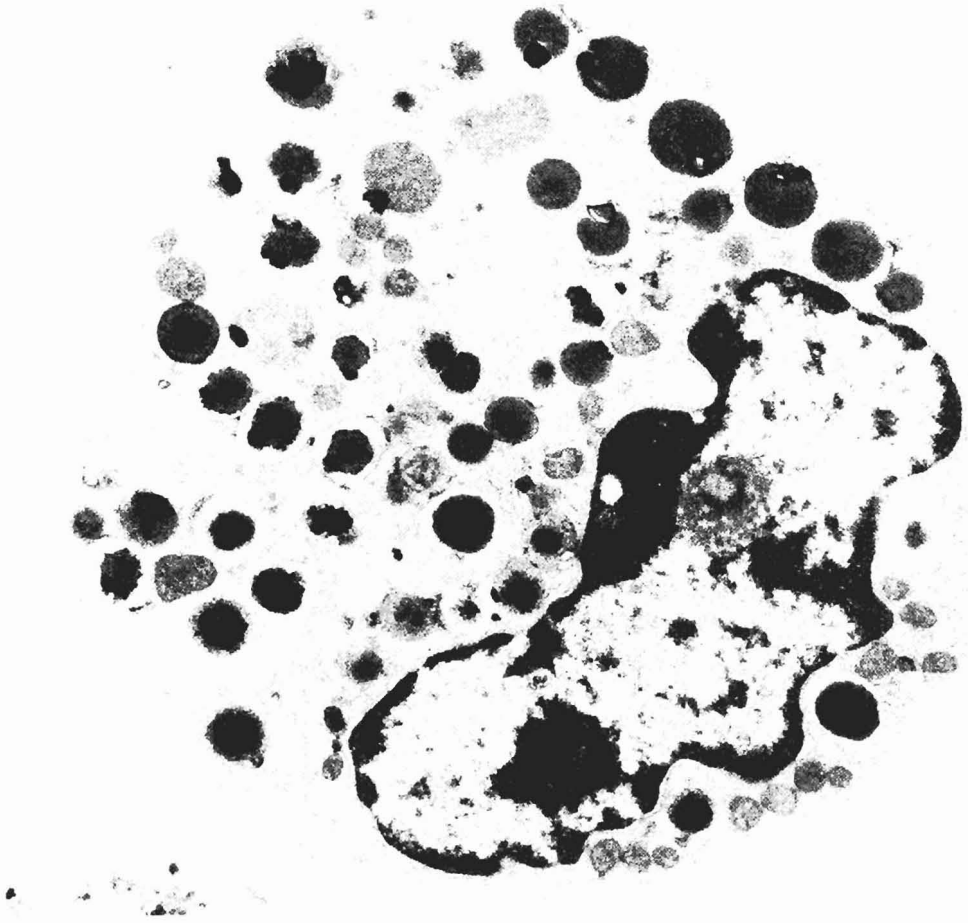


Fig. 17. An eosinophilic myelocyte (UCBC suspension culture with 3T3 fibroblast culture supernatant for three weeks) shows an eccentric, single lobular nucleus with partially dispersed chromatin and a large nucleolus, dilated cisterns of RER, and numerous immature granules. Some of these contain small vesicles and irregular dense membranes. x 16,000

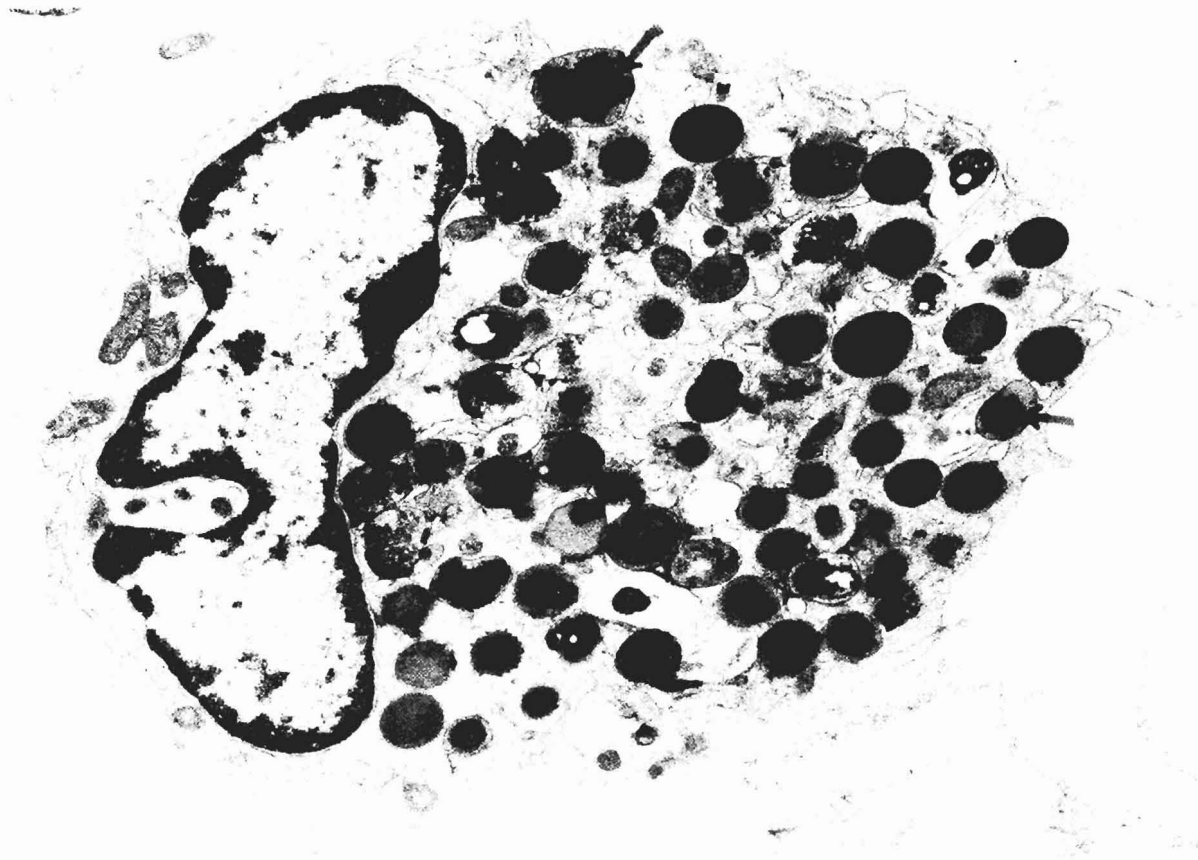


Fig. 18. An eosinophilic myelocyte (UCBC suspension culture with rhSCF for three weeks) displays features similar to those seen in Figs. 3 (bone marrow sample), 8 (rhIL-3-), 9 (rhIL-5-), and 17 (3T3 fibroblast supernatant-containing cultures of UCBC). Note the condensation of dense core material in several immature secondary granules (arrows). x 14,500

membrane in these activated cells and did not appear to be extensively released from cells, as determined by immunogold ultrastructural preparations (Fig. 15b,c) (Dvorak et al., 1992).

5. 5. c-kit ligand-containing additives to suspension cultures of umbilical cord blood cells are associated with the development, maturation, activation and secretion of eosinophils

c-kit ligand, a recently described human mast cell growth factor (reviewed in Mitsui et al., 1993) (and also known as stem cell factor (SCF), kit ligand (KL), steel factor (SF), mast cell growth factor (MCGF)) is a product of fibroblasts. We have examined a large number of suspension cultures of human cord blood cells that were supplemented with c-kit ligand from a variety of sources. These include fibroblast culture supernatants (or a partially purified fraction thereof) and recombinant human stem cell factor (rhSCF) or mouse mast cell growth factor (rmMCGF) (Mitsui et al., 1993; Dvorak et al., 1993b,c,e, 1994a,b). In all of these cultures, morphologically identifiable eosinophils with the following morphologies were found.

a. Mature eosinophils with a complete complement of secretory granules (Fig. 16)

Mature, eosinophils, developing *in vitro* and having a full complement of secretory granules, are readily recognized by their polylobed nuclei with condensed chromatin, irregular, blunt surface processes and secretory granules. The large, membrane-bound secretory granules differ somewhat from the same secondary granule population in peripheral blood eosinophils. The central crystalline core compartment, for example, is irregularly shaped and located within these bicompartmental granules; in many granules, a well-defined core compartment is not evident.

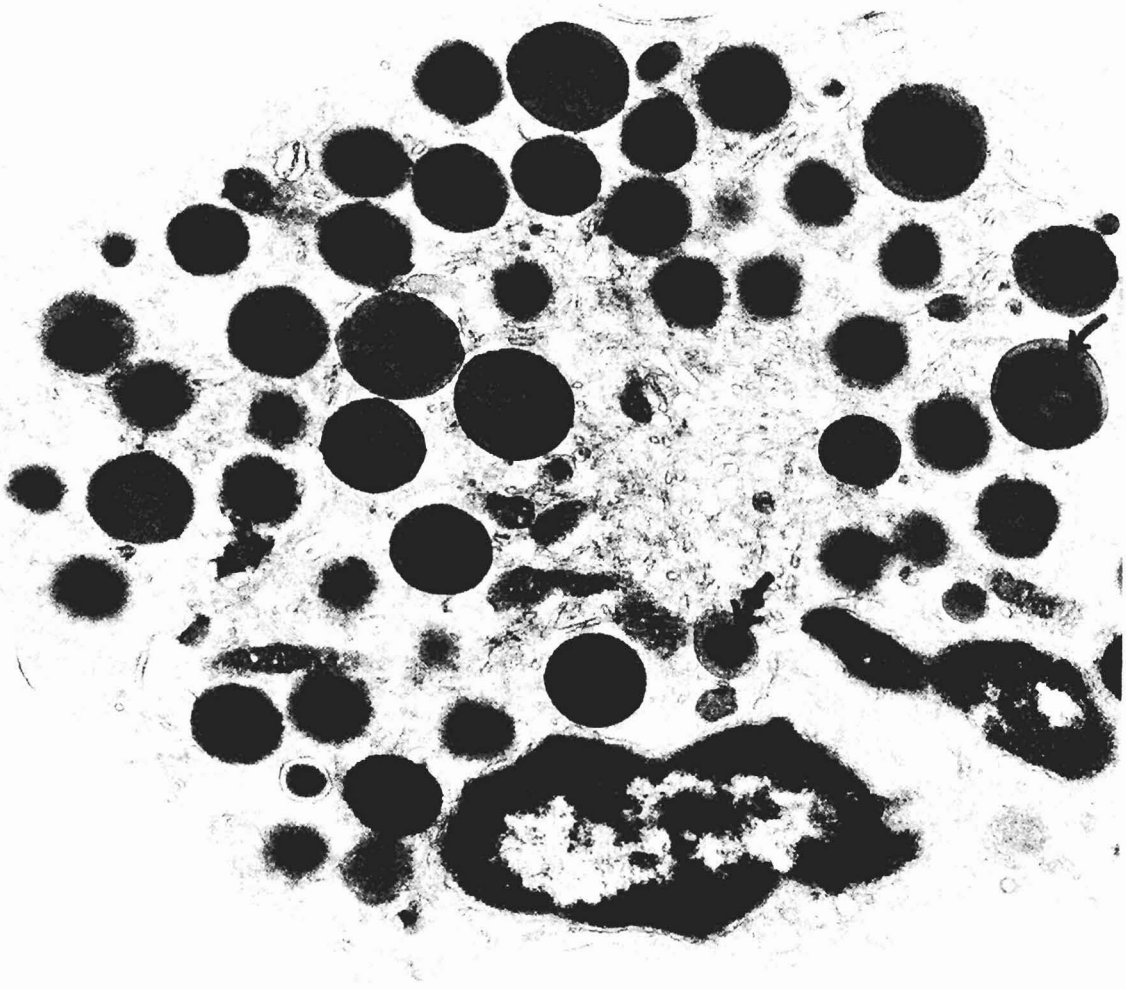


Fig. 19. A portion of the cytoplasm of a late eosinophilic myelocyte (UCBC suspension culture with rhSCF, three weeks) shows separation of nuclear lobes, one lipid body, and numerous large, round, immature secondary granules. Note that central cores are poorly visible in most of these granules. Several, however, show central, small and very large core compartments (arrows). x 13,500

