THE ENTRY OF GRANULE-ASSOCIATED PEROXIDASE INTO THE PHAGOCYTIC VACUOLES OF EOSINOPHILS*

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Since the unique granules of the eosinophil probably hold the key to whatever specialized functions the cell may have, their composition has been of particular interest.

Histochemical analysis has indicated the presence of protein (1), lipid (2), and metals (3); and biochemical analysis of isolated granules has revealed the presence of hydrolytic enzymes and a particularly high content of peroxidase (4). The peroxidase has been localized by electron microscopy to the external matrix, rather than the internal crystalloid core (5–8). Insofar as they are subcellular organelles containing hydrolytic enzymes within their limiting membrane, eosinophil granules may be classified as lysosomes (9, 10).

Recent studies have shown that the lysosomes of the polymorphonuclear leukocytes are involved in a variety of physiological and pathological events (9, 10). In particular, the granules are intimately associated with the process of phagocytosis (9–14). Electron microscopic studies have established that within minutes after ingestion of a particle, the membranes of the granules fuse with those of the phagocytic vacuoles, and the lysosomal enzymes of the granule gain access to the vacuole (10, 11). In the case of the neutrophil, acid phosphatase (5) and, more recently, peroxidase (15) have been localized by ultrastructural histochemistry within phagocytic vacuoles.

Although the phagocytic efficiency of eosinophils is less than that of neutrophils, they are capable of phagocytizing all classes of microorganisms (16), and the phenomena observed in neutrophils occur also in eosinophils. Thus, when the eosinophil granule contacts the ingested particle, it joins its membrane to that of the phagocytic vacuole (10, 11), changes its refractive index (17), and appears to explode (18). Materials of the same electron density as the granule appears within the vacuole (11), and the evidence suggests that the granule's contents, and hence its enzymes, enter the phagocytic vacuole.

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In this communication, we report on the fine structural distribution of peroxidase in eosinophilic leukocytes during phagocytosis. The relative homogeneity (with respect to peroxidase) of the eosinophil's granules facilitated the tracing of the enzyme's fate.

Materials and Methods

Collection of Leukocytes.—Peritoneal exudate cells were lavaged from the peritoneal cavity of guinea pigs (19), concentrated by centrifugation for 3 min at 750 rpm (Sorvall RC2B), resuspended, and counted in an hemocytometer. The solution used for lavage and resuspension was Medium 199, Hanks' base, with 10% calf serum (Baltimore Biological Labs., Baltimore, Md.). When exudates were obtained from normal 275 g animals, the eosinophil content ranged from 0 to 24%, but was usually less than 5%. Exudates containing much higher concentrations of eosinophils (28–66%) were obtained from animals which had been immunized by weekly intraperitoneal injections of 0.5 ml of horse serum over the course of 15 to 18 months. The cells were collected 2 days after the last injection, a time at which the eosinophil content is high and the neutrophil content is low (less than 1%). Most of the experiments were carried out with cells derived from immunized animals, because of the advantages of working with high concentrations of eosinophils. Essentially similar results were obtained with cells from nonimmunized animals.

Phagocytosis of Microorganisms.—

Escherichia coli: A strain isolated from the urine of a patient was grown on nutrient agar for 16 hr, transferred to nutrient broth for 3 hr, washed twice in saline and once in Medium 199, and enumerated in a Petroff-Hauser counting chamber. Killed bacteria were used for some experiments: following a 16 hr exposure to 0.4% formalin, the cells were washed as above, and a sample was plated on nutrient agar to verify killing.

Zymosan: 2.5 mg of zymosan (Nutritional Biochemicals Corp., Cleveland, Ohio), suspended in 1 ml of water, were kept in a boiling water bath for 30 min, washed three times in saline and once in Medium 199, and counted in an hemocytometer. For some experiments, boiling was omitted.

Incubation mixtures: 1×10^7 leukocytes were mixed with sufficient viable *E. coli* organisms to yield bacillus/leukocyte ratios of 10:1, 20:1 and 50:1, respectively. The mixture in 2 ml total volume was agitated in 12 x 75 mm polystyrene tubes at 37°C in a reciprocating shaker operating at 150 strokes per minute, and samples were removed at 0, 2, 5, 15, 30, and 60 min. When killed bacilli were used, the ratio was 50:1 and the sampling times were 5, 30, and 60 min. When leukocytes from unsensitized animals were used, the ratio was 50:1 and the sampling times were 0, 2, 5, and 30 min. Zymosan/leukocyte ratios were 3:1, 5:1 and 10:1 and the sampling time was 5 and 30 min.

Preparation of smears for light microscopy: Cell suspensions were diluted to a concentration of 2×10^5 cells/ml and one drop was transferred to a slide in a Cytocentrifuge (Shandon Scientific Co., London, England). The degree of phagocytosis was assessed on Wright-stained smears by enumerating the proportion of leukocytes containing microorganisms and the number of microorganisms per cell.

Electron Microscopy.—Suspensions of cells were centrifuged at 750 rpm for 3 min and the pellet resuspended by means of gentle shaking in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, with 0.01% calcium chloride added. The cells, kept in suspension by periodic shaking, were fixed for 40 min. They were then washed twice in cacodylate buffer and kept in the same medium for 16 to 48 hr at 4°C. For the cytochemical demonstration of peroxidase, the buffer was decanted and the fixed cells were resuspended in 10 ml of incubation medium, containing 0.05% diaminobenzidine tetrahydrochloride, 0.01% H_2O_2 in 0.05 M tris(hydroxy-

methyl)aminomethane (Tris) buffer at pH 7.6 (20). After incubation for 5 to 20 min, the cells were washed three times in buffer and then postfixed at 4°C for 1 hr in 1.3% osmium tetroxide in pH 7.4 collidine buffer, with 0.01% calcium chloride. They were then dehydrated in increasing concentrations of alcohol, infiltrated with 1:1 propylene oxide–Epon 812 mixture for 1 hr, and embedded in Epon 812 at the bottom of conical polypropylene centrifuge tubes (Nalgene Piping Systems, Rochester, N. Y.) or B.E.E.M. capsules (Bronx, N. Y.). When the pellets were over 1 mm in thickness, portions were distributed into different tubes or capsules. Thin sections were cut with a diamond knife on an LKB III Ultrotome, and some were stained with either lead citrate or a combination of uranyl acetate and lead citrate. Observations were made with a Philips EM 200 electron microscope.

