MICROENDOCYTOSIS IN EOSINOPHILIC LEUKOCYTES

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ABSTRACT

Rat peritoneal eosinophils were examined after intraperitoneal infusion either of a mixture of phosphate-buffered saline (PBS) and colloidal gold or of fetal calf serum. These cells characteristically contained vesiculotubular structures, cuplike structures, and small granules in addition to the crystalloid granules which stratified to the opposite pole from the small granules during centrifugation. The cup-shaped structures and elaborate labyrinths of vacuole-like spaces increased markedly after injection of the PBS-colloidal gold mixture, presumably as features of heightened microendocytic activity. The vesiculotubular structures increased greatly after infusion of fetal calf serum. A few crystalloid granules exhibited fine-structural changes after the PBS-colloidal gold injection, and more numerous crystalloid granules appeared altered after fetal calf serum. Infrequent small granules contained a lucent, crystal-like silhouette after the fetal calf serum injection. Eosinophils evidenced microendocytic uptake of gold spherules into coated vesicles, the cup-shaped structures, and the small granules, but not into the vesiculotubular structures or crystalloid granules after intraperitoneal infusion of the PBS-gold mixture. Strong unmasked acid phosphatase activity in small granules contrasted with the general lack of activity in normal-appearing crystalloid granules and moderate activity in apparently altered crystalloid granules, presumably reflecting active and latent forms of enzyme in the different granules.

Eosinophil granulocytes are known to function as phagocytes in the inflammatory process (3). These cells apparently migrate chemotactically in the same way as neutrophils (16, 31) except that they respond to the influence of IgG_1 but not of IgG_2 (17). Eosinophils have been shown to ingest bacteria (9, 10, 13, 14, 21), fungi (10, 21), zymosan (2, 13), latex spheres (10, 19, 21), sensitized red blood cells (2), antigen-antibody complexes (2, 20, 35), and other materials (1, 8, 12, 35). Ingestion of relatively large particles clearly constitutes an important function of eosinophils as of neutrophils and macrophages. The numerous eosinophils commonly encountered in noninflamed tissues or the peritoneal cavity afford little or no evidence of such activity, however, and their role remains unexplained.

Ultrastructural characteristics have suggested that human tissue eosinophils (see reference 25 and footnote 1), like macrophages, might be engaged in uptake either of dissolved macromolecules or of minute particles present in the environs of the cell. This possibility was investigated in the present

[1] R. T. Parmley, and S. S. Spicer. Submitted for publication.

study in which rat peritoneal eosinophils were examined after intraperitoneal injection of fetal calf serum or colloidal gold. Ingestion by a cell of materials from the environment has classically been referred to as either pinocytosis (cell drinking), or phagocytosis (cell eating), depending upon whether the material ingested was in a liquid or a solid phase. However, both solid and liquid materials are frequently ingested in the same vesicle or vacuole. The weight of recent evidence, as well as observations from the present study, indicates that a more functional categorization may be made on the basis of the size of the quantum of material taken into the cell (see 23, 29). Furthermore, energy requirements and mechanisms of uptake depend more upon the size of endosome than upon the material ingested. Therefore, we will use the term microendocytosis to indicate uptake into vesicles less than 1,000 Å in diameter, and macroendocytosis for engulfment into vacuoles exceeding 1,000 Å in diameter. Evaginations from the cell surface apparently mediate macroendocytosis (type A endosome formation as diagramed in reference 29) whereas small surface invaginations effect various microendocytic mechanisms including the more specific type C formation of rough-coated vesicles (29). Eosinophils appear from the present findings to ingest material by both mechanisms.

The ultrastructure of developing eosinophils and their content of characteristic crystalloid granules has been well elucidated in many species including man (15, 32). Several newly recognized organelles distinct from the crystalloid granule or its precursors have recently been demonstrated in mature eosinophils (references 25, 27, and 30; see also footnote 1). These membrane-limited organelles, generally measuring less than 0.1 μm in narrow dimension and having vesicular, tubular, ring-shaped, or cup-like contour, have been referred to as vesicular and tubular structures in human (25, and see footnote 1) and rat (1) eosinophils, as secondary granules in cat eosinophils (30), and as microgranules in eosinophils of man and other species (27). Additionally, a somewhat larger, morphologically distinct structure with unmasked, fixative-resistant acid phosphatase has been designated the small granule in human eosinophils (25).[1] The studies reported here provide evidence that several of the aforementioned organelles function in microendocytosis and that the uptake of different materials can follow alternative paths, one leading to the small granule and the other to the crystalloid granule.

MATERIALS AND METHODS

Eosinophils were obtained from the peritoneal cavity of female Sprague-Dawley rats weighing about 150 g. Peritoneal fluid was harvested immediately after an intraperitoneal injection of 10 ml of phosphate-buffered saline (PBS) at pH 7.2, and 30 min and 60 min after an injection of 10 ml of fetal calf serum (Difco Laboratories, Detroit, Mich.), or of a mixture of equal parts of PBS and a commercial (Harleco, Gibbstown, N. J.) colloidal suspension of ca. 200 Å gold particles (34). This suspension was made approximately isotonic by a ninefold concentration (24) with drying as described previously (18). Heparin was added at a concentration of 10 U/ml to each of the wash solutions to minimize fibrin formation in the peritoneal fluid and was assumed not to alter endocytosis at this level. Specimens of peritoneal wash cells were obtained and processed for morphological and cytochemical examination as detailed previously (18). Acid phosphate activity was localized with the Barka-Anderson method (5) in the specimens obtained after injection of the colloidal gold mixture. Control sections were incubated in medium lacking the glycerophosphate substrate. Ultrathin sections of morphological preparations were examined in a Hitachi HS-8 electron microscope with and without uranyl acetate and lead citrate staining. Thin sections of the cytochemical preparations were examined without heavy metal staining.