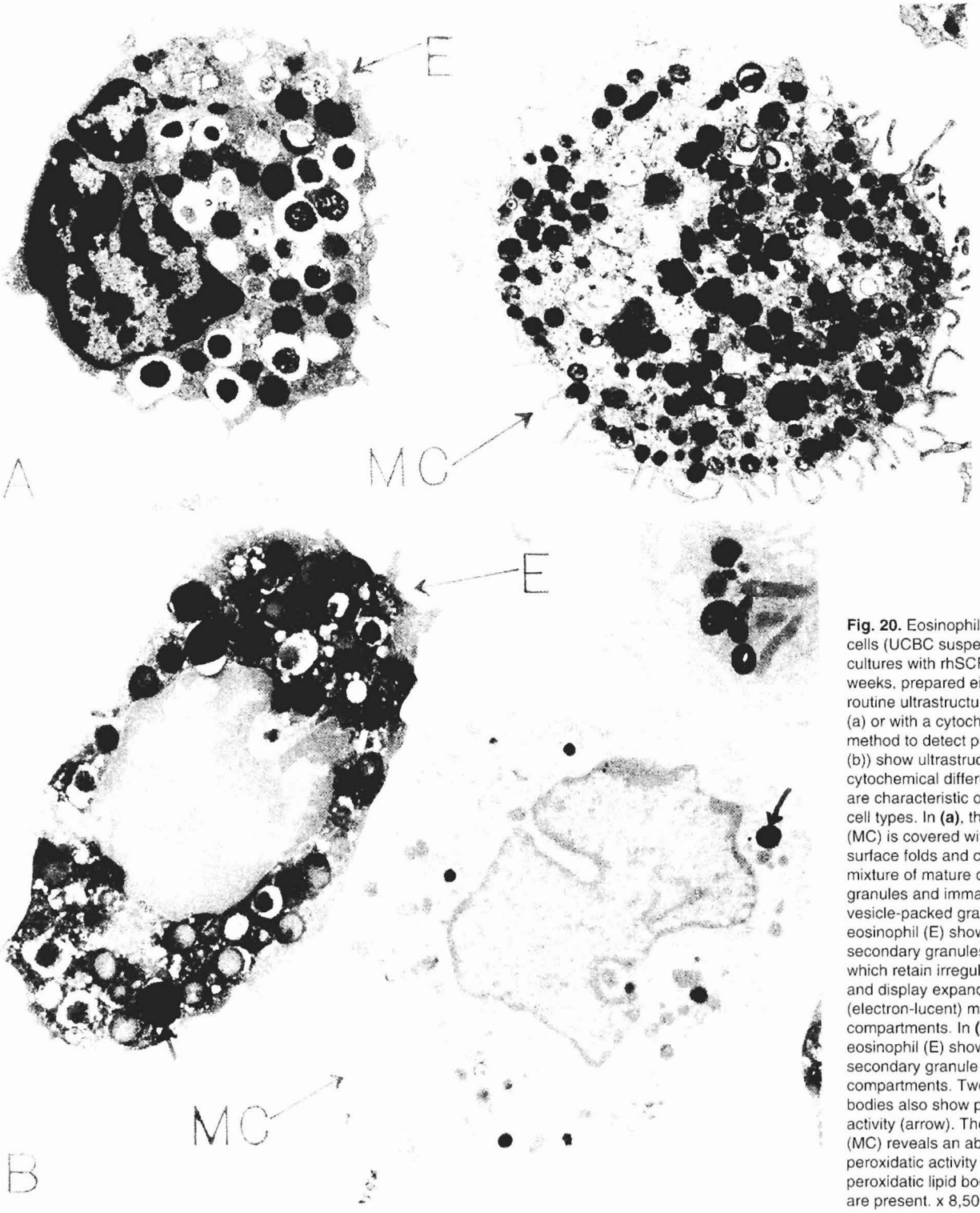


Fig. 20. Eosinophils and mast cells (UCBC suspension cultures with rhSCF, six weeks, prepared either with a routine ultrastructural method (a) or with a cytochemical method to detect peroxidase (b)) show ultrastructural and cytochemical differences which are characteristic of these two cell types. In **(a)**, the mast cell (MC) is covered with narrow surface folds and contains a mixture of mature dense granules and immature, vesicle-packed granules. The eosinophil (E) shows secondary granules, many of which retain irregular cores and display expanded, empty (electron-lucent) matrix compartments. In **(b)**, the eosinophil (E) shows EPO in secondary granule matrix compartments. Two lipid bodies also show peroxidatic activity (arrow). The mast cell (MC) reveals an absence of peroxidatic activity in granules; peroxidatic lipid bodies (arrow) are present. x 8,500

b. Eosinophilic myelocytes (Figs. 17-19).

Eosinophilic myelocytes are also present in *in vitro* samples. These immature eosinophils (Figs. 17-19) are approximately three-times larger than mature eosinophils. Their nuclei are not polylobed; rather, large, lobular, monolobed nuclei with dispersed chromatin and large nucleoli characterize eosinophilic myelocytes. The surface architecture consists of irregular, broad processes. Eosinophilic myelocytes have large amounts of rough endoplasmic reticulum with distended cisternae, as well as expanded Golgi structures. Their cytoplasm is filled with large, dense immature granules, some of which display centrally condensing crystalline cores. Some immature granules contain less dense materials, often surrounded by small intragranular vesicles. Intragranular, dense membranous structures can also be found. Recognizing these subcellular features will prevent confusion with immature human mast cells, which are regularly present in the same culture samples (Fig. 20). The use of naturally occurring c-kit ligand, which is present

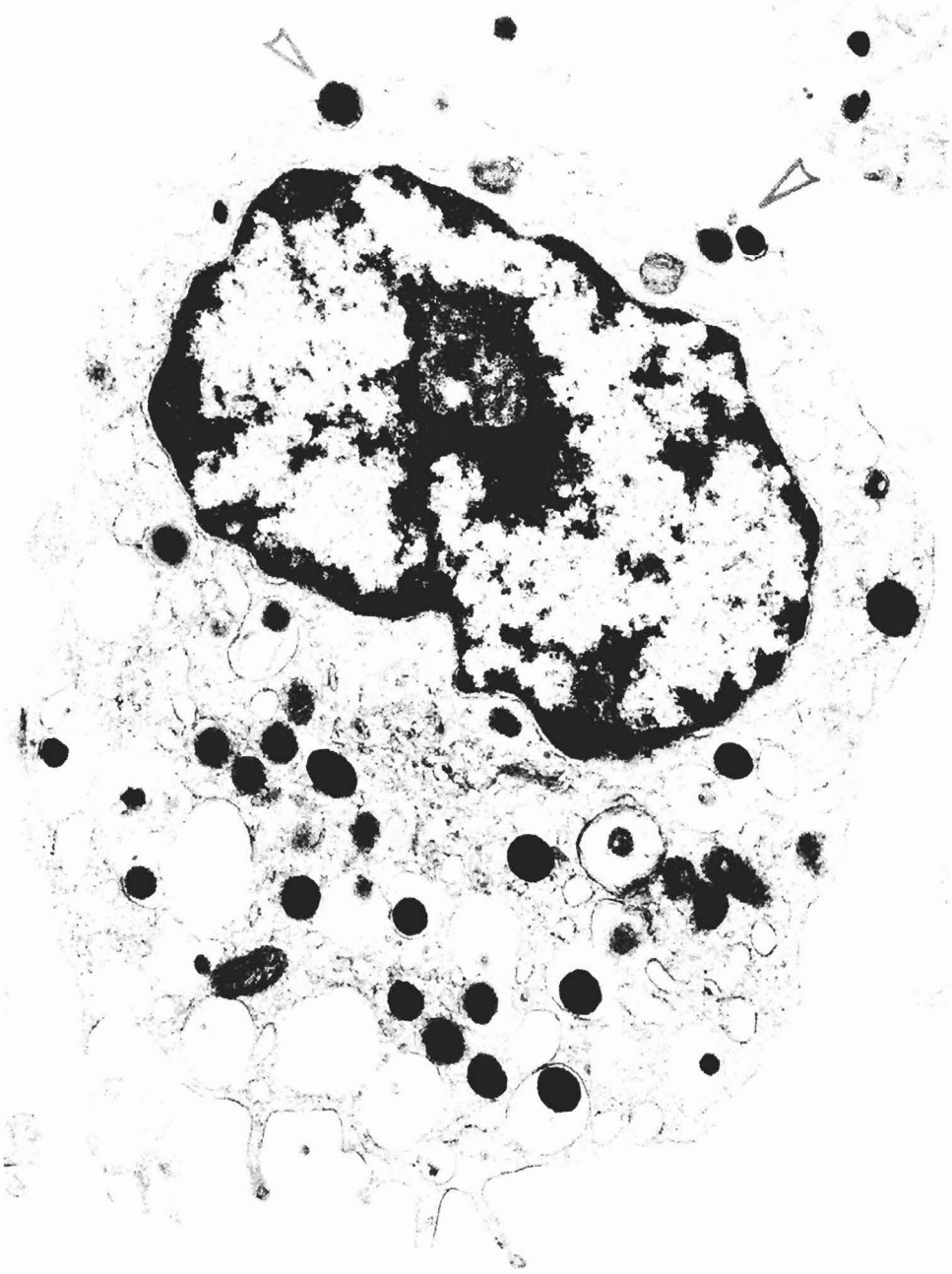


Fig. 21. An eosinophilic myelocyte (UCBC suspension culture with rhSCF, three weeks) shows extrusion of nonmembrane-bound, dense primary granules (arrowheads) through the plasma membrane. x 17,500