The prime control for the specificity of the peroxidase reaction was the omission of hydrogen peroxide from the histochemical incubation medium. In addition, observations were made on cells which had been (*1*) fixed in osmium without prior exposure to the histochemical incubation medium; (*2*) exposed for 5 to 30 min to Medium 199 without organisms and then reacted for peroxidase in the usual manner; (*3*) fixed in glutaraldehyde and then boiled for 10 min either in the fixative or in buffer before incubation in the complete histochemical medium; (*4*) incubated in complete histochemical medium containing in addition (*a*) 10^{-1}, 10^{-2}, or 10^{-3} M potassium cyanide, (*b*) high concentrations (1%) of H_2O_2, (*c*) 2×10^{-2} M triamino 1,2,4-triazole (Mann Research Labs., N. Y.), or (*d*) 10^{-1} or 10^{-2} M sodium azide; and (*5*) incubated in diaminobenzidine for 10 min and then in both diaminobenzidine and 3×10^{-3} M potassium ferricyanide for an additional 10 min (20).

RESULTS

Light Microscopy.—Bacteria were seen inside eosinophils within 5 min after the start of incubation. At this time, 10% of the eosinophils contained one to two bacilli each, when the bacillus/leukocyte ratio was 10:1. After 15 min, 75% of the eosinophils contained two to three bacilli each, and after 30 min, virtually all eosinophils contained an average of seven bacilli each. Since granules lyse in less than 1 sec (12), the chance of witnessing granule-vacuole interaction should be enhanced by a high content of organisms per cell. This was brought about by increasing the bacillus/leukocyte ratio to 50:1. Under this circumstance, each eosinophil contained at least five bacilli after 5 min and 10–20 bacilli after 30 min.

The phagocytosis of zymosan proceeded more rapidly. Within 5 min, 75% of the eosinophils contained one to four particles and after 30 min, virtually every eosinophil contained a like number of particles.

Electron Microscopy.—

Peroxidase in granules: Peroxidase activity, indicated by a black reaction product, was localized to the matrix of almost all the granules (Figs. 1 and 2). The density of the central crystalloid varied depending on the length of incubation in the histochemical medium. After incubation for 10 min or less, the crystalloid appeared virtually unstained (Fig. 3 *a*), while after incubation for 20 min the crystalloid appeared moderately dense and the granules fractured on sectioning. Occasionally there was fine punctate black speckling over the crystalloids (Fig. 3 *a*); this was attributed to the use of lead or uranyl acetate stains, since no such speckling was seen when the stains were omitted (Fig.

3 *b*). Occasional granules with no central crystalloid were positive in their entirety. In preparations in which hydrogen peroxide had been omitted from the histochemical medium or in preparations not exposed to the histochemical medium at all, the central crystalloid appeared more electron dense than the peripheral matrix in over 90% of the granules (Fig. 4). An occasional granule had a totally lucent internum and grey externum. Several studies have emphasized that the comparative electron density of internum and matrix in eosinophil granules (20) varies according to fixation procedures, incorporation of stains, and other factors (21–24). With the routine method which we adopted,

TABLE I

Controls for specificity of peroxidase reaction in granules of guinea pig peritoneal eosinophils

Incubation mixture			Black reaction product in matrix of granule
DAB*	H_2O_2 (0.01%)	Special conditions	
0	0	0	−
+	0	0	−
+	+	0	+
+	+‡	0	+
+	+	Triaminotriazole (2×10^{-2} M)	+
+	+	KCN (10^{-2} M)	+
+	+	KCN (10^{-1} M)	±
+	+	NaN_3 (10^{-2} M)	±
+	+	NaN_3 (10^{-1} M)	−
+	+	Boiled cells	−
+	0	$K_3Fe(CN)_6$, (3×10^{-3} M)	−

* Diaminobenzidine tetrahydrochloride (Sigma Chemical Co.), 0.05% in 0.05 M Tris buffer at pH 7.6.

‡ Concentration was 1%.

however, no dense black matrix was observed unless the specimens had been exposed to the complete histochemical medium. In nonphagocytizing eosinophils, the reaction product was limited to the granules proper; while many eosinophils exhibited golgi complexes and small arrays of endoplasmic reticulum, these organelles were free of peroxidase activity.

The specificity of the histochemical reaction was explored in various ways (Table I). Neither excess H_2O_2, nor triamino 1,2,4-triazole eliminated the black reaction product in the matrix of eosinophil granules. In some preparations reacted in the presence of 10^{-1} M cyanide, the black reaction product seemed less intense, but this effect could not be strictly quantitated. Prior boiling of the cells inhibited the reaction. Sodium azide (10^{-1} M) markedly reduced the intensity of the reaction, but 10^{-2} M azide reduced the reaction only in a minor proportion of the granules. Diaminobenzidine which had been oxidized by ferricyanide showed no affinity for the granules.

Phagocytosis of zymosan granules: All stages of phagocytosis of zymosan granules by eosinophils were encountered. One to four zymosan particles were found in a single cell, and, at times several others were attached to the surface or were being engulfed by pseudopods (Fig. 5). The intracellular organisms were bound by a membrane which was within a distance of 100 to 1000 A of the zymosan capsules. Some, but not all of the phagocytic vacuoles exhibited peroxidase activity, which was most commonly seen (Figs. 5 and 6) as a rim of black material filling the space between the zymosan capsule and the vacuole membrane along part or all of the perimeter of the capsule. Many images suggesting fusion of granules and phagocytic vacuoles were encountered. The most common appearance was that of one or several granule-shaped masses of peroxidase within the limiting membrane of the vacuole and continuous with the rim of the reaction product surrounding the zymosan particle (Figs. 7 and 8). A crystalloid was rarely encountered in the granules at this stage. Some phagocytic vacuoles showed peroxidase-containing outpouchings and tortuous channels (Fig. 8). The internal core was sometimes absent also from some of the granules which lay extremely close to, but not within, the phagocytic vacuoles.

Some granules, which were not in apparent association with a phagocytic vacuole, exhibited other morphologic changes. They were larger than the normal granule, at times occupying more than three times the normal area. These larger granules generally had no crystalloid core, and the black-staining matrix was intercalated with less dense material or, in the case of the larger granules, with no apparent material at all. Some of these structures communicated, as sequential sections showed, with a phagocytic vacuole, but most did not. These structures did not appear to reflect a methodologic artifact, since other granules in the same cell and adjacent cells exhibited quite normal morphology (Fig. 8). Nor were these alterations a specific consequence of phagocytosis, as they were seen in eosinophils which had been incubated without organisms for 30 min.