To evaluate the response of certain organelles to different peritoneal injections, their prevalence was determined by numerical counts on the eosinophils in each group (Table I). Vesicular and tubular structures (Figs. 1–4) were counted in several cells, and in each profile vesicular structures outnumbered tubular five- to sixfold. Vesicular structures thus varied in abundance among the several cells in parallel with tubular structures, as would be expected if these profiles represented different planes of section of the same organelle. For this reason and because vesicular structures were very numerous and difficult to distinguish from Golgi vesicles, tubular structures alone were counted to assay the prevalence of the vesiculotubular structures in the remainder of the cells (Table I). Cup-shaped structures were enumerated separately because of their distinctive arched contour and lucent focus. They were found to vary independently of tubular structures in different specimens (Table I) and were considered not simply to represent profiles of bent tubular structures. The presence of crowded vacuole-like spaces, termed labyrinthine spaces, was also tabulated. The prevalence of small-type granules was not determined because they were stratified at one pole by the centrifugation, unlike the tubulovesicular and cup-shaped structures which appeared to be distributed relatively uniformly throughout the cytoplasm.

TABLE I

Changes in Organelles of Peritoneal Eosinophils after Intraperitoneal Infusions

Solutions injected intraperitoneally	Time after injection				
	Less than 5 min	30 min		60 min	
	PBS	PBS and colloidal gold mixture	100% fetal calf serum	PBS and colloidal gold mixture	100% fetal calf serum
No. of cells	41	46	40	36	39
No. of crystalloid granules per cell profile	35.7	34.4	39.2	33.0	31.7
Percent of cells with altered crystalloid granules	4.9	37.0	75.0	25.0	69.2
No. of altered crystalloid granules					
without membranous lamellae	1	25	57	7	40
with membranous lamellae	1	18	35	4	12
No. of small membrane-limited structures					
average no. of the tubular type per cell profile	9.5	11.0	20.9	10.5	18.3
average no. of the cup-shaped type per cell profile	0.7	17.6	8.1	14.8	7.8
Percent of cells with labyrinthine spaces	0	21.7	15.0	52.8	2.5

RESULTS

Immediately after Infusion of PBS

Eosinophils comprised approximately 10% of the peritoneal wash cells, and characteristically contained numerous granules with an axial crystalloid surrounded by slightly less dense matrix (Fig. 1). Frequent eosinophils also revealed 1–12 small granules which generally occupied the cytoplasmic pole opposite the crystalloid granules, apparently as a result of stratification by centrifugation as previously noted (25) (Figs. 1, 3). These small granules measured up to 0.5 μm in diameter and had uniform or heterogeneous content devoid of crystalloid.

In addition, these eosinophils contained small membrane-limited structures which measured less than 0.1 μm in their narrow dimension and possessed content which appeared slightly more dense than that of Golgi vesicles. On the basis of morphology and variable prevalence under different conditions, these small membrane-limited structures could be divided morphologically into two varieties: a vesicular or tubular type, and a cup-shaped type. The vesicular and tubular structures had a round or elongated profile; the cuplike structures had an arched contour usually curving around a lucent cytoplasmic focus.

Eosinophils harvested quickly after injection contained numerous membrane-limited structures of the vesicular and tubular type but very few of the cup-shaped variety (Figs. 1–4) (Table I). They possessed no labyrinthine spaces and revealed little variation in the fine structure of the crystalloid granules.

30–60 Min after Injection of PBS and Colloidal Gold

The density of the matrix often exceeded that of the crystalloid in many otherwise normal-looking granules in these eosinophils (Figs. 5 and 7). In about one-third of the cells, one to several crystalloid granules appeared more altered (Table I). This alteration was characterized by the lessened density and more grainy texture of the matrix, by

partial to complete dissolution of the crystalloid, or by the presence of membranous lamellae (Figs. 5 and 7). The limiting membrane of these granules was obscured in many cases, usually near the pole of the crystalloid where the granules often contacted or were bordered by small vesicular or tubular structures.

Cup-shaped structures typically embracing a lucent focus (Figs. 7–9) increased markedly 30–60 min after injection of PBS and colloidal gold, but vesicular and tubular structures remained little changed (Table I). A labyrinth of largely empty-appearing spaces, which were limited by membrane and often separated only by a thin curved septum, occupied one or more peripheral regions of the cytoplasm or filled a larger area near the nucleus of many of these eosinophils (Figs. 8–10, see also Fig. 11). Cytoplasmic veils and thin membrane-bounded arches or loops frequently projected from the surface of these cells and often comprised the most superficial part of the smaller peripheral labyrinths appearing to function in their genesis (Figs. 6, 19). Coated buds, i.e. caveolae, often evaginated the membranes of the labryinthine spaces (Fig. 9, see also Fig. 11) and cuplike structures bordered them (Figs. 8–10). The vacuolar labyrinths near the nucleus were larger than those at the periphery and occasionally contained finely particulate matter (Fig. 8) or forms suggestive of degraded micro-organisms (Fig. 10). These labyrinths were most numerous in eosinophils harvested 60 min after injection of PBS and gold (Table I) and were not encountered in the other peritoneal cell types.