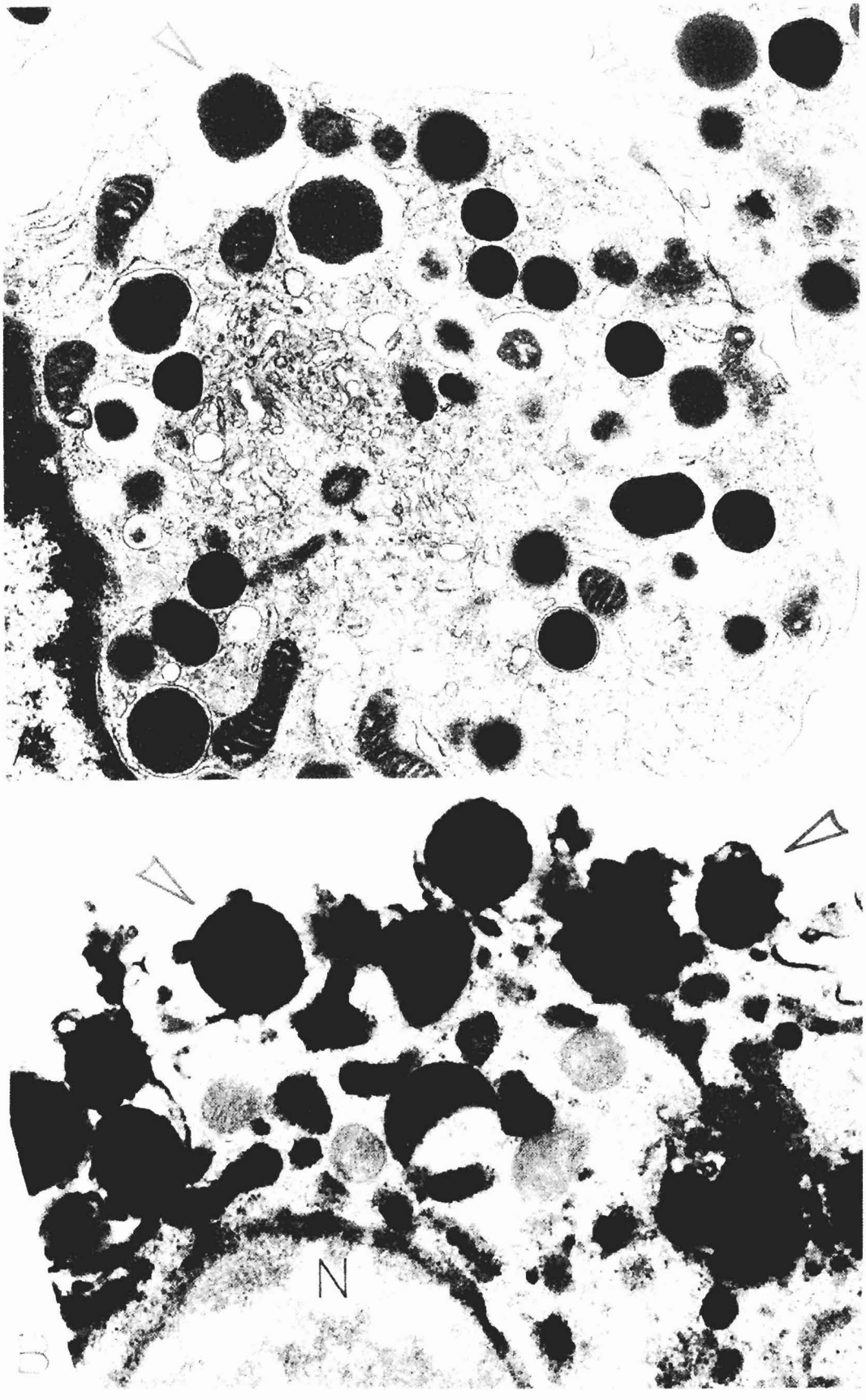
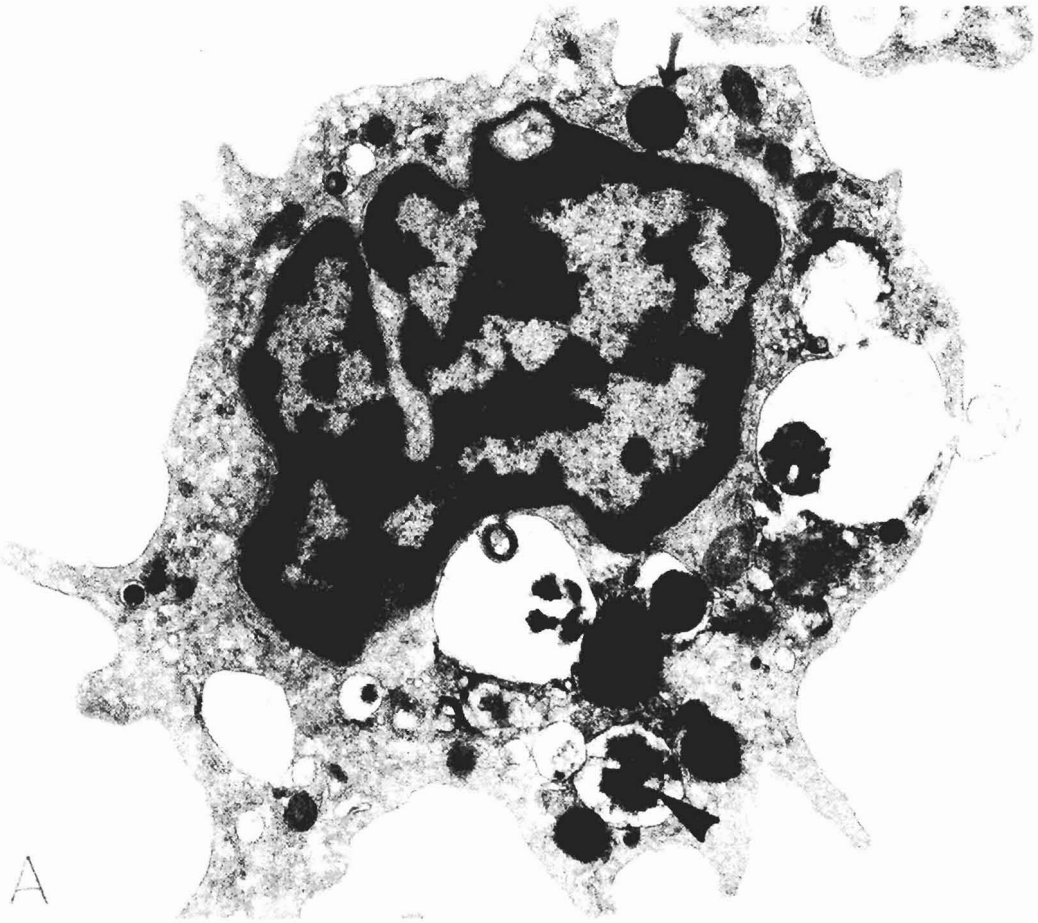


Fig. 22. Eosinophilic myelocytes (UCBC suspension cultures with fibroblast culture supernatant (a) or rhSCF (b), three weeks, prepared either by a routine ultrastructural (a) or by a cytochemical method to demonstrate endogenous peroxidase (b)) show secretion of primary granules by extrusion (arrowheads). In **(a)**, a single granule is being released through the plasma membrane; in **(b)**, numerous extruded, peroxidase-containing granules remain adjacent to the cell surface. The perinuclear cistern and RER cisternae contain EPO in (b) (N = nucleus). (a) x 25,000; (b) x 35,000



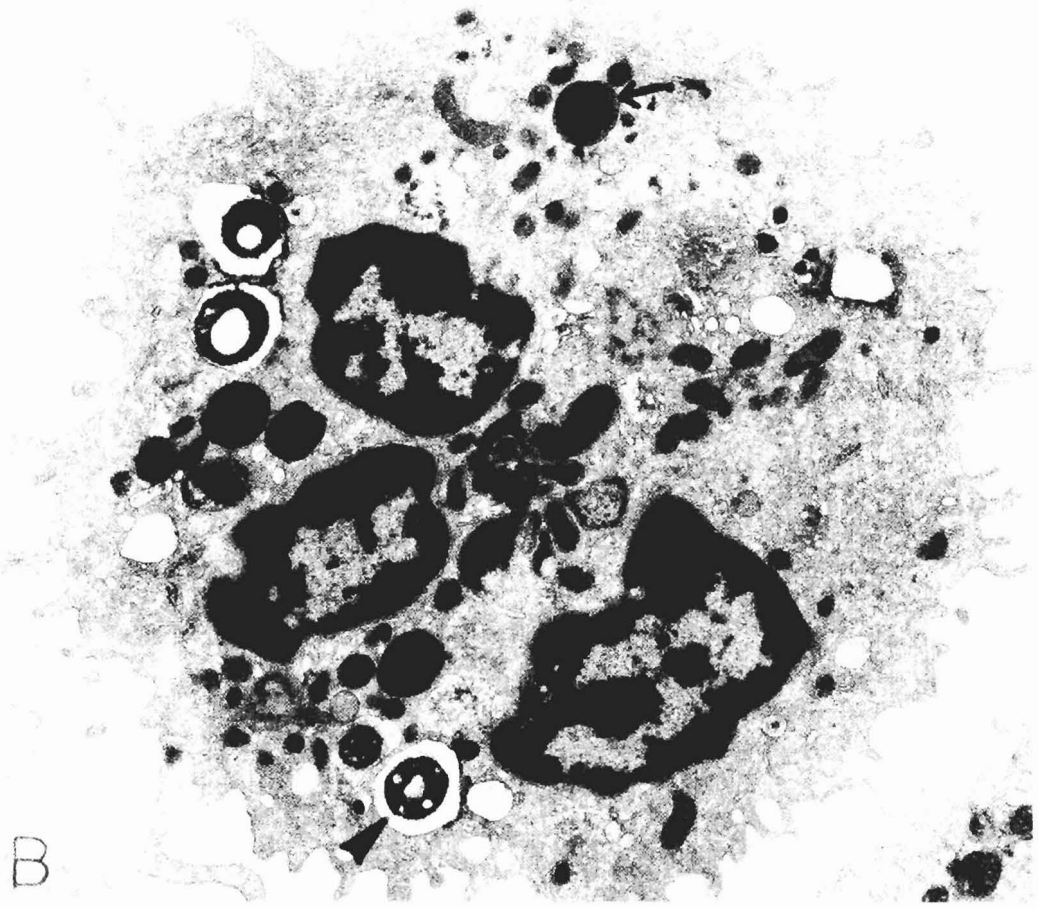


Fig. 23. Mature eosinophils (UCBC suspension cultures with rhSCF, six weeks) show PMD, characterized by expanded, empty, electron-lucent matrix compartments of secondary granules. Several unaltered, bicompartmental secondary granules remain (arrows). Residual, irregular dense core material is present in some granules (arrowheads). (a) x 16,000; (b) x 13,000

in fibroblast supernatants, or the use of rhSCF, as a human cord blood cell culture additive, resulted in the development of eosinophilic myelocytes and their mature progeny, which did not differ morphologically.

c. Members of the eosinophil lineage differ from immature mast cells.