In some preparations, the reaction product was found extracellularly between the surface membrane of contiguous cells. We did not determine whether this observation reflected an artifact induced by mechanical disruption of the cells during processing, or whether it represented a biological phenomenon induced by phagocytosis. In any case, the enzyme was seen outside cells only when eosinophils had been incubated with zymosan.

The cytoplasmic granules of peritoneal macrophages only rarely contained peroxidase; reaction product was present in the endoplasmic reticulum, golgi apparatus, and perinuclear cisternae of some of these cells, but the specificity of this reaction for peroxidase was not determined. Zymosan was also phagocytized by macrophages, but in contrast to eosinophils, there was no peroxidase activity in the phagocytic vacuoles (Fig. 9). Occasional membrane-bound vacuoles containing eosinophil granules were seen within macrophages, and these were considered to represent phagocytosis of disrupted eosinophils.

Phagocytosis of E. coli: Phagocytic vacuoles containing bacteria and a rim of peroxidase were seen as early as 5 min after incubation (Figs. 10 and 11). These were similar to those described for zymosan, but they were encountered with much less frequency, and no images showing clear-cut fusion of granules with vacuoles were seen. The lesser incidence of peroxidase in vacuoles may simply be related to the smaller surface area occupied by phagocytized bacteria, but we did not pursue this or any other biological or technical explanation further.

DISCUSSION

The observations recorded in this paper provide direct evidence for the release of a granule-associated enzyme into the phagocytic vacuoles of eosinophils, a phenomenon which had been strongly suggested by previous biochemical and morphologic studies. The enzyme studied was peroxidase and advantage was taken of the availability of a simple, reproducible and sensitive method for the localization of this enzyme by electron microscopy (18). The presence of a black reaction product is ascribed to peroxidase activity, since the reaction was consistently negative in the absence of H_2O_2, was inhibited by boiling and by azide, and was not elicited nonspecifically by oxidized diaminobenzidine. However, it should be noted that cyanide, a peroxidase inhibitor, did not abolish the reaction. In addition, triamino 1,2,4-triazole, a catalase inhibitor (25), did not affect the reaction. These compounds inhibit exogenous (horse radish) peroxidase and (beef liver) catalase, respectively; the reasons for their failure to act on endogenous enzymes in eosinophils are not apparent.

In contradistinction to the neutrophil's granules, those of the eosinophil appear to be homogeneous in peroxidase content. In our studies of guinea pig eosinophils, peroxidase was limited to the matrix of the granule, except upon prolonged (20 min) reaction with substrate. This is in keeping with the results of other studies, which showed that peroxidase is present only in the external matrix of the granules of rabbits (8), humans (5) and rats (6, 7). The same localization has been described for acid phosphatase (26–28). In addition, non-crystalloid-containing granules were positive in their entirety, an observation which is similar to that reported by Bainton and Farquhar (8) in mature and immature rabbit eosinophils.

In our experiments with zymosan particles, phagocytosis took place in the absence of added immune (anti-zymosan) serum. The morphologic appearance of granule fusion and rupture was almost identical with that recorded by Zucker-Franklin and Hirsch in rabbit peritoneal eosinophils during ingestion of zymosan (11). These authors did not use any enzyme-specific cytochemical method, but their micrographs clearly show the grey contents of eosinophilic granules encircling zymosan particles. The actual indentification of an enzyme in phagocytic vacuoles was made by Enomoto and Kitani (5), who demonstrated acid phosphatase in the vacuoles of human neutrophils. More recently, Baehner

et al. (15) have demonstrated peroxidase in the vacuoles of human neutrophils following the ingestion of zymosan, and our electron microscopic findings show that a similar process occurs in the case of the eosinophil leukocyte.

We found no clear-cut image of intact crystalline cores within phagocytic vacuoles of guinea pig eosinophils, even in preparations not reacted for peroxidase (in which the crystalloids would be relatively electron dense). In addition, cores were often absent in those granules that appeared to be in the process of fusing with a vacuole. At present, we can offer no explanation for this phenomenon. It is unlikely that only noncrystal containing granules fuse, since the latter are infrequent in normal eosinophils. The crystal may become solubilized during degranulation, but no data is available on this point. The fate of the crystal may well be related to the species of origin. In a preliminary experiment with human peripheral blood eosinophils, apparently intact crystalloids (as well as peroxidase) were seen in phagocytic vacuoles surrounding zymosan particles. Zucker-Franklin et al. (29) also reported intact crystals in *human* eosinophil phagosomes after ingestion of mycoplasma; this was in contrast to the presence of disrupted crystalloids in *rabbit* peritoneal eosinophils after phagocytosis of zymosan particles.

Neutrophils and eosinophils are rich in peroxidase, but the function of this enzyme is unknown. Klebanoff (30) recently suggested that neutrophil peroxidase may play a role in the bactericidal activity of neutrophils by catalyzing the iodination of bacteria in the presence of iodide ions and hydrogen peroxide. Whether such a mechanism may also apply to eosinophils is conjectural. Eosinophil peroxidase differs from neutrophil peroxidase (31), but studies on bactericidal action within eosinophils are not available (32). In any event, bactericidal activities cannot be a major function of the eosinophil, because under ordinary circumstances this cell is not a respondent to bacterial invasion.

SUMMARY

The ultrastructural localization of peroxidase was studied in guinea pig eosinophils which had phagocytized zymosan or *Escherichia coli*. After phagocytosis, the membrane of the granule was joined to the membrane of the phagocytic vacuole, and the enzyme, which is ordinarily restricted to the matrix of the granule, was seen in the vacuole surrounding the ingested particle.