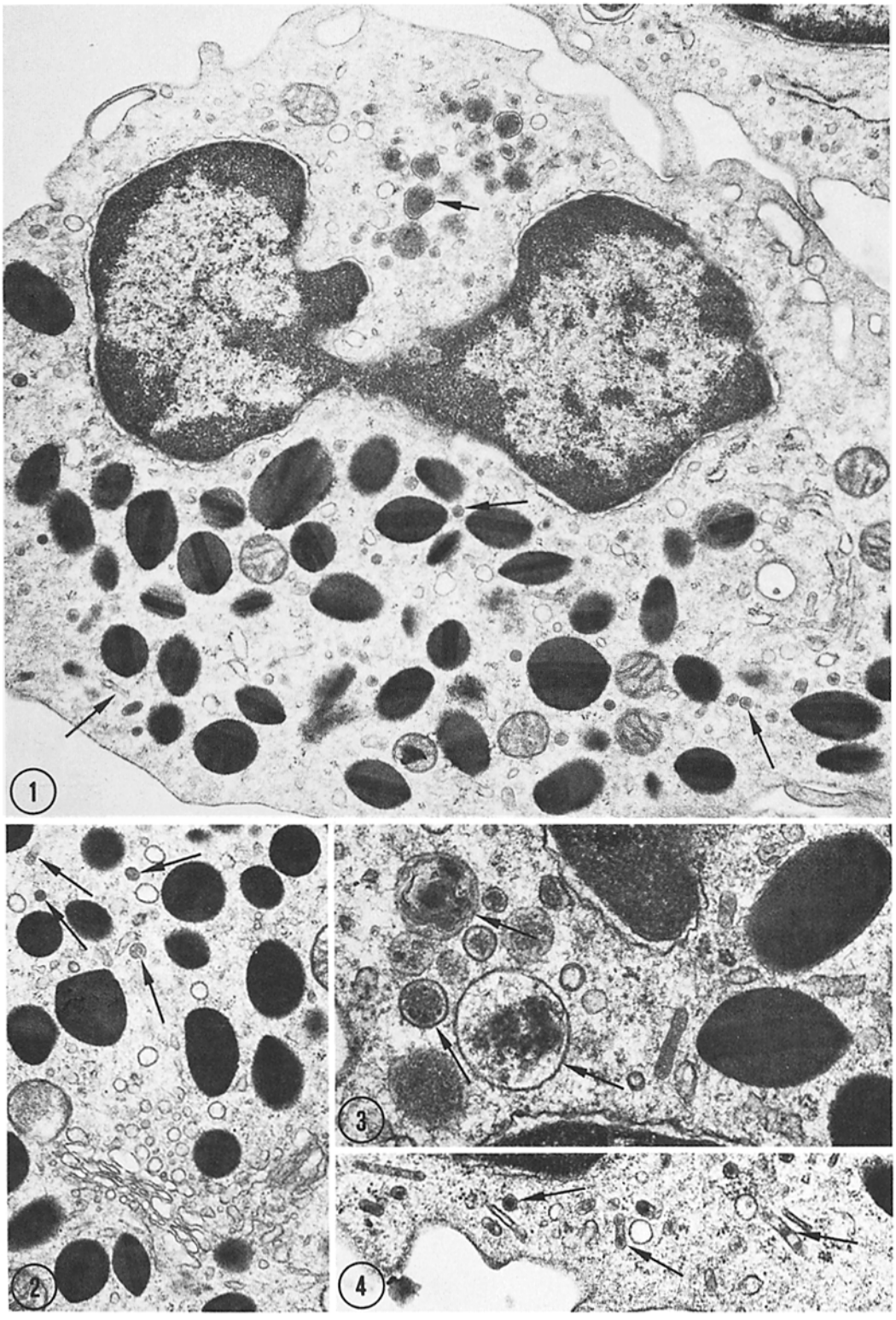
Gold spherules contacted points on the eosinophil surface and occupied coated buds and peripheral coated vesicles which apparently had budded from the surface of labyrinthine or other subsurface spaces (Figs. 12, 13). Gold particles were also observed in cup-shaped structures, some of which were larger than the usual cuplike structures which prevailed in such abundance in these cells (Figs. 8, 9, 12, 14, and 17). In addition, some of the small-type granules enclosed endocytosed gold spherules (Figs. 8, 10, 14–16). However, gold particles were not seen in unaltered or altered crystalloid granules of the eosinophils or in the small membrane-limited structures which bordered or contacted the altered crystalloid granules.

In occasional eosinophils, a few to several of the crystalloid granules which appeared morphologically intact disclosed acid phosphatase activity, but usually such granules lacked reaction product (Figs. 19–22). However, some granules which had a lucent matrix suggestive of extraction disclosed acid phosphatase activity in or around the crystalloid or, in some instances, only in the matrix (Figs. 19, 22–24). Some of the altered crystalloid granules containing membranous lamellae revealed light deposits, but others appeared unreactive (Fig. 21). In addition, heavy reaction product filled profiles which corresponded in location, size, shape, and prevalence to the small granules visualized morphologically (Figs. 19, 20, and 22). Reaction product was superimposed on endocytosed gold particles in a number of these small granules (Figs. 19, 20). Moderate deposits indicative of acid phosphatase activity occupied the Golgi cisternae of these eosinophils (Fig. 21). The labyrinthine spaces at the cell periphery lacked reaction product (Fig. 20); but in the larger, more central ones, a few sparse deposits occasionally adhered to the surface of the spaces. The vesiculotubular and cuplike structures were devoid of reaction product. Cells incubated in substrate-free control medium lacked precipitates in Golgi cisternae and cytoplasmic granules.

Structures revealing incorporation of gold spherules in other cells included heterophagic dense bodies of macrophages, specific granules of mast cells, small cytoplasmic bodies of mast cells, and lymphocytes and small endocytic vesicles in all of these cells. On the other hand, neutrophils failed to incorporate endocytosed gold particles into cytoplasmic granules and afforded no evidence of uptake into endosomes except for a rare phagocytic vacuole containing gold particles along with bacteria (Fig. 18).

30–60 Min after Injection of Fetal Calf Serum

Morphological changes of crystalloid granules in these eosinophils resembled those observed after PBS injection but were more frequent and more pronounced (Table I). Some large granules clearly representing altered crystalloid granules were filled with membranous laminae and lacked a crystalloid (Figs. 25, 27–29). Several vesicular and tubular structures often closely bordered or contacted the altered granules, especially near the poles of the crystalloids where they distorted the granule contour (Figs. 25–27). A lucent focus indented the pole of a rare crystalloid granule. More than half of the altered crystalloid granules were located adjacent to the nucleus.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The small granules in these eosinophils often had heterogeneous content and occasionally enclosed a uniformly lucent crystal (Figs. 30, 31). These crystals greatly elongated some of the presumed small granules. Similar crystals were very common in the heterophagic dense bodies of neighboring macrophages as previously noted (18).