Eosinophils can be distinguished from immature mast cells, which regularly develop from suspension cultures of human cord blood cells supplemented with the c-kit ligand (Fig. 20). In routine electron microscopic preparations, the surface architecture and the immature granules of developing mast cells clearly differ from those in eosinophils (Fig. 20). Mast cells, for example, are characterized by narrow surface folds and large numbers of variably dense immature granules. Ultrastructural cytochemical preparations to detect endogenous peroxidase show peroxidase only in immature mast cell; eosinophil granules, lipid bodies and synthetic organelles stain positively with this technique (Fig. 20).

d. Morphological evidence of regulated secretion from eosinophilic myelocytes which develop in vitro (Figs. 21, 22).

Regulated secretion from secretory cells is defined morphologically by the extrusion of membrane-free granules from granulated secretory cells. We previously described this process *in vivo* in bone marrow eosinophilic myelocytes (Dvorak et al., 1991a) and in mature tissue eosinophils (Dvorak et al., 1993d). Eosinophilic myelocytes developing *in vitro* also have the capacity for regulated secretion. For example, and as in bone marrow *in vivo*, eosinophilic myelocytes extrude immature primary granules, which are moderately dense, finely granular, nonmembrane-bound structures devoid of crystalline cores, through pores in the plasma membrane (Figs. 21, 22). This secretory mechanism of immature primary granules may account for the small numbers (~5%) of retained primary granules that are seen in mature circulating eosinophils (Dvorak et al., 1988). The primary granule is the only eosinophil granule storage site of Charcot-Leyden crystal protein, as determined by immunogold ultrastructural studies (Dvorak et al., 1988).

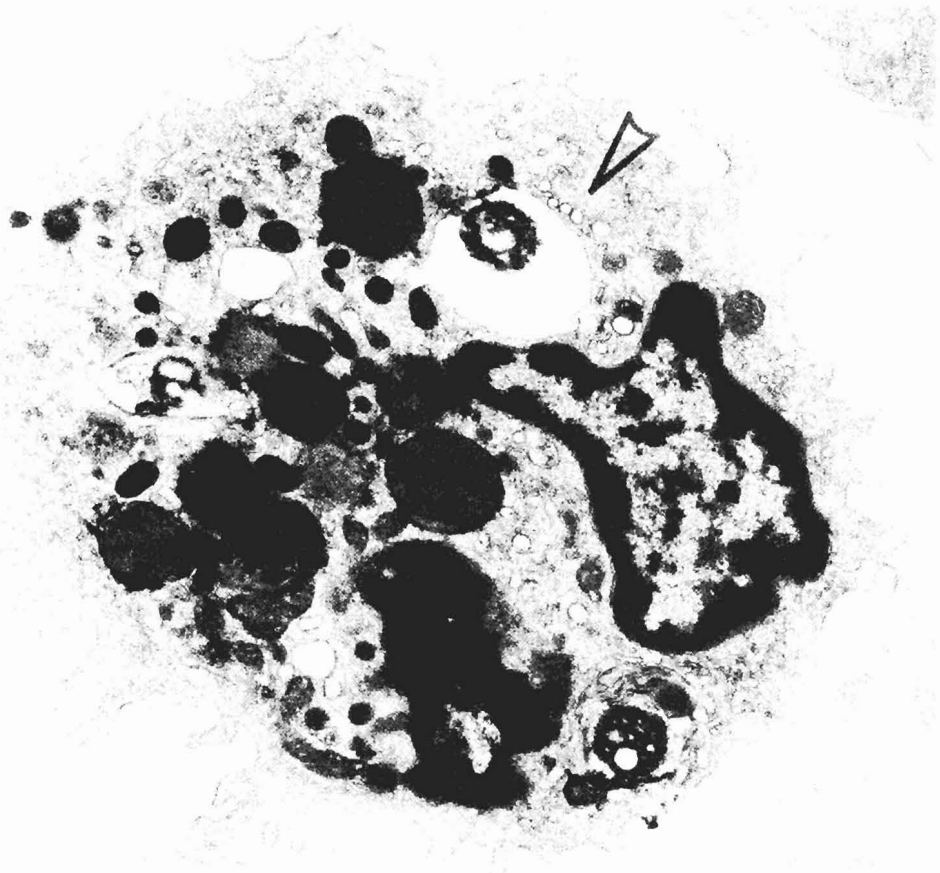


Fig. 24. A mature eosinophil (UCBC suspension culture with rhSCF, six weeks) undergoing PMD shows clusters of electron-lucent vesicles (arrowhead) adjacent to an enlarged secondary granule which has residual core material and an empty matrix compartment containing several vesicles. x 17,500

e. PMD of eosinophils in vitro (Figs. 23-26).

PMD is a term we chose to define a secretory process in granulated secretory cells that differs morphologically from the regulated extrusion of entire granules from secretory cells (Dvorak, 1991a, 1992). In PMD, for example, granule contents are released in the absence of granule-to-granule or granule-to-plasma membrane fusions, with subsequent retention of granule containers in the cytoplasm. Furthermore, we used a cytochemical method to demonstrate that EPO-loaded vesicles effected PMD in mature and immature eosinophils *in vitro* (Dvorak et al., 1992, 1993f). Mature eosinophils, developing in c-kit ligand-supplemented cultures of human cord blood cells, fulfil the ultrastructural morphological criteria for PMD (Figs. 23-26). Thus, variable numbers of secondary granules with empty, full, and partially empty granule containers remain in the cytoplasm. Some secondary granules with dense inner cores and less dense matrix compartments are unchanged (Fig. 23a), whereas other secondary granules are enlarged and partially or completely empty (Fig.

Fig. 25. A mature eosinophil (UCBC suspension culture with rhSCF, six weeks) is undergoing extensive PMD of ~50% of the secondary granules. Note the clustered, electron-lucent smooth vesicles near releasing granules (arrowheads). Full, dense vesicles of similar size are also present near and attached to (arrows) secondary granules. x 14,000

Fig. 26. Mature eosinophils (UCBC suspension cultures with rhSCF for three weeks **(a)** or six weeks **(b)**), undergoing extensive PMD, show release from virtually all secondary granules. Their expanded containers remain in the cytoplasm and are surrounded by smooth vesicles. Irregularly shaped dense core material (arrow) remains in some of the enlarged, electron-lucent granule chambers. (a) x 12,000; (b) x 15,000

Fig. 27. An eosinophil (UCBC suspension culture with rhSCF, six weeks) shows apoptosis, characterized by a pyknotic nucleus filled with a crescent of dense chromatin. Note the absence of chromatin leakage and the absence of damaged cellular membranes, in contrast to what is seen in necrosis *in vivo* (compare with Fig. 7). x 13,000

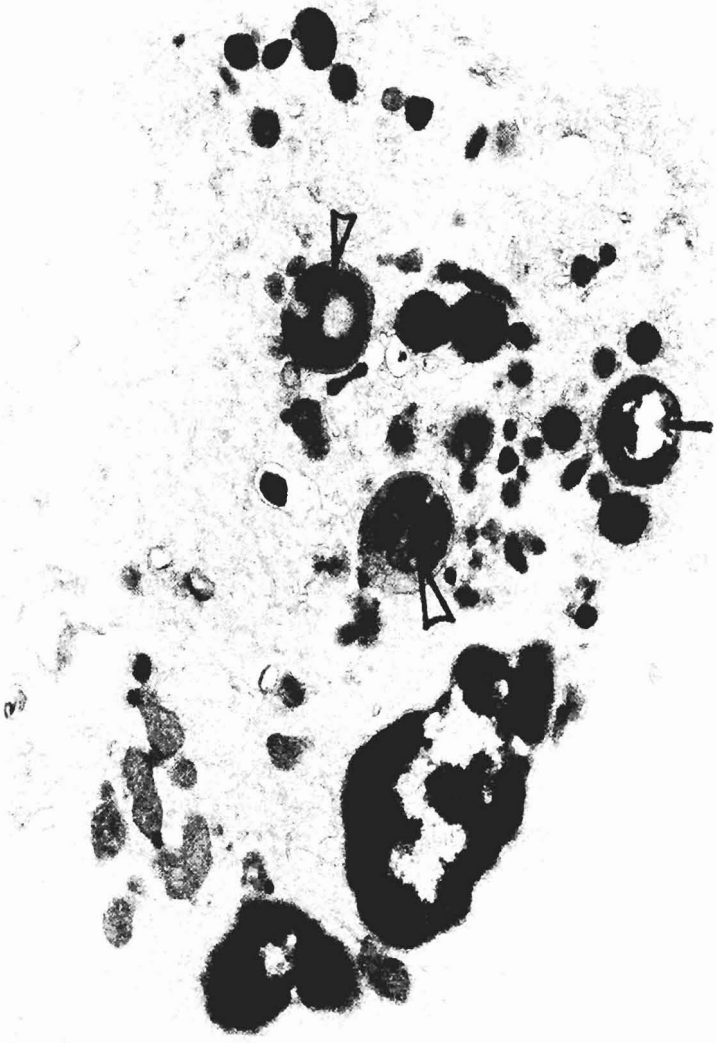


Fig. 28. A mature eosinophil (UCBC suspension culture with rhSCF, six weeks) shows increased, abnormally shaped aggregates of core material in two remaining secondary granules (arrowheads) and focal release of the dense core from another (arrow). Increased numbers of small granules and tubulovesicular structures are present. x 17,000

23b). Retention of core material, despite completely empty matrix compartments, was regularly observed (Fig. 23b): focal losses from core compartments of secondary granules with retention of matrix contents, as regularly occurs in tissue eosinophils from some diseased tissues *in vivo*, were not regular features of PMD that we observed *in vitro*. Clusters of electron-lucent and electron-dense vesicles adjacent to secondary granules were regular features of PMD *in vitro* (Figs. 24-26). Some of these vesicles were fused to granules. Some eosinophils undergoing extensive PMD could be definitively recognized by the retention of irregularly shaped core material within electron-lucent secondary granules (Fig. 26b).

f. Apoptosis

Apoptosis (programmed cell death) was evident in two cell lineages developing in c-kit ligand-supplemented cultures of human umbilical cord cells. Thus, in addition to neutrophils (Dvorak et al., 1993e), some eosinophils morphologically expressed apoptosis (Fig. 27). These cells were characterized by extensive condensation of nuclear chromatin. Chromatolysis of nuclei and lysis of membranes - features of necrosis - were not prominent in these cultures. Other lineages simultaneously present (viz., mast cells, basophils, macrophages, endothelial cells) did not show apoptosis or necrosis.

g. Qualitative morphologic changes in the secondary granules of mature eosinophils in vitro (Figs. 28-31).