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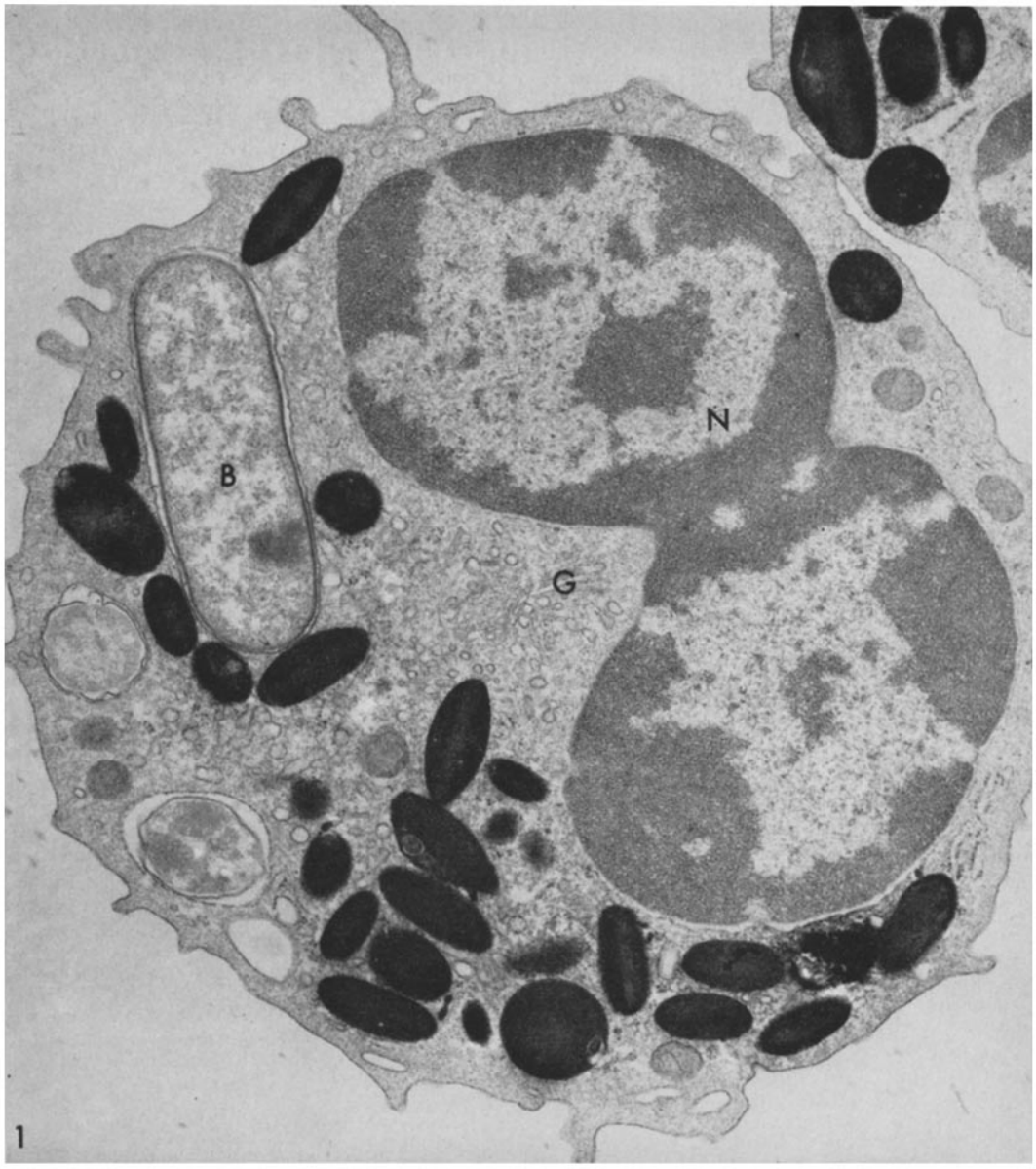
BIBLIOGRAPHY

1. Vercauteren, R. 1951. A cytochemical approach to the problem of the significance of blood and tissue eosinophilia. *Enzymologia*. **14**:340.
2. Sheehan, H. L. 1939. The study of leucocyte granules by Sudan Black B. *J. Pathol. Bacteriol.* **49**:580.

3. McNary, W. F. 1960. The histochemical demonstration of trace metals in leucocytes. *J. Histochem. Cytochem.* **8**:124.
4. Archer, G. T., and J. G. Hirsch. 1963. Isolation of granules from eosinophil leukocytes and study of their enzyme content. *J. Exp. Med.* **118**:277.
5. Enomoto, T., and T. Kitani. 1966. Electron microscopic studies on peroxidase and acid phosphatase reaction in human leukocytes. *Acta Haematol. Jap.* **29**:554.
6. Kelenyi, Von G., E. Zombai, and A. Nemeth. 1965. Histochemische und elektronenmikroskopische Beobachtungen an der spezifischen Granulation der eosinophilen Granulozyten. *Acta Histochem.* **22**:77.
7. Yamada, E., and R. Yamauchi. 1966. Some observations on the cytochemistry and morphogenesis of the granulocytes in rat bone marrow as revealed by electron microscopy. *Acta Haematol. Jap.* **29**:530.
8. Bainton, D. F., and M. G. Farquhar. 1967. Segregation and packaging of granule enzymes in eosinophils. *J. Cell Biol.* **35**:6A.
9. Weissmann, G. 1965. Lysosomes. *N. Engl. J. Med.* **273**:1084, 1143.
10. Zucker-Franklin, D. 1968. Electron microscopic studies of human granulocytes: structural variations related to function. *Seminars Hematol.* **5**:109.
11. Zucker-Franklin, D., and J. G. Hirsch. 1964. Electron microscope studies on the degranulation of rabbit peritoneal leukocytes during phagocytosis. *J. Exp. Med.* **120**:569.
12. Hirsch, J. G. 1962. Cinemicrophotographic observations on granule lysis in polymorphonuclear leukocytes during phagocytosis. *J. Exp. Med.* **116**:827.
13. Horn, R. G., S. S. Spicer, and B. K. Wetzel. 1964. Phagocytosis of bacteria by heterophil leukocytes. Acid and alkaline phosphatase cytochemistry. *Amer. J. Pathol.* **45**:327.
14. Lockwood, W. R., and F. Allison. 1964. Electron micrographic studies of phagocytic cells. II. Observations of the changes induced in the cytoplasmic contents of human granulocytes by the ingestion of rough pneumococcus. *Brit. J. Exp. Pathol.* **45**:294.
15. Baehner, R. L., M. J. Karnovsky, and M. L. Karnovsky. 1969. Degranulation of leukocytes in chronic granulomatous disease. *J. Clin. Invest.* **48**:187.
16. Weinberg, M., and P. Séguin. 1914. Recherches biologiques sur l'éosinophilie. *Ann. Inst. Pasteur Paris* **28**:470.
17. Hardy, W. B. 1898. Further observations upon the action of the oxyphil and hyaline cells of frog's lymph upon bacilli. *J. Physiol.* **23**:359.
18. Archer. G. T., and J. G. Hirsch. 1963. Motion picture studies on the degranulation of horse eosinophils during phagocytosis. *J. Exp. Med.* **118**:287.
19. Litt, M. 1960. Studies in experimental eosinophilia. I. Repeated quantitation of peritoneal eosinophilia in guinea pigs by a method of peritoneal lavage. *Blood.* **16**:1318.
20. Graham, R. C., and M. J. Karnovsky. 1966. The early stage of absorption of horse-radish peroxidase in the proximal tubule of the kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291.
21. Miller, F., E. DeHarven, and G. E. Palade. 1966. The structure of eosinophil leukocyte granules in rodents and man. *J. Cell Biol.* **31**:349.
22. Faller, A. 1966. Zur Frage von Struktur und Aufbau der eosinophilen Granula. *Z. Zellforsch.* **69**:551.

