The American Journal of PATHOLOGY

May 1979 • Volume 95, Number 2

Peroxisomes of Rat Peritoneal Macrophages During Phagocytosis

M. Eguchi, P. L. Sannes, PhD, and S. S. Spicer, MD

The peroxisomes of resident macrophages in the rat peritoneal cavity were examined during the phagocytosis of latex microbeads, employing the alkaline diaminobenzidine (DAB) technique. Peroxisomes generally were located in close proximity to phagosomes and were often observed in a process of apparent fusion with phagosomes. Cytochemical evidence was also obtained for discharge of catalase from peroxisomes to phagosomes. The profiles indicating fusion were observed after 10 minutes of incubation with microbeads. The number of peroxisomes was increased in macrophage profiles examined 30 minutes after exposure to microbeads. Acid phosphatase was localized in small vesicles that were distinct from peroxisomes, and peroxidase was not demonstrable in peroxisomes. A method for ultrastructural localization of periodate reactive complex carbohydrate demonstrated glycoproteins in numerous small vesicles or granules, some of which possibly represented peroxisomes. The possible function of peroxisomes during phagocytosis in rat peritoneal macrophages is considered. (Am J Pathol 95:281–294, 1979)

THE PEROXISOME is a commonly encountered organelle of animal cells,[1,2] functioning in lipid metabolism [3-7] and protection from toxic effects of H_2O_2.[8] However, enzyme composition of peroxisomes varies in different tissues,[9] suggesting that the organelle may function in different capacities in the various sites.[8]

Peroxisomes have been demonstrated cytochemically in macrophages [10,11] but their biologic significance in these cells remains unclear. Macrophages function in phagocytic defense against microorganisms and