Some secondary granules in mature eosinophils revealed abnormally increased amounts of dense core material (Fig. 28). This material was arranged as large, irregular blocks and intertwined threads, as we noted in rhIL-3- or IL-5- supplemented cultures (Dvorak et al., 1989). Rarely, we also found focal core losses in these granules (Figs. 28, 29). At times, the matrix

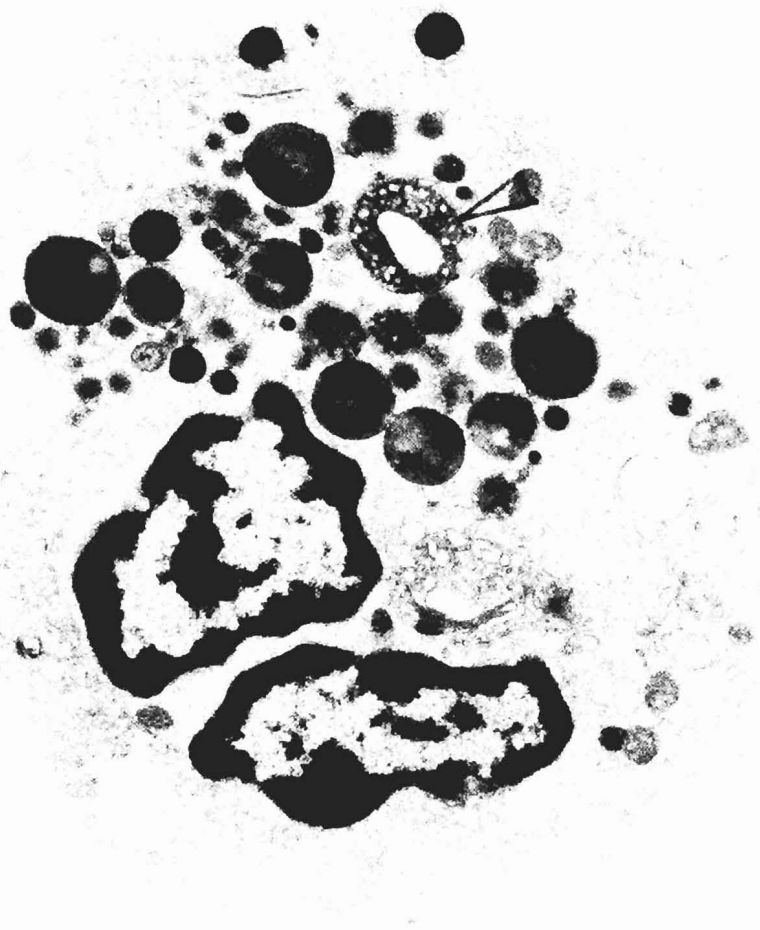


Fig. 29. A mature eosinophil (UCBC suspension culture with rhSCF, six weeks) shows diminished numbers of secondary granules and increased small granules. One secondary granule (arrowhead) has released its dense core, and the matrix compartment contains dense contents and numerous vesicles. x 16,500

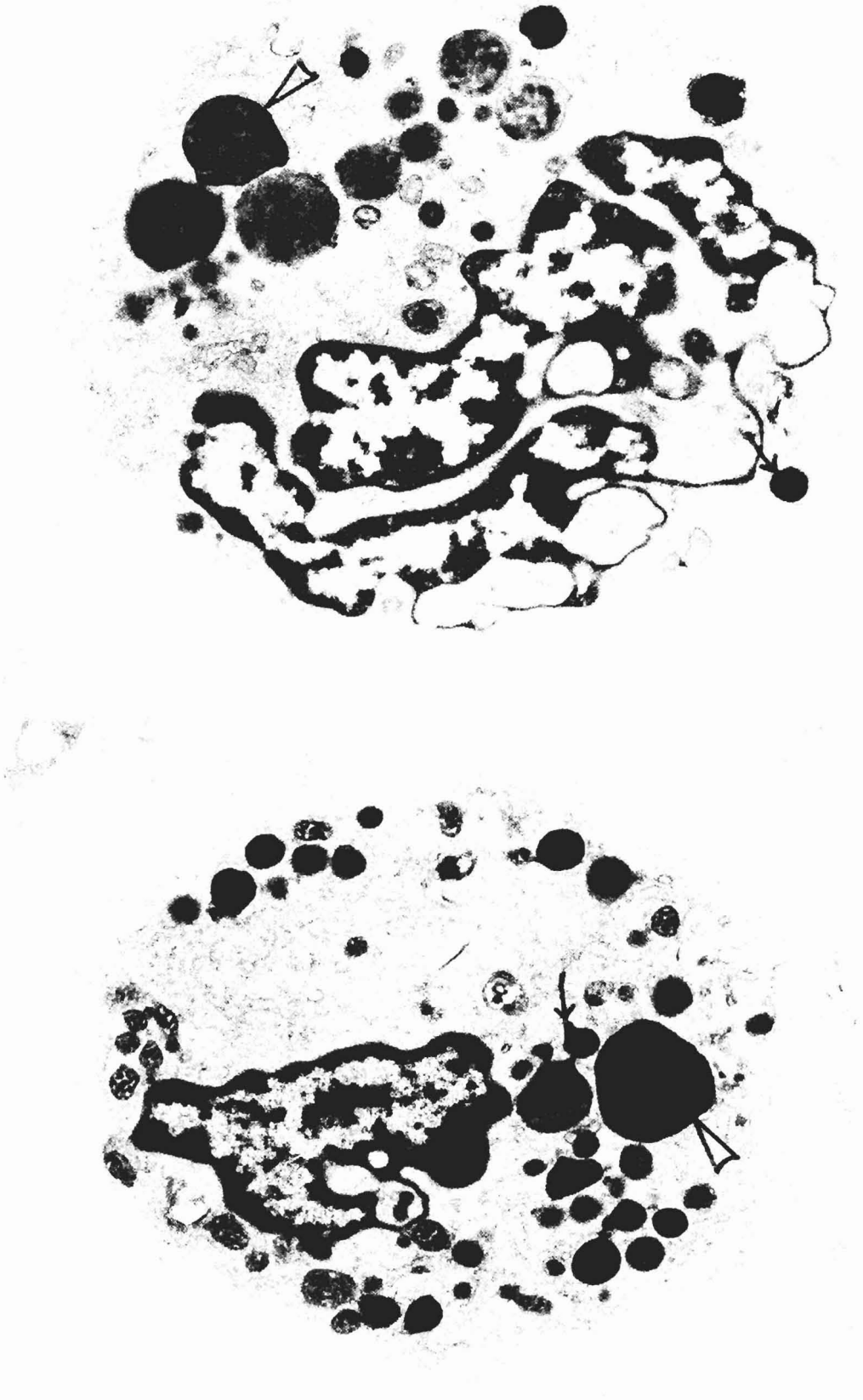


Fig. 30. Mature eosinophils (UCBC suspension cultures with rhSCF, six weeks) show markedly enlarged secondary granules (arrowheads). In **(a)**, all secondary granules are enlarged, and many have irregular collections of dense core material. Several osmiophilic lipid bodies (arrow), surrounded by tubules of smooth endoplasmic reticulum (SER), are present. In **(b)** secondary granules have electron-dense protrusions, suggesting electron-dense vesicle attachments (arrow). (a) x 20,500; (b) x 14,000

compartment of secondary granules was filled with electron-lucent vesicles within the dense components of this compartment (Fig. 29). Some mature eosinophils contained only enlarged secondary granules, some of which displayed irregular, dense aggregates of core material (Fig. 30); other mature eosinophils had either one or several enlarged secondary granules in addition to granules of normal size (Figs. 30, 31). Often, the enlarged secondary granules were round, completely electron-dense, and surrounded by numerous small, dense, membrane-bound structures (Fig. 31a). Appropriate cytochemical preparations for the demonstration of EPO revealed endogenous enzyme in both the large and the normal-sized secondary granules (Fig. 31b).

h. Quantitative changes in the secondary granules of mature eosinophils in vitro (Figs. 32, 33).

Mature eosinophils with decreased numbers of secondary granules were noted *in vitro* (Figs. 32, 33). The preservation of dense core material in one or more of the remaining secondary granules is their identifying hallmark (Fig. 32b). Some mature eosinophils that were completely devoid of secondary granules were also present (Fig. 33). These cells were recognizable as granulocytes by their polylobed nuclei with condensed chromatin and by their irregular surface processes. Secretory granules, characteristic of basophils and neutrophils, were absent; the behaviour of these two additional grnaulocyte lineages differed dramatically in similar cultures (Dvorak et al., 1993c,e). Thus, basophils generally displayed signs of PMD (Dvorak et al., 1993c), and neutrophils did not mature beyond the distinctive myelocyte stage of development (Dvorak et al., 1993e). Some additional details, which are helpful in recognizing paucigranular, polynuclear cells as eosinophils, include the presence of large amounts of cytoplasmic smooth endoplasmic reticulum and