23. Hudson, G. 1967. Eosinophil granules and uranyl acetate: an electron microscopy study of guinea pig bone marrow. Exp. Cell Res. **46**:121.
24. Ghadially, R. N., and E. W. Parry. 1965. Probable significance of some morphologic variations in eosinophil granule revealed by the electron microscope. *Nature.* **206**:632.
25. Fahimi, H. D. 1968. Cytochemical localization of peroxidase activity in rat hepatic microbodies (peroxisomes). *J. Histochem. Cytochem.* **16**:547.
26. Miller, F. 1966. Electron microscopic cytochemistry of leukocyte granules. *In* Proceedings Sixth International Congress of Electron Microscopy. R. Uyeda, editor. Maruzen Co., Ltd., Tokyo. **2**:71.
27. Ghidoni, J. J., and A. F. Goldberg. 1966. Light and electron microscopic localization of acid phosphatase activity in human eosinophils. *Amer. J. Clin. Pathol.* **45**:402.
28. Seeman, P. M., and G. E. Palade. 1967. Acid phosphatase localization in rabbit eosinophils. *J. Cell Biol.* **34**:745.
29. Zucker-Franklin, D., M. Davidson, and L. Thomas. 1966. The interaction of mycoplasmas with mammalian cells. I. Hela cells, neutrophils and eosinophils. *J. Exp. Med.* **124**:521.
30. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **126**:1063.
31. Archer, G. T., G. Air, M. Jackas, and D. B. Morell. 1965. Studies on rat eosinophil peroxidase. *Biochim. Biophys. Acta.* **99**:96.
32. Hirsch, J. G. 1965. Neutrophil and eosinophil leukocytes. *In* The Inflammatory Process. B. W. Zweifach, L. Grant, and R. T. McClusky, editors. Academic Press, New York. 245.



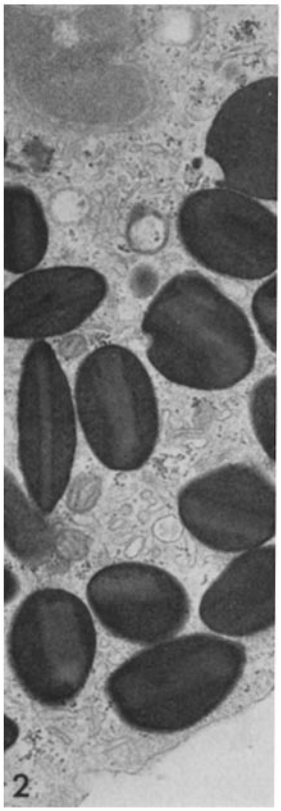
All eosinophils were derived from the peritoneal cavity of guinea pigs. Unless otherwise stated, micrographs are from sections stained with uranyl acetate and lead citrate. Magnifications are approximate.

FIG. 1. Eosinophil incubated with *E. coli* for 15 min and reacted for peroxidase; there are three intracellular bacteria (one labeled B). The matrix of all the granules stains for peroxidase, the crystalloids are less intensely stained. In this cell, there is no peroxidase in the phagocytic pouch. N, nucleus; G, golgi. × 20,000.

FIG. 2. Eosinophil granules after 15 min reaction for peroxidase. The matrix is electron dense; the crystalloids in some granules stain with moderate intensity. × 25,000.

FIG. 3. *a*. Eosinophil granules after 7 min reaction for peroxidase; the section was stained with uranyl acetate and lead. The positive matrix contrasts with the central crystalloid which is negative, except for faint stippling (see *b*). × 22,000. *b*. Eosinophil granules after 7 min reaction for peroxidase. Same preparation as *a*, except that the section has not been stained with uranyl and lead. The crystalloid is nonreactive. × 39,000.