The number of vesicular and tubular structures in these eosinophils greatly exceeded the number in eosinophils of the other specimens, but the prevalence of cup-shaped structures and labyrinthine spaces was not as increased as after the PBS-colloidal gold injection (Table I). Thin cytoplasmic arches or loops did not project from these cells as from those exposed to PBS and gold.

DISCUSSION

Rat peritoneal eosinophils in this study like those of human tissues (25)[1] appeared little involved in the macroendocytic activity for which these cells are noted. However, the peritoneal eosinophils evidenced ingestion of both small particles and solute by a process referred to here as microendocytosis. This process entails uptake of small quantities by sequestration of the substance in coated surface buds which pinch into the cytoplasm as coated vesicles (29), and depends on invagination of the plasmalemma (23).

Under some conditions, the microendycytic process in eosinophils apparently occurs as a second step after formation of a lybyrinth of spaces by surface evaginations of the type that are thought to initiate macroendocytosis (18, 23, 29). Thus, the more peripheral and presumably less-developed labyrinths were associated with uniquely thin cytoplasmic veils that appeared to envelop extracellular space. These labyrinthine spaces, encountered here particularly after injection of the PBS-colloidal gold solution, appear not to have been described before. Their occasional content of flocculent matter or micro-organisms indicates that the deeper labyrinths lack surface connections and themselves represent a form of phagosome. Caveolae protruding from the labyrinthine spaces or from single subsurface spaces give rise to coated vesicles in the cytoplasm. The observation that cuplike structures increased in prevalence with labyrinths and bordered them closely raises the possibility that the cuplike structures arise as endosomes from the labyrinthine spaces or that they develop from the vesicles budding from these spaces. The uptake of gold into both vesicles and cuplike structures supports the latter consideration. However, many of the gold-laden, cup-shaped structures appeared larger than those without gold and either became enlarged on acquiring gold or represented a different functional entity.

Microendocytosis possibly is mediated in eosinophils by more than one mechanism, since cell organelles presumably involved in the process varied independently under various conditions. Thus, the opposite responses of vesiculotubular structures and cup-shaped structures to the two bathing solutions suggest that these organelles participate in endocytosis of different substances. Their incorporation of gold spherules implicates both coated vesicles and cup-shaped structures in the endocytosis of small particulates. The vesicular and tubular structures failed to incorporate gold

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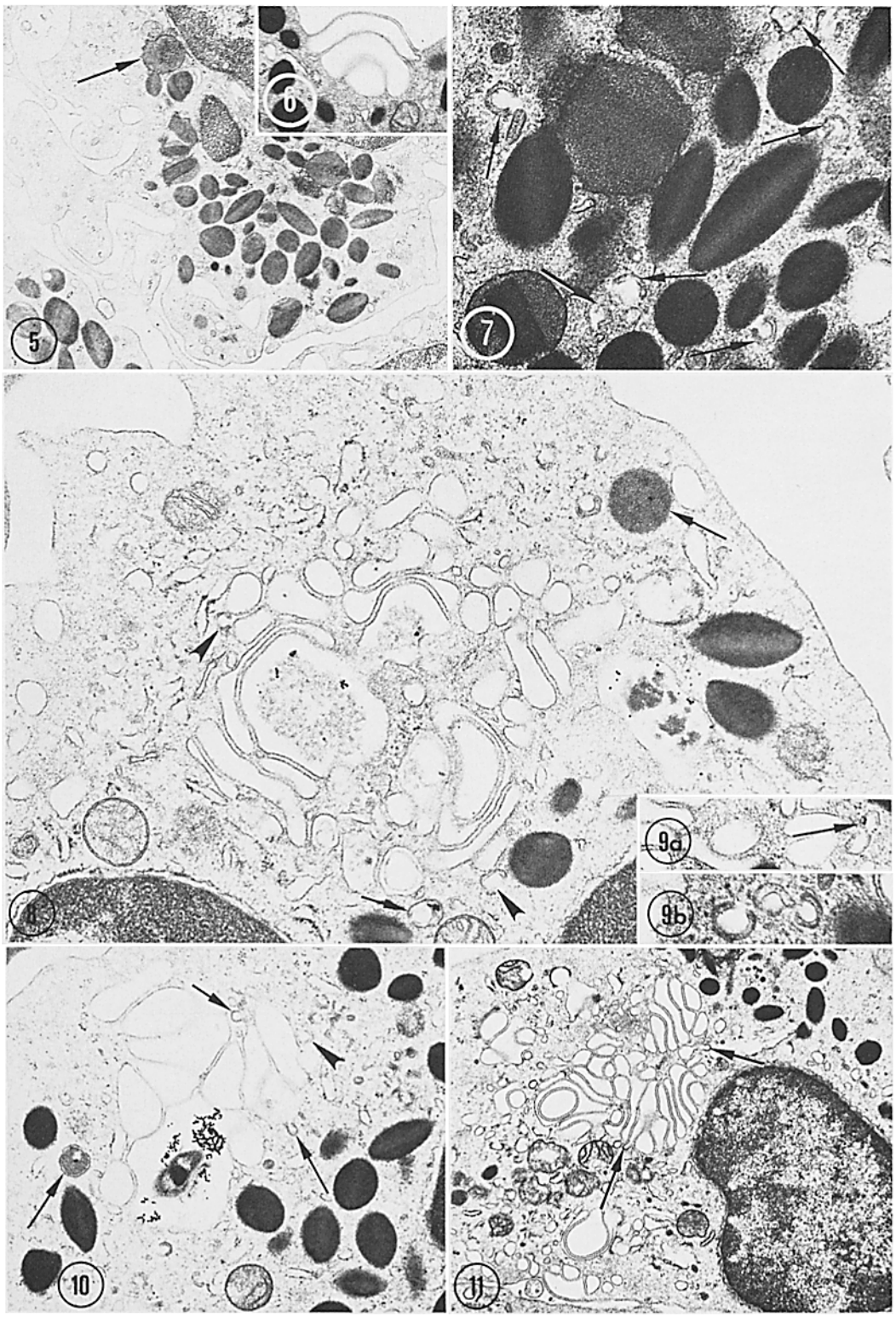
All figures illustrate rat peritoneal eosinophils except for the neutrophil of Fig. 18. The ultrathin sections of the morphological preparations shown in Figs. 1–11, 13–18, and 25–31 were stained with uranyl acetate-lead citrate. The thin sections of the morphological specimen illustrated in Fig. 12 and of the acid phosphatase preparations in Figs. 19–24 were unstained.