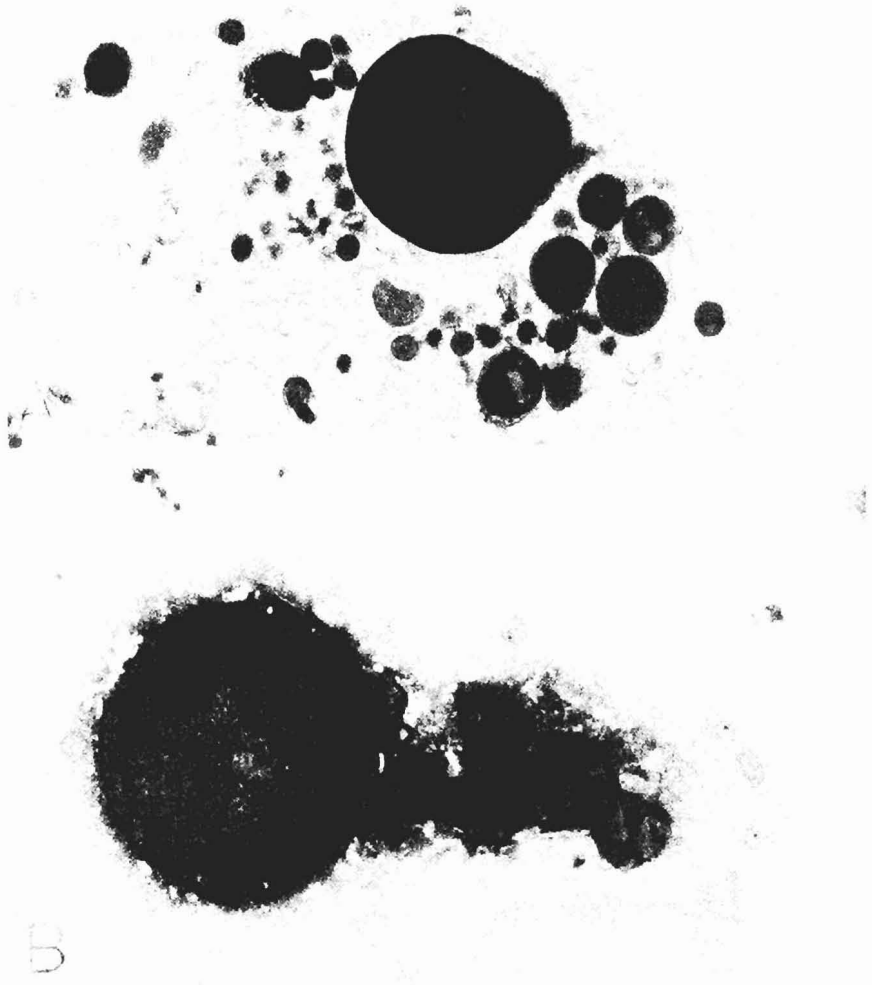


Fig. 31. Portions of the cytoplasm of mature eosinophils (UCBC suspension cultures with rhSCF, six weeks, prepared with either a routine ultrastructural method (a) or a cytochemical one to detect endogenous peroxidase (b)) show giant granules. In **(a)**, the giant granule is surrounded by small, dense granules and vesicles; in **(b)**, the giant granule and smaller secondary granules contain EPO. (a) x 14,500; (b) x 13,000

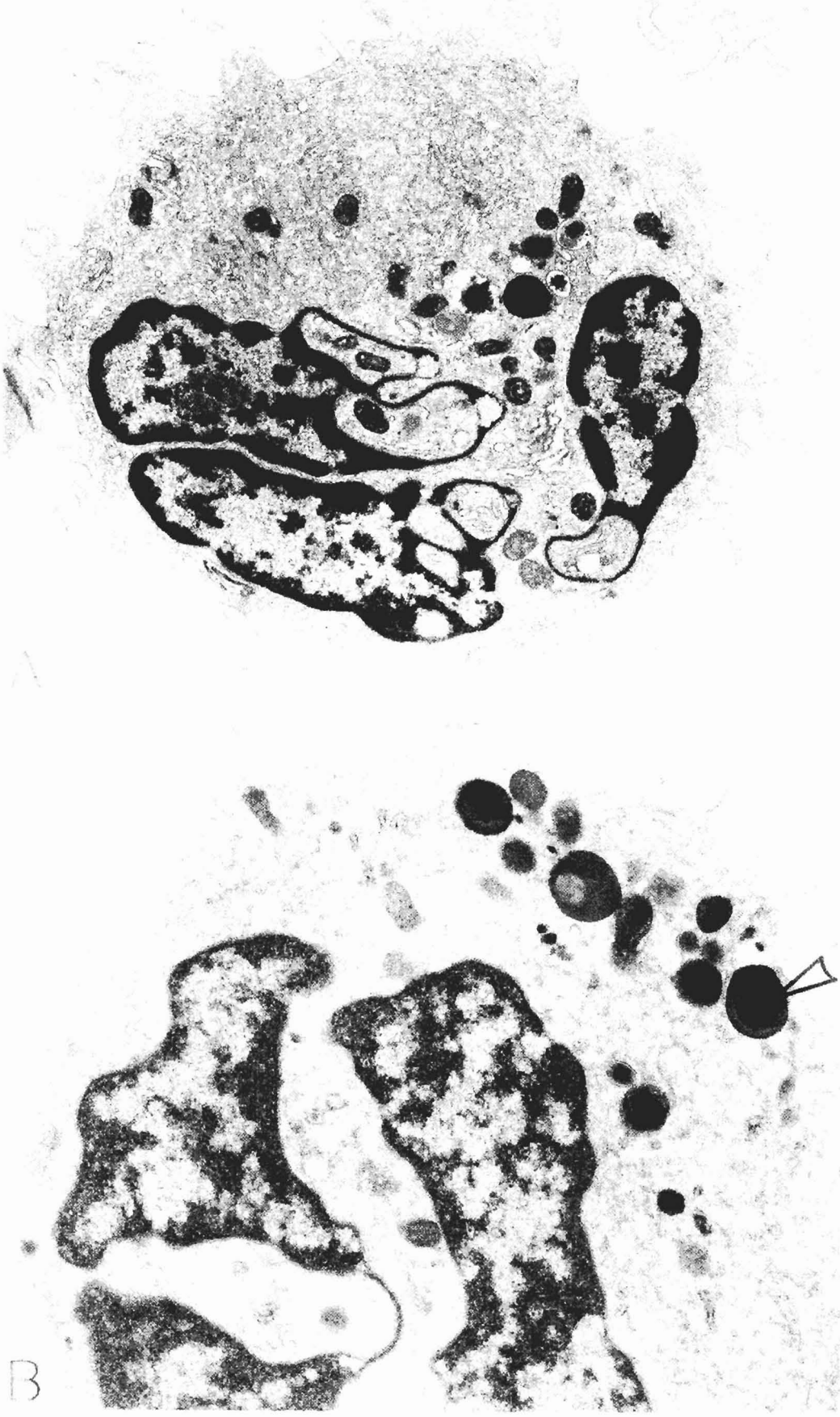


Fig. 32. Mature eosinophils (UCBC suspension cultures with rhSCF, six weeks) show diminished numbers of secondary granules. In **(a)**, the granule-poor cytoplasm is filled with SER and tubulovesicular structures. In **(b)**, core material is preserved in one remaining granule (arrowhead).
(a) x 14,500;
(b) x 17,500

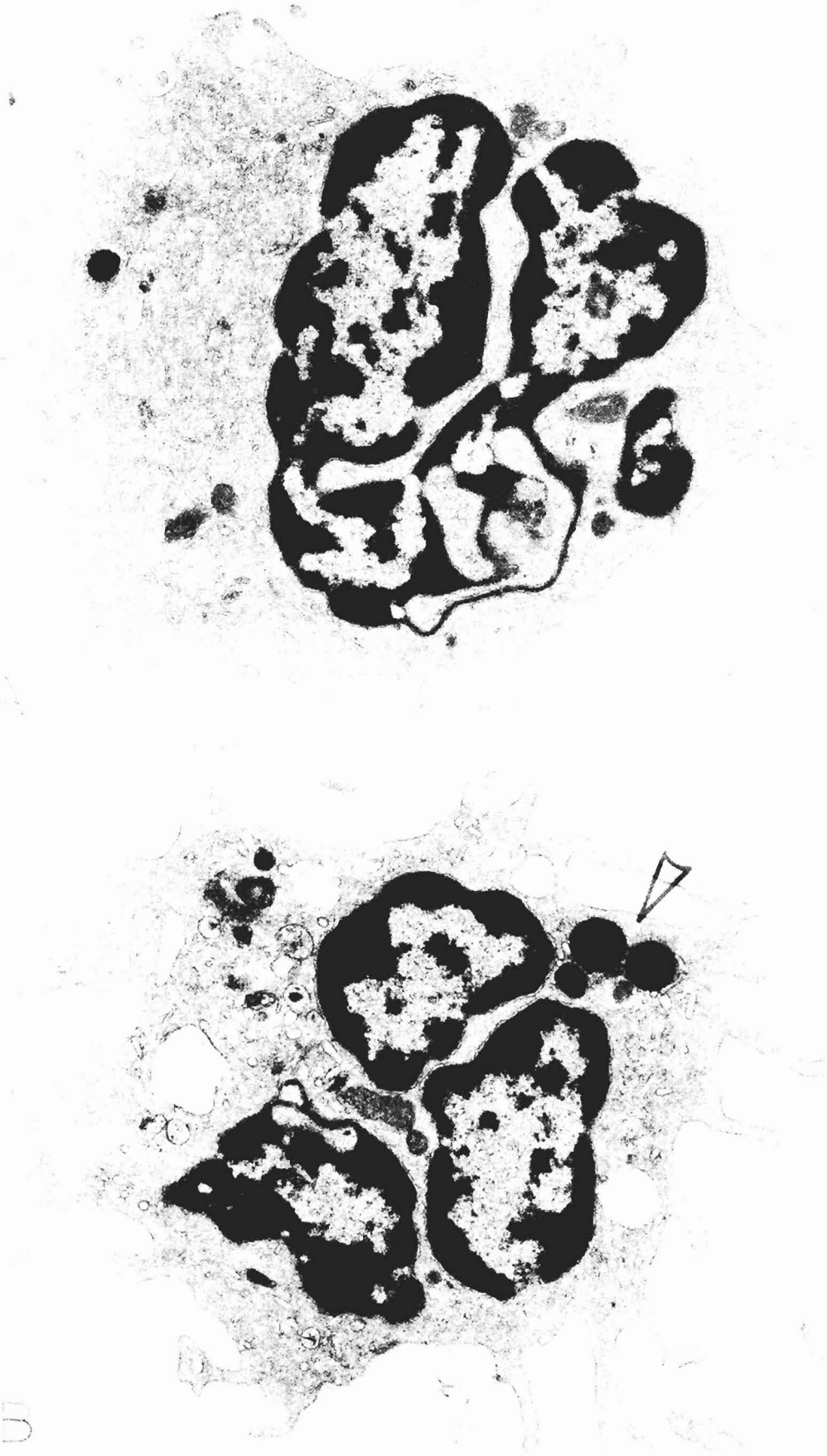


Fig. 33. Mature, granule-free eosinophils (UCBC suspension cultures with rhSCF, six weeks) are recognizable by their typical polylobed granulocyte nuclei. In **(a)**, a single primary granule remains; the cytoplasm is filled with SER and tubulovesicular structures. In **(b)** two lipid bodies (arrowhead) are seen. (a,b). x 16,000