FIG. 4. An eosinophil after exposure to *E. coli* for 15 min and incubation in histochemical medium from which H_2O_2 was omitted. The central crystalloid is dense while the peripheral matrix is light gray. B, phagocytized bacterium. × 26,000.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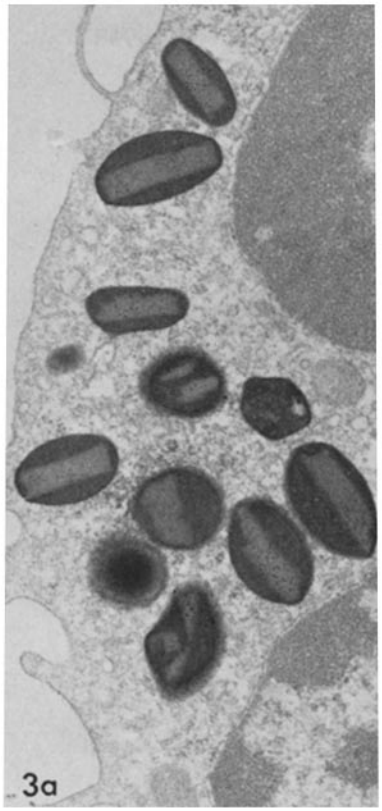
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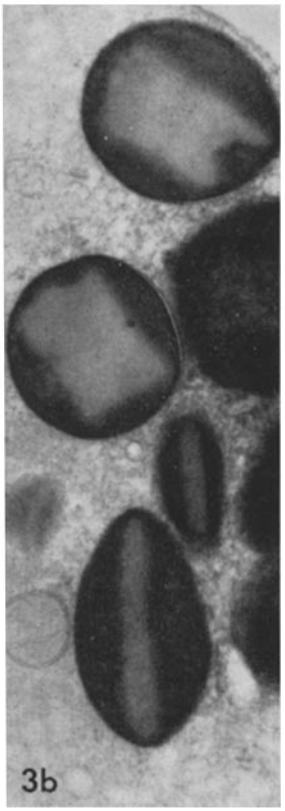
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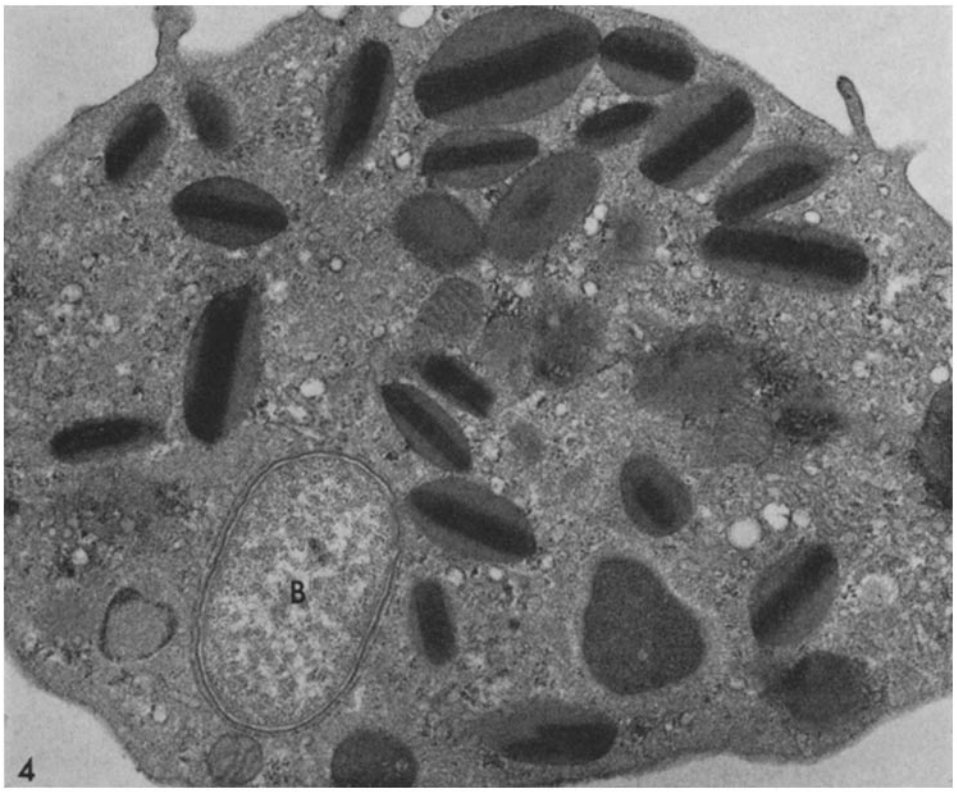
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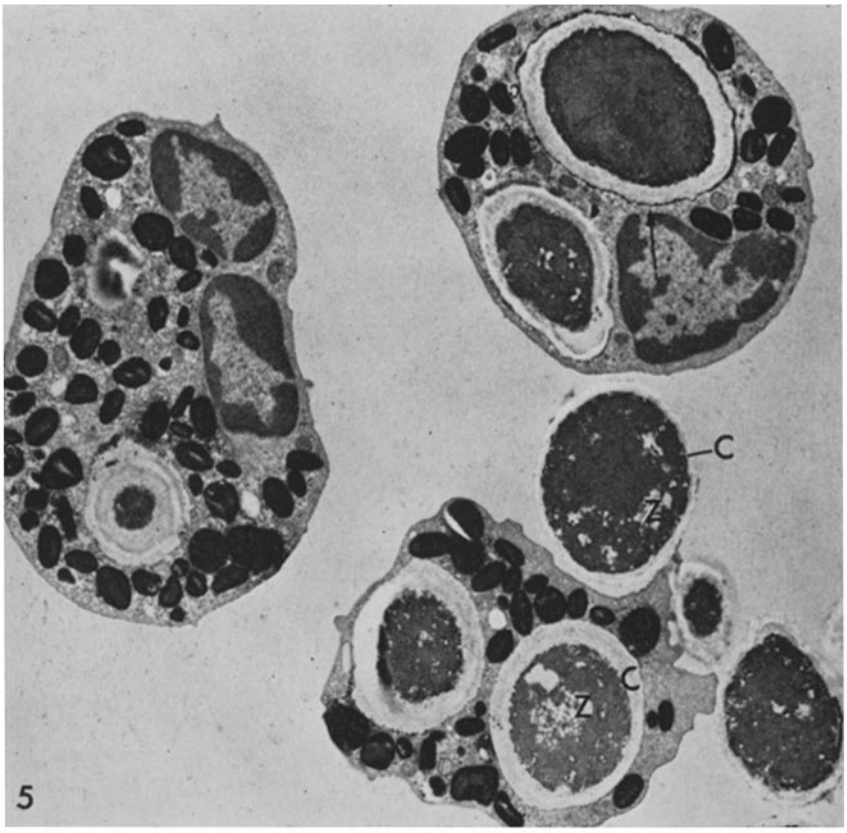


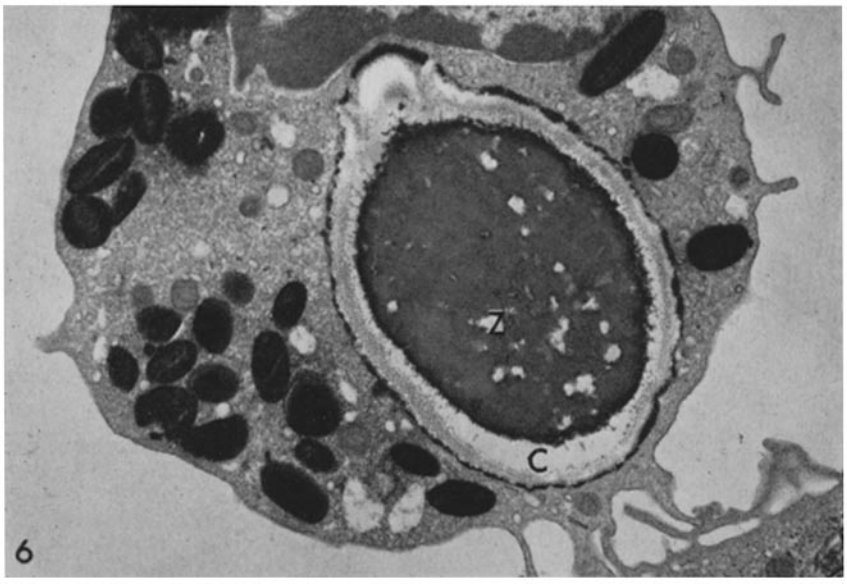


FIG. 5. Survey micrograph of three eosinophils incubated with zymosan for 30 min and reacted for peroxidase. Each zymosan particle consists of a body (Z) and a capsule (C). The arrow points to a phagocytized zymosan particle which is surrounded by a rim of reaction product (peroxidase). × 7000.