FIGURE 1 This eosinophil harvested immediately after PBS injection reveals membrane-limited small granules (short arrow) at the cytoplasmic pole opposite the crystalloid granules. The vesicular or tubular type of small membrane-limited structure (long arrow) is scattered through the cytoplasm but cup-shaped structures are absent. × 16,600.

FIGURE 2 A cell like that in Fig. 1. In contrast to the Golgi-associated vesicles with a lucent content, the vesicular-tubular structures (arrows) have a moderately dense content. × 17,500.

FIGURE 3 In another cell obtained immediately after the PBS infusion, some of the small granules (arrows) display heterogeneous content. × 37,500.

FIGURE 4 Like Fig. 1, showing vesicular and tubular structures (arrows) with variably dense content, lucent vesicles, and no cup-shaped structures. × 23,800.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and apparently do not function in microendocytosis of such small particles. Their marked increase after exposure to fetal calf serum implies a role in uptake of dissolved macromolecules for these organelles.

An unusual feature of the cup-shaped structures was the lucent cytoplasmic focus which they partially enveloped. Such low-density foci of unknown nature possibly result from endocytic accumulation of extracellular solute or fluid. This consideration seems consistent with the very marked increase in gold-free cuplike structures after intraperitoneal injection of the PBS-colloidal gold mixture. Vesicles with antimonate-precipitable cations in eosinophils fixed with Komnick's antimonate-osmium tetroxide solution (15 and 25) are consistent with endocytosis of fluid-containing electrolyte.

Small granules of rat eosinophils resemble those in human eosinophils (25)[1]. The gold particles demonstrated in the small granules are apparently transported to them by vesicles and cuplike structures. Their content of gold spherules after injection of colloidal gold and of lucent crystalline material after injection of fetal calf serum testifies to the heterophagic nature of these bodies. The extracted appearance of the latter crystal-like profiles and the similarity of these silhouettes to crystalline profiles in human Leydig cell tumors (7) suggest that eosinophils endocytose a lipid component of the calf serum.

The small granules of the eosinophil resemble the heterophagic dense bodies of rat peritoneal macrophages and infrequent small granules of lymphocytes in their unmasked acid phosphatase and uptake of gold spherules (18). The term microendosome would conveniently encompass these similar heterophagic organelles that function in microendocytosis. Neutrophils, on the other hand, lacked caveolae, endocytic vesicles, and microendosomes. These cells appear to function only in macroendocytosis (23 and 24), and in the cells of this study contained colloidal particles exclusively in phagocytic vacuoles (macroendosomes) which presumably acquired the spherules in "piggyback" fashion (26).

The demonstration of acid phosphatase in gold-containing small granules identifies these bodies as secondary lysosomes. Small granules presumably acquire acid phosphatase from nearby reactive Golgi vesicles as do heterophagic bodies in macrophages (11, 18, 19). Strong, unmasked acid phosphatase activity in eosinophil small granules contrasts with the absence of cytochemically demonstrable acid phosphatase activity in most crystalloid granules of this and previous studies (4, 14, 25, 28 and 33)[1]. This difference perhaps reflects the active state of hydrolases in secondary lysosomes

FIGURE 5 Thick processes protrude from the surface of an eosinophil harvested 30 min after the PBS-gold infusion. Several altered crystalloid granules (arrows) are partially occupied by membranous lamellae. × 9,600.

FIGURE 6 Cytoplasmic veils bridge an indented surface area in a configuration suggesting a mechanism for genesis of the labyrinths. × 13,300.