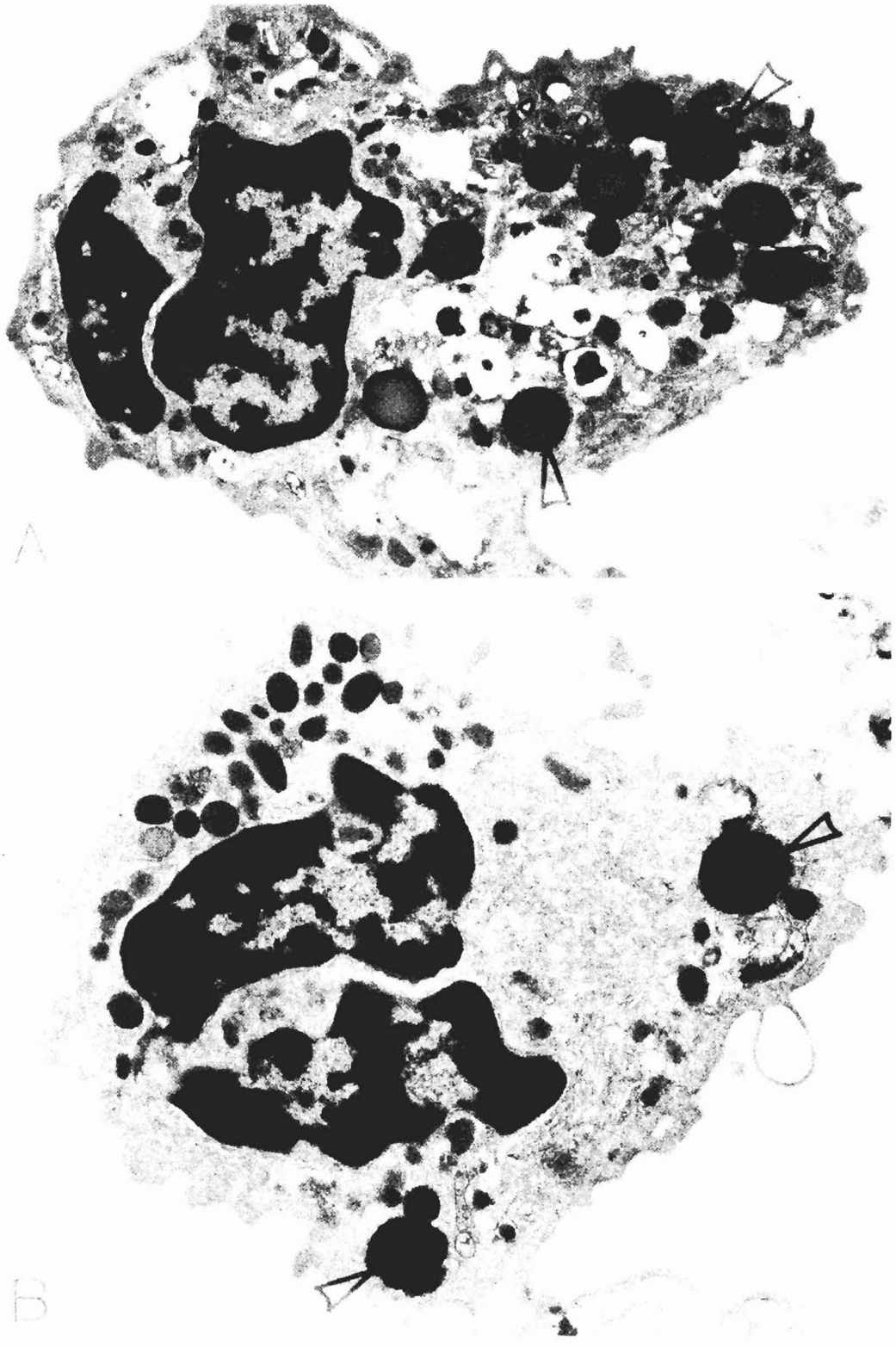


Fig. 34. Mature eosinophils (UCBC suspension cultures with rhSCF, eight weeks) show condensed, polylobed granulocyte nuclei. In **(a)**, the eosinophil has large numbers of round, osmiophilic lipid bodies (arrowheads) and piecemeal losses from secondary granules. In **(b)**, the eosinophil has two large lipid bodies (arrowheads). Nearly all remaining granules are homogeneously dense primary granules. Bicompartmental secondary granules are absent.
(a) x 12,000; (b) x 17,500

tubulovesicular structures (Figs. 32a, 33b). Rarely, a single primary granule (Fig. 33a) or several osmiophilic lipid bodies (Fig. 33b) remained in the cytoplasm.

i. Lipid bodies, primary granules, and small granules increase in mature eosinophils in vitro (Figs. 34-36).

Mature eosinophils have variable numbers of non-membrane-bound, osmiophilic, round lipid bodies that show qualitative and quantitative changes *in vitro*. Thus, we often noted increased numbers of these organelles in mature cells arising from cultured human cord blood cells (Fig. 34). Lipid bodies were generally round; some were enlarged (Fig. 34b), and many showed focal electron-lucent areas and dense particles within them.

Some mature eosinophils with typical mature granulocyte nuclei were devoid of bicompartmental secondary granules but contained increased numbers of homogeneously dense primary granules (Fig. 34b). The primary granules in eosinophils arising in culture contained Charcot-Leyden crystal protein, as demonstrated by ultrastructural immunogold stains (Fig. 35). Gold label for this protein, as we have previously demonstrated in mature eosinophils arising in rhIL-5-supplemented human cord blood cultures (Dvorak et al., 1991b), was also present in the cytoplasm and nucleus of eosinophils *in vitro* (Fig. 35).

Other eosinophils with diminished secondary granules displayed increased numbers of small, dense, membrane-bound granules (Fig. 36) - structures that are known to contain hydrolytic enzymes (reviewed in Dvorak et al., 1991a).

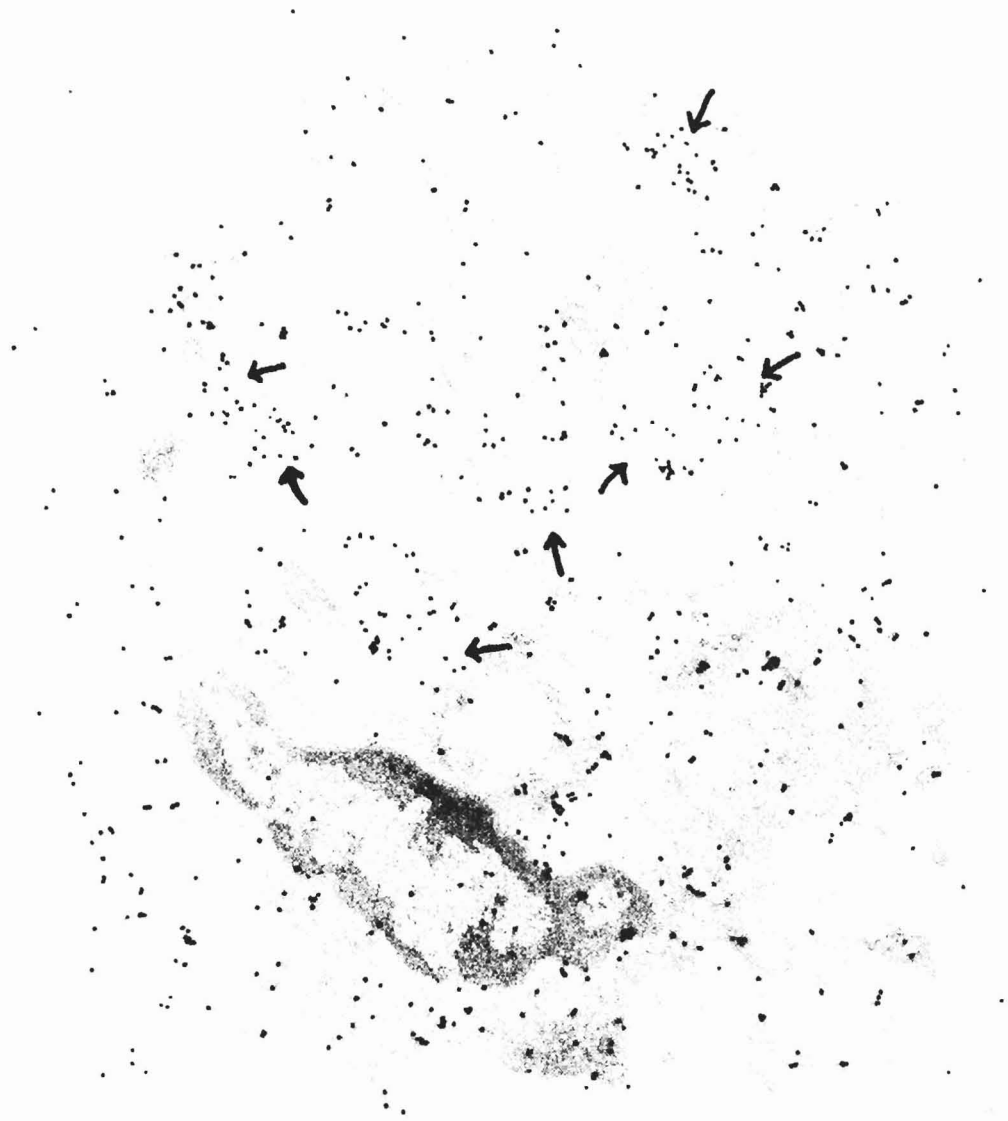


Fig. 35. Eosinophil (UCBC suspension culture with rhSCF, six weeks, and prepared with an immunogold method to detect CLC-P) shows gold-labeled primary granules (arrows). Diffuse CLC-P is also present in the nucleus and cytoplasm. x 15,500