FIG. 6. Portion of an eosinophil which has phagocytized a zymosan particle. Note the rim of peroxidase between the capsule (C) of the zymosan and the vacuolar limiting membrane. × 12,000.

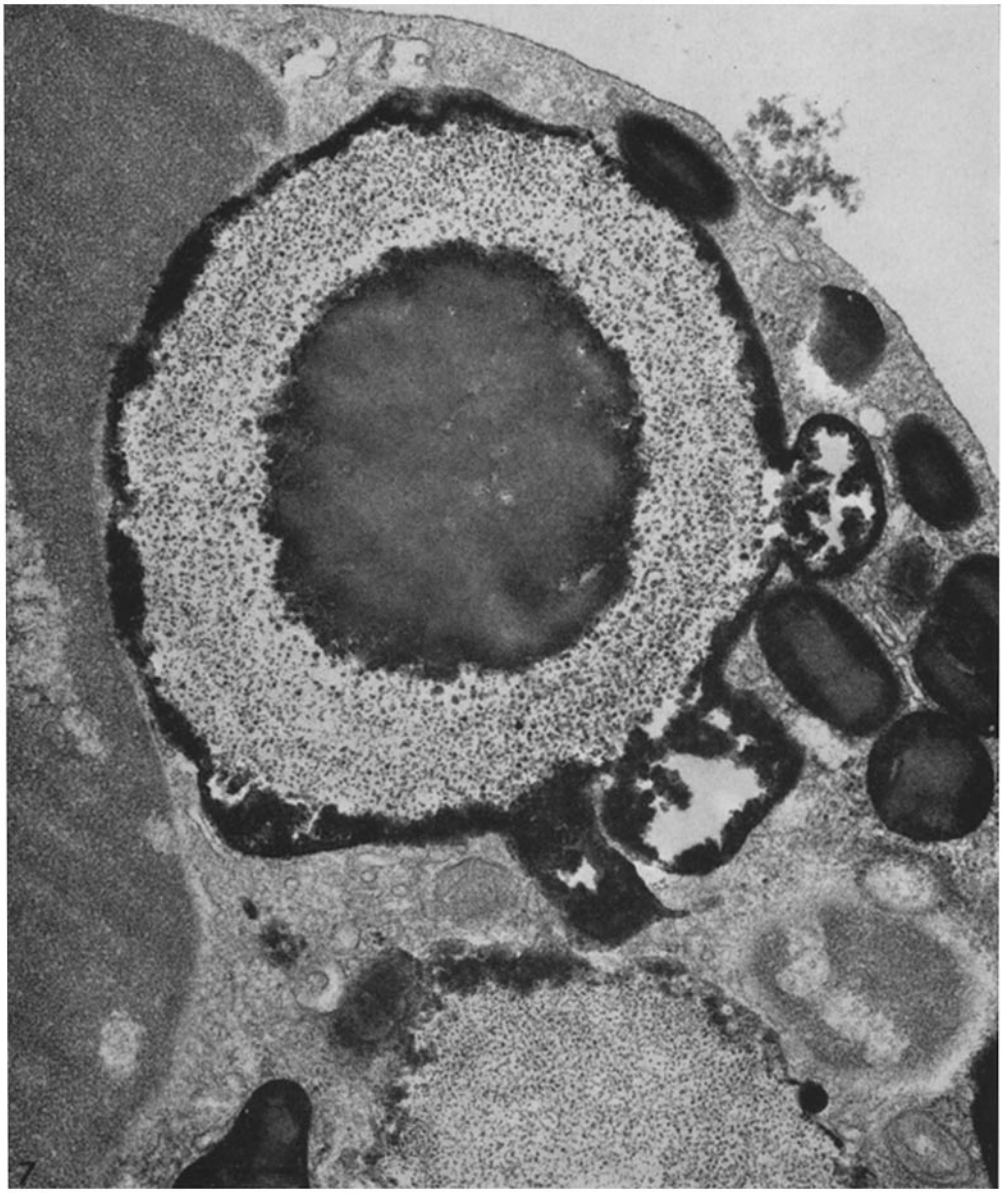
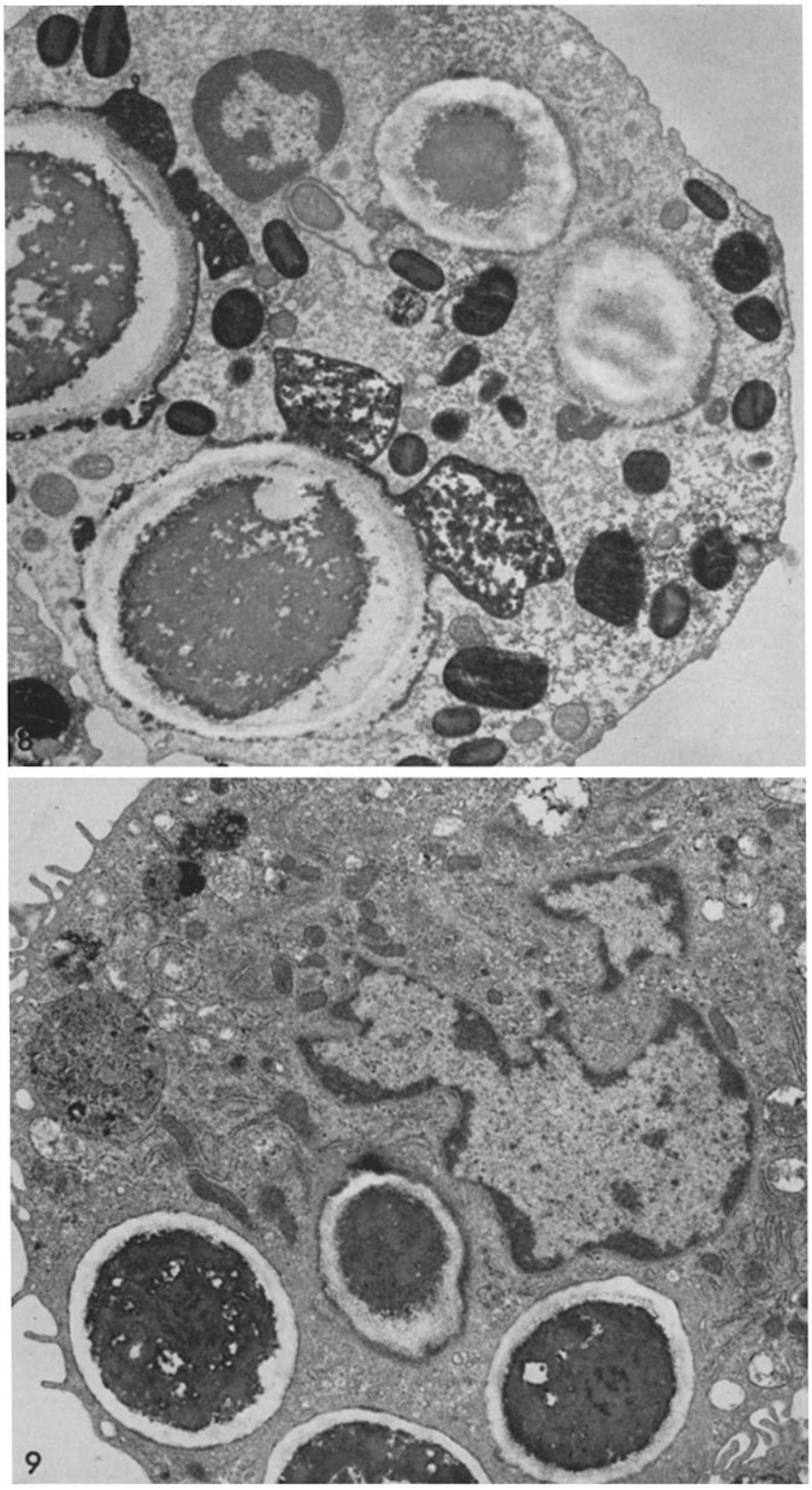
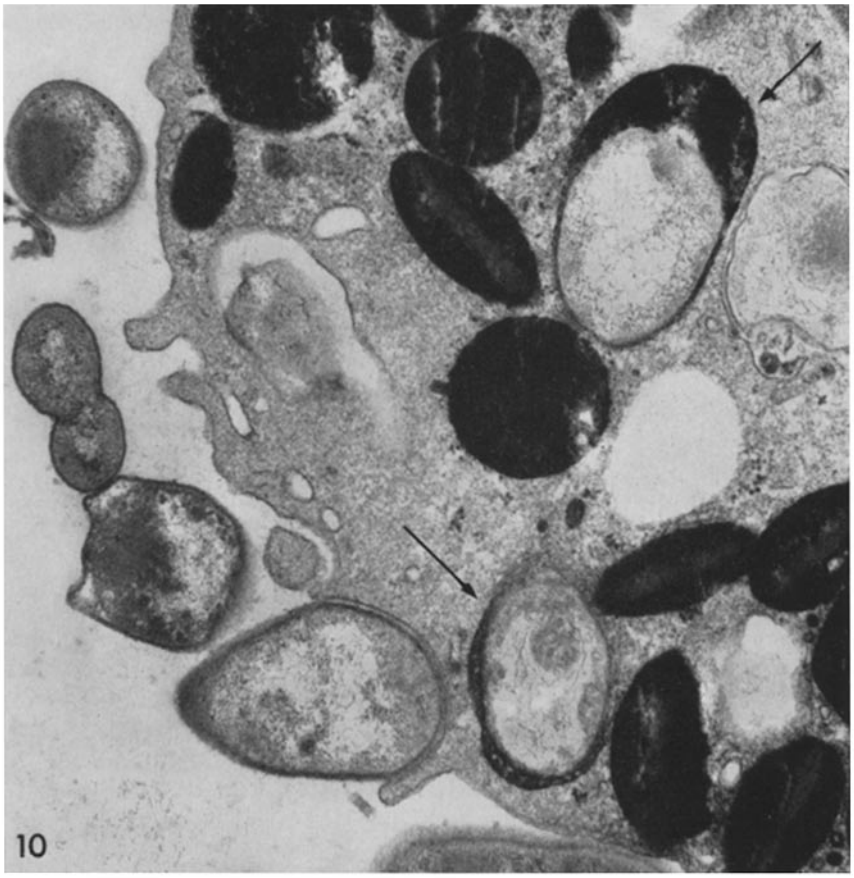