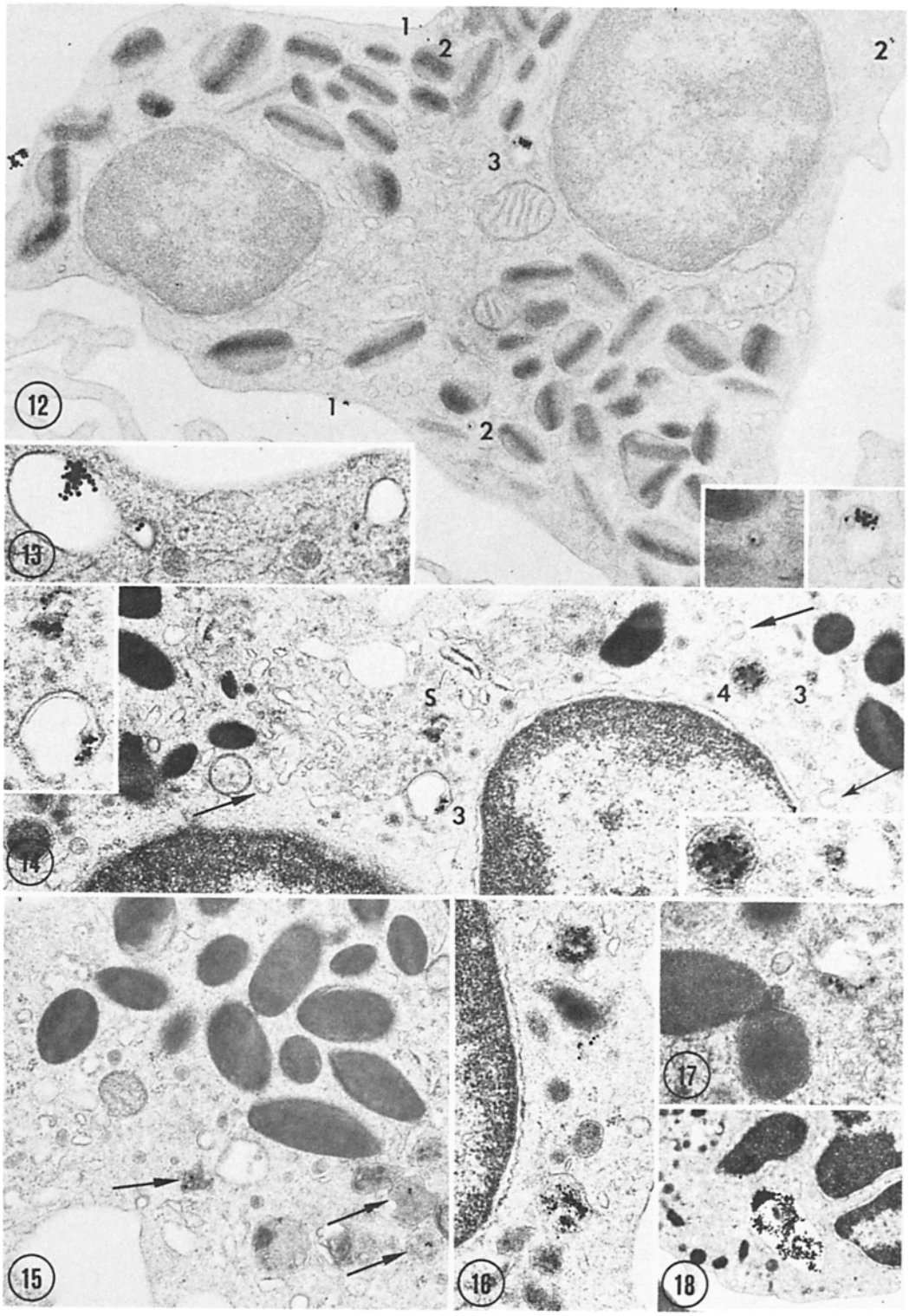
FIGURE 7 Like Fig. 5. Cup-shaped (arrows) and vesiculotubular structures are present. Two crystalloid granules reveal altered density, incipient dissolution of the crystalloid, and surface irregularity. × 36,000.

FIGURE 8 This eosinophil harvested 60 min after injection of PBS and colloidal gold displays a labryinth of vacuolar profiles. Some of these vacuoles contain amorphous material and gold particles. Cup-shaped structures (arrowheads) border the labyrinthine spaces. Another cup-shaped structure (short arrow) and a small type of granule (long arrow) contain gold spherules. × 23,800.

FIGURE 9 Cup-shaped structures are shown in (*a*) lying near labyrinthine spaces and enclosing gold spherules, and in (*b*) typically embracing a lucent focus. Preparation as in Fig. 8. (*a*) × 23,800. (*b*) × 39,200.

FIGURE 10 Like Fig. 8. Another labryinth of vacuoles contains gold aggregates and a structure suggestive of a degraded micro-organism. Caveolae (short arrows) bud from the vacuoles. A cup-shaped structure (arrowhead) borders the vacuolar apparatus. A small granule (long arrow) encloses a gold particle. × 16,700.

FIGURE 11 An eosinophil harvested 30 min after injection of fetal calf serum shows a well-developed vacuolar labyrinth. Caveolae (arrows) bud from the vacuoles. × 11,500.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as compared to the latent state of hydrolases in primary lysosomes in which the enzymes are in storage. Cytochemical visualization of acid phosphatase in the crystalloid granules is thought to require unmasking of the enzyme through an undefined disruptive process (4, 14, 22, 25, 28 and 33). Unmasking of acid phosphatase observed in some of the crystalloid granules in this study could in part at least be a result of conversion of the granule to a heterophagic organelle. The unusual localization of acid phosphatase in the crystalloid of some granules may reflect activation of normally latent enzyme in the crystalloid or may result from diffusion of activated enzyme from matrix into the crystalloid as a consequence of alteration in vivo or during processing.

The alterations of crystalloid granules observed previously in human (25)[1] and here in rat eosinophils, cannot be readily reconciled with the conventional view that the crystalloid granules of these cells function only as do granules of neutrophils, by fusing with phagocytic vacuoles. These alterations occurred after injection of fetal calf serum in eosinophils which failed to evidence phagocytic activity. The changes in crystalloid granules were most prominent after infusion of fetal calf serum and correlated strikingly with an increase in the population of vesiculotubular structures. This correlation, and the numerous profiles in which these latter structures bordered and appeared to fuse with altered (but not with normal-appearing) crystalloid granules, support the possibility that the vesiculotubular structures react with crystalloid granules possibly in transporting endocytosed material to them. Uptake of extracellular substance into the crystalloid granule has been shown to occur in horse eosinophils (6). Such activity in eosinophils would parallel that in mast cells whereby microendocytosis converts specific granules to heterophagic bodies (24), except that in mast cells, solid particles such as gold spherules are transported to the specific granules. Neutrophils revealed no alteration of their cytoplasmic granules in the present study and appeared to preserve their granules as primary lysosomes storing enzymes for fusion with phagocytic vacuoles.