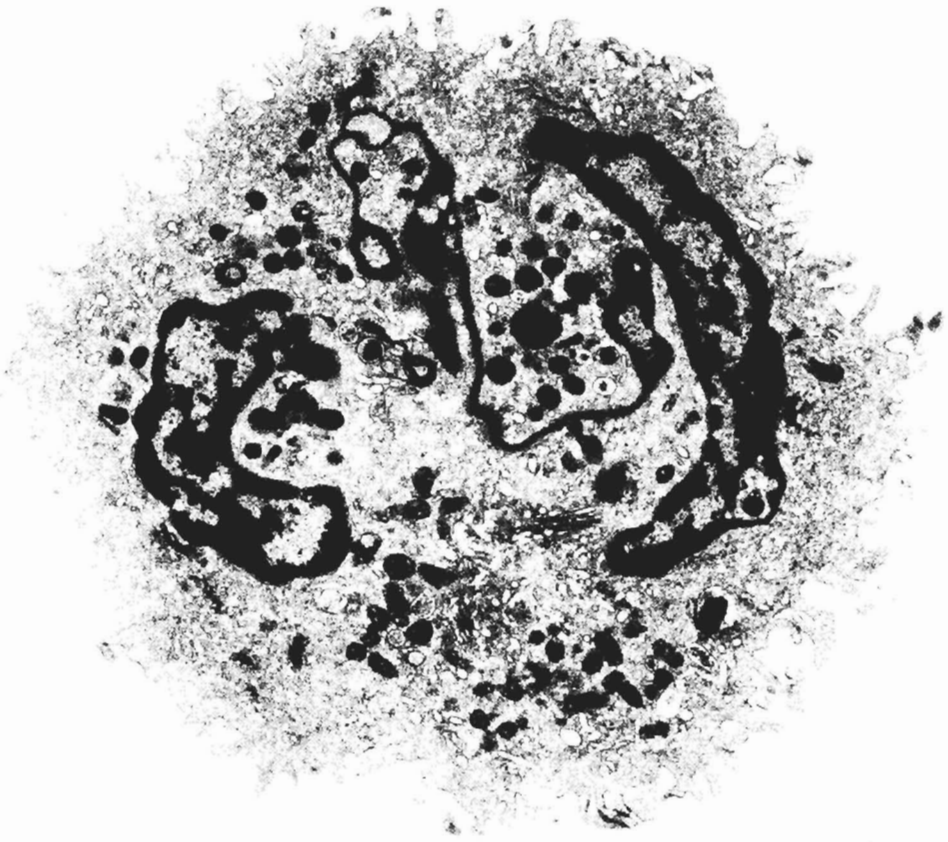


Fig. 36. Eosinophil (UCBC suspension culture with rhSCF, six weeks) shows increased numbers of small granules and no secondary granules. Note the typical polylobed granulocyte nucleus. x 11,000

Acknowledgements. We thank Peter K. Gardner for editorial assistance in the preparation of the manuscript; Kara Levine and Elizabeth Gillis for photographic assistance, and Susan Kissell-Rainville for electron microscopy technical assistance. This work was supported by U.S.P.H.S. Grant AI-33372 and the Lillian Gong Memorial Research Fund.

References

Beil W.J., Weller P.F., Tzizik D.M., Galli S.J. and Dvorak A.M. (1993). Ultrastructural immunogold localization of tumor necrosis factor-α to the matrix compartment of eosinophil secondary granules in patients with the idiopathic hypereosinophilic syndrome. J. Histochem. Cytochem. 41, 1611-1615.

Dvorak A.M. (1980). Ultrastructural evidence for release of major basic protein-containing crystalline cores of eosinophil granules *in vivo*: Cytotoxic potential in Crohn's disease. J. Immunol. 125, 460-462.

Dvorak A.M. (1988). Ultrastructural pathology of Crohn's disease. In: Inflammatory bowel diseases. Basic Research and clinical implications. Goebell H., Peskar B.M. and Malchow M. (eds). MTP Press, Ltd. Lancaster. pp 3-41.

Dvorak A.M. (1991a). Degranulation of basophils and mast cells. In: Blood Cell Biochemistry, vol. 4: Basophil and mast cell degranulation and recovery. Dvorak A.M. (vol. ed), Harris J.R. (series ed). Plenum Press. New York. pp 101-275.

Dvorak A.M. (1991b). Ultrastructure of human gastrointestinal system. Interactions among mast cells, eosinophils, nerves and muscle in human disease. In: Effects of Immune cells and inflammation on smooth muscle and enteric nerves. Snape Jr W.J. and Collins S.M. (eds). CRC Press, Inc. Boca Raton, FL. pp 139-168.

Dvorak A.M. (1992). Basophils and mast cells: Piecemeal degranulation *in situ* and *ex vivo*: A possible mechanism for cytokine-induced function in disease. In: Granulocyte responses to cytokines. Basic and clinical research. Coffey R.G. (ed). Marcel Dekker, Inc. New York. pp 169-271.

Dvorak A.M. (1993). Ultrastructural studies on mechanisms of human eosinophil activation and secretion. In: Eosinophils in allergy and inflammation. Gleich G.J. and Kay A.B. (eds). Marcel Dekker. Inc. New York. pp 159-209.

Dvorak A.M., Monahan R.A., Osage J.E. and Dickersin G.R. (1980). Crohn's disease: Transmission electron microscopic studies. II. Immunologic inflammatory response. Alterations of mast cells, basophils, eosinophils, and the microvasculature. Hum. Pathol. 11, 606-619.

Dvorak A.M., Mihm Jr. M.C., Osage J.E., Kwan T.H., Austen K.F. and Wintroub B.U. (1982). Bullous pemphigoid, an ultrastructural study of the inflammatory response: Eosinophil, basophil and mast cell granule changes in multiple biopsies from one patient. J. Invest. Dermatol. 78, 91-101.

Dvorak A.M., Ishizaka T. and Galli S.J. (1985). 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. 53, 57-71.

Dvorak A.M., Letourneau L., Login G.R., Weller P.F. and Ackerman S.J. (1988). Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. Blood 72, 150-158.

Dvorak A.M., Saito H., Estrella P., Kissell S., Arai N. and Ishizaka T. (1989). 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. 61, 116-132.

Dvorak A.M., Letourneau L., Weller P.F. and Ackerman S.J. (1990a). 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. 632, 608-615.

Dvorak A.M., Weller P.F., Monahan-Earley R.A., Letourneau L. and Ackerman S.J. (1990b). 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. 62, 590-607.

Dvorak A.M., Ackerman S.J. and Weller P.F. (1991a). Subcellular morphology and biochemistry of eosinophils. In: Blood cell biochemistry. Vol. 2. Megakaryocytes, platelets, macrophages and eosinophils. Harris J.R. (ed). Plenum Publishing Corp. London. pp 237-344.

Dvorak A.M., FuritsuT., Letourneau L., Ishizaka T. and Ackerman S.J. (1991b). 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. 138, 69-82.

Dvorak A.M., Ackerman S.J., Furitsu T., Estrella P., Letourneau L. and Ishizaka T. (1992). 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. 140, 795-807.

Dvorak A.M., Ishizaka T., Weller P.F. and Ackerman S.J. (1993a). 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: Eosinophils: Biological and clinical aspects. Makino S. and Fukuda T. (eds). CRC Press. Boca Raton, FL. pp 13-22.

Dvorak A.M., Mitsui H. and Ishizaka T. (1993b). Human and murine recombinant *c-kit* ligands support the development of human mast cells from umbilical cord blood cells: Ultrastructural identification. Int. Arch. Allergy Immunol. 101, 247-253.

Dvorak A.M., Mitsui H. and Ishizaka T. (1993c). 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. 54, 465-485.

Dvorak A.M., Onderdonk A.B., McLeod R.S., Monahan-Earley R.A., Antonioli D.A., Cullen J., Blair J.E., Cisneros R., Letourneau L., Morgan E., Silen W. and Cohen Z. (1993d). Ultrastructural identification of exocytosis of granules from human gut eosinophils *in vivo*. Int. Arch. Allergy Immunol. 102, 33-45.

Dvorak A.M., Estrella P., Mitsui H. and Ishizaka T. (1993e). *c-kit* ligand induction of immature neutrophils in cultures of human umbilical cord blood. Eur. J. Cell Biol. 62, 422-431.

Dvorak A.M., Estrella P. and Ishizaka T. (1993f). Vesicular transport of peroxidase in human eosinophilic myelocytes. Clin. Exp. Allergy. (in press).

Dvorak A.M., Mitsui H. and ishizaka T. (1994a). Stimulation of partial development of human mast cell progenitors by supernatant fluid from mouse fibroblast cultures. Clin. Exp. Allergy (in press).

Dvorak A.M., Ishizaka T., Letourneau L., Albee E.A., Mitsui H. and Ackerman S.J. (1994b). 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. 42, 251-263.

Dvorak A.M., Morgan E.S., Tzizik D.M. and Weller P.F. (1994c). Ultrastructural localization of prostaglandin endoperoxide synthase (cyclooxygenase) to nonmembrane-bound cytoplasmic lipid bodies in human eosinophils and 3T3 fibroblasts. (submitted).

Egesten A., Aluments J., von Mecklenburg C., Palmegren M. and Olsson I. (1986). Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. J. Histochem. Cytochem. 34, 1399-1403.

Ishizaka T., Dvorak A.M., Conrad D.H., Niebyl J.R., Marquette J.P. and Ishizaka K. (1985). Morphologic and immunologic characterization of human basophils developed in cultures of cord blood mononuclear cells. J. Immunol. 134, 532-540.

Jabara H.H., Ackerman S.J., Vercelli D., YokotaT., Arai K.-I., Abrams J., Dvorak A.M., Lavigne M.C., Banchereau J., De Vries J., Leung D.Y.M. and Geha R.S. (1988). Induction of interleukin-4-dependent IgE synthesis and interleukin-5 dependent eosinophil differentiation by supernatants of a human helper T-cell clone. J. Clin. Immunol. 8, 437-446.

Mitsui H., Furitsu T., Dvorak A.M., Irani A.-M.A., Schwartz L.B., Inagaki N., Takei M., Ishizaka K., Zsebo K.M., Gillis S. and Ishizaka T. (1993). Development of human mast cells from umiblical cord blood cells by recombinant human and murine c-kit ligand. Proc. Natl. Acad. Sci. USA 90, 735-739.

Peters M.S., Rodriguez M. and Gleich G.J. (1986). Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. Lab. Invest. 54, 656-662.

Saito H., Hatake K., Dvorak A.M., Leiferman K.M., Donnenberg A.D., Arai N., Ishizaka K. and Ishizaka T. (1988). Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc. Natl. Acad. Sci. USA 85, 2288-2292.

Weller P.F. and Dvorak A.M. (1985). Arachidonic acid incorporation by cytoplasmic lipid bodies of human eosinophils. Blood 65, 1269-1274.

Weller P.F. and Dvorak A.M. (1994). Human eosinophils: development, maturation and functional morphology. In: Mechanisms in asthma and rhinitis. Implications for diagnosis and treatment. Busse W. and Holgate S. (eds). Blackwell Scientific Publishers, Inc. Cambridge, MA. pp 255-274.

Weller P.F., Monahan-Earley R.A., Dvorak H.F. and Dvorak A.M. (1991a). Cytoplasmic lipid bodies of human eosinophils. Subcellular isolation and analysis of arachidonate incorporation. Am. J. Pathol. 138, 141-148.

Weller P.F., Ryeom S.W. and Dvorak A.M. (1991b). Lipid bodies:

Structurally distinct, nonmembranous intracellular sites of eicosanoid formation. In: Prostaglandins, leukotrienes, lipoxins and PAF. Bailey J.M. (ed). Plenum Press. New York. pp 353-362.

Weller P.F.Marshall W.L., Lucey D.R., Rand T.H., Dvorak A.M. and Finberg R.W. (1994). Infection, apoptosis and killing of mature human eosinophils by human immunodeficiency virus-1. (submitted).