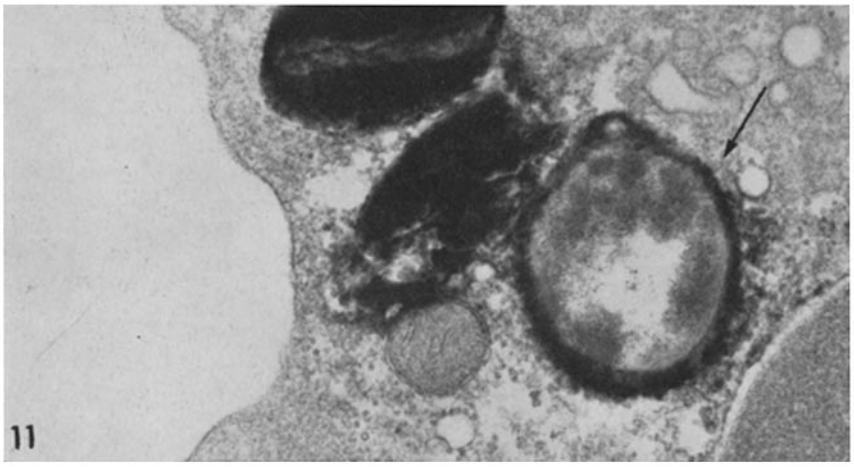
FIG. 7. Portion of an eosinophil showing a phagocytized zymosan particle and structures resembling granules apparently fusing with the phagocytic vacuole. There is reaction product within the granules and the phagocytic vacuole. × 40,000.

FIG. 8. An eosinophil that has phagocytized four zymosan particles. The two particles of the lower left are surrounded with peroxidase. Note the large peroxidase-containing outpouchings of the lower phagocytic vacuole (see text). × 13,000.

FIG. 9. A macrophage containing four zymosan particles and other phagocytic bodies and vacuoles. Reaction product is not seen within the zymosan containing vacuoles. × 10,000.







FIGS. 10 and 11. Portion of an eosinophil which has phagocytized *E. coli*. The arrows point to phagocytic vacuoles which exhibit peroxidase around bacteria. × 39,000; × 55,000.