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FIGURE 12 This eosinophil recovered 30 min after the PBS-gold injection contains gold spherules on the cell surface (*1*), in endocytic vesicles (*2*), and in a cup-shaped structure (*3*). The insets enlarge one of the vesicles and the cup-shaped structure enclosing gold. × 19,300. *Inset*, × 37,500.

FIGURE 13 Gold-bearing caveolae in a cell like that in Fig. 12 bud from subsurface vacuole-like profiles. × 43,900.

FIGURE 14 An eosinophil like that in Fig. 12 reveals clumps of gold particles in cup-shaped structures (*3*), a small granule (*4*), and an undefined structure (*S*). These cup-shaped structures carrying gold spherules are larger than the usual ones (arrows). Insets enlarge the gold-containing structures. × 24,100. *Inset*, × 36,500.

FIGURE 15 Like Fig. 12. Gold particles lie in the small granules (arrows) but not in the crystalloid granules. Vesiculotubular structures and cup-shaped structures are abundant. × 21,400.

FIGURE 16 Like Fig. 12. Aggregates of gold particles occupy small granules. × 22,300.

FIGURE 17 Like Fig. 12. Gold particles lie in a body possibly related to or derived from a cup-shaped structure. × 30,000.

FIGURE 18 This neutrophil harvested 60 min after the PBS-gold perfusion reveals clumps of gold particles in a large cytoplasmic vacuole, probably a phagosome, but is typically devoid of gold spherules elsewhere. × 9,500.

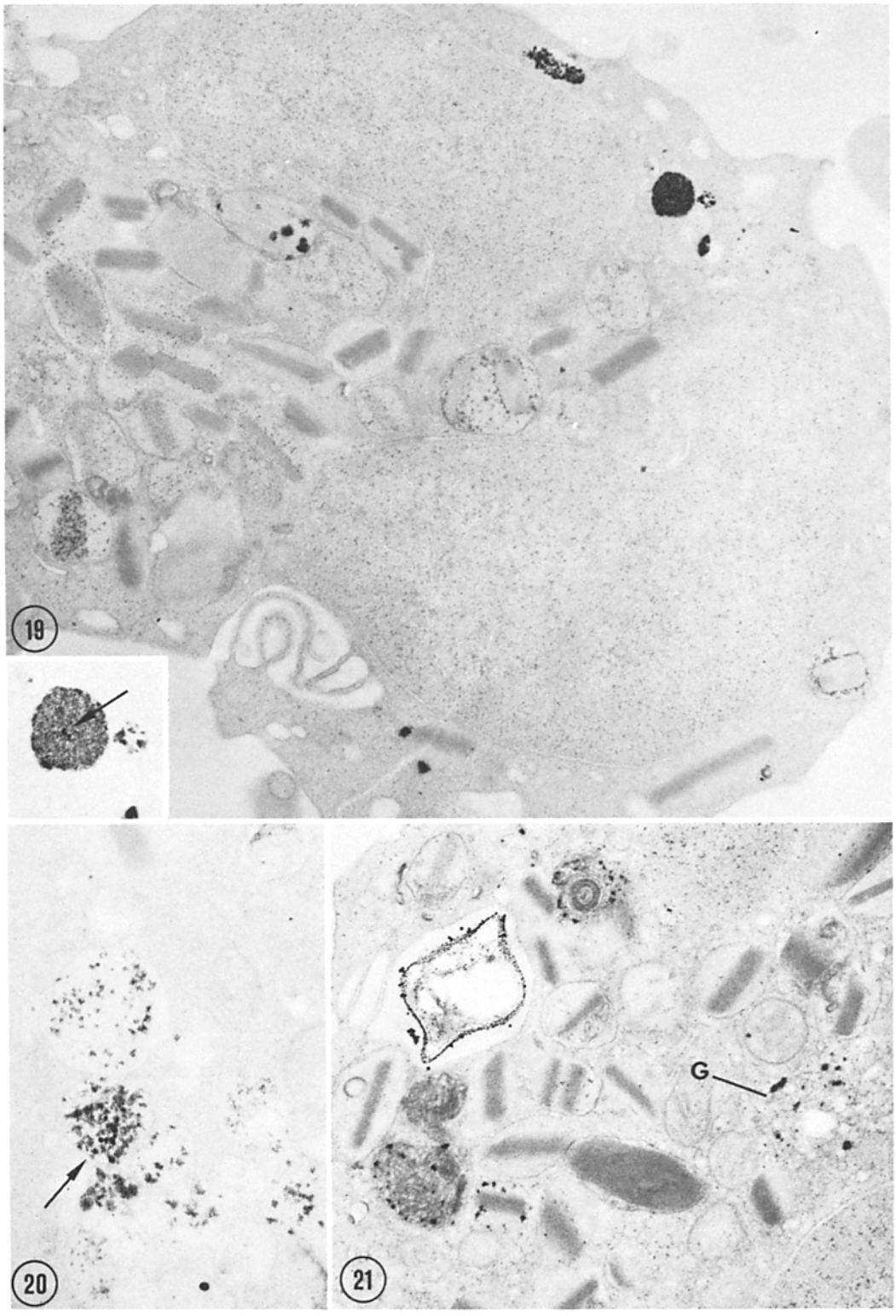
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FIGURE 19 Small granules (upper right) containing intense acid phosphatase activity contrast with normal crystalloid granules which display weak or no reactivity. Altered crystalloid granules reveal somewhat more reaction product in the matrix or crystalloid. The inset enlarges one of the small granules in which gold particles (arrow) and heavy reaction product coexist. The cell was harvested 60 min after the PBS-gold injection. × 20,800. *Inset*, × 42,000.

FIGURE 20 Like Fig. 19. Several small granules (arrow) display acid phosphatase activity and contain both gold particles and reaction product. × 47,500.

FIGURE 21 Like Fig. 19. Acid phosphatase is evident in the Golgi apparatus (*G*) and some of the normal or altered crystalloid granules. A small labyrinth of vacuole-like spaces encloses a structure containing gold particles and fine granular material possibly comprised in part as least of reaction product. × 23,800.

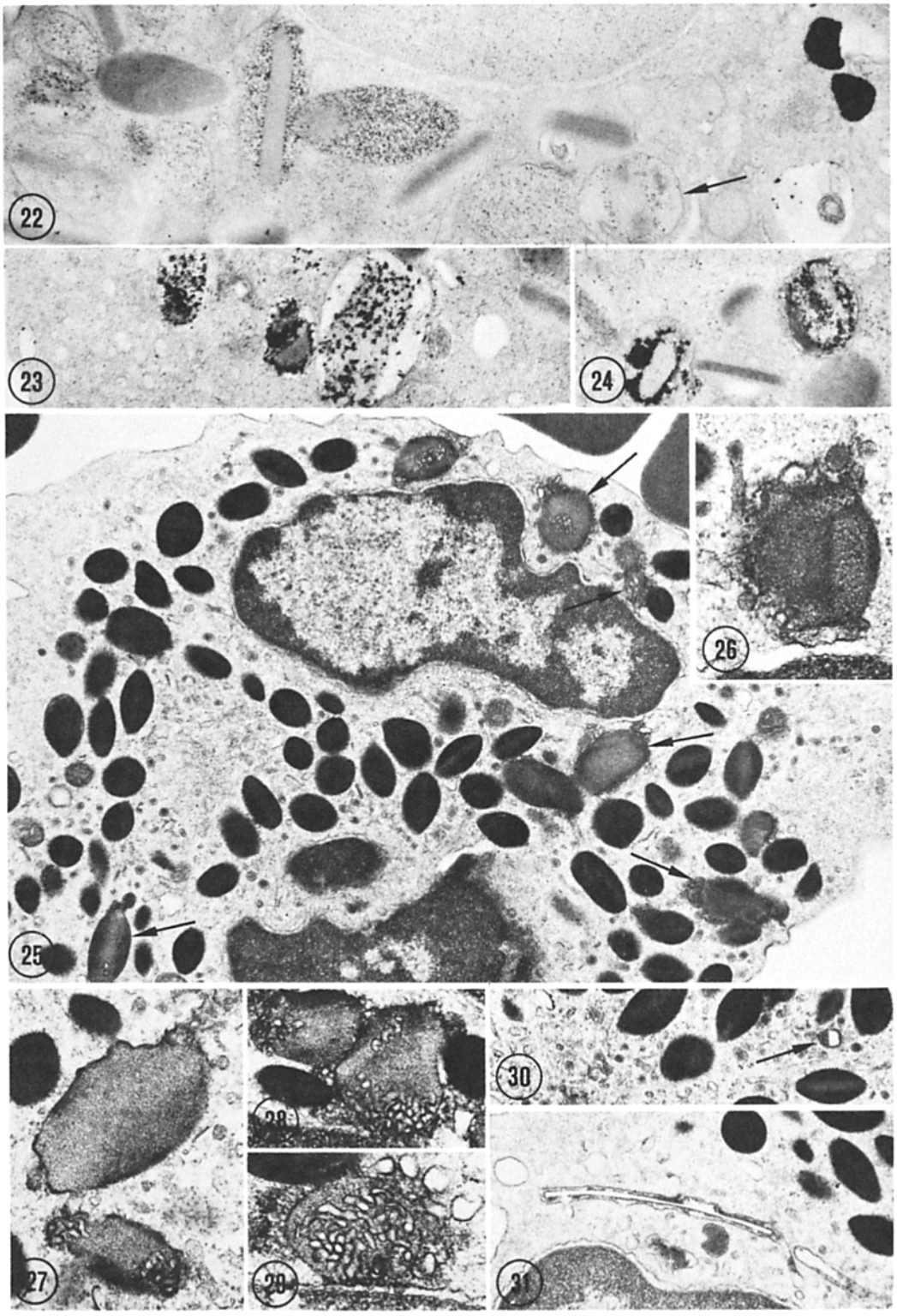
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FIGURE 22 Like Fig. 19. In an altered crystalloid granule (arrow), reaction product indicative of acid phosphatase borders the crystalloid, and in some of the others deposits fill the matrix. Two small granules at upper right reveal strong acid phosphatase activity. × 23,800.

FIGURE 23 Like Fig. 19. A large altered granule with extracted-appearing matrix displays heavy reaction product exclusively in the crystalloid. Several vesicular structures at the lower left lack acid phosphatase activity. × 22,600.

FIGURE 24 Like Fig. 19, showing altered crystalloid granules with strong acid phosphatase reaction product. × 24,000.

FIGURE 25 This eosinophil harvested 30 min after injection of fetal calf serum displays altered crystalloid granules (arrows), several of which lie adjacent to the nucleus. Vesiculotubular structures of moderate density border the altered granules and are distributed throughout the cytoplasm in abundance. × 17,500.

FIGURE 26 Like Fig. 25, demonstrating another altered granule which is bordered by vesicular and tubular structures, and vesicles with a clear content. The limiting membrane of this granule is obscured. × 37,500.

FIGURES 27–29 Like Fig. 25. Membranous lamellae occupy a small-to-large portion of the altered crystalloid granules. The altered granules are bordered by vesicular-tubular structures (Fig. 27) and empty-appearing vesicles (Fig. 29). Fig. 27, × 34,500. Fig. 28, × 27,000. Fig. 29, × 34,500.

FIGURE 30 Like Fig. 25. A small granule (arrow) encloses an electron-lucent crystal. Vesiculotubular structures are crowded in this field. A few cup-shaped structures are also present. × 17,500.

FIGURE 31 Small-type granules are elongated by lucent crystals. One small granule lacks such a lucent silhouette. The cell was harvested 60 min after the fetal calf serum injection. × 19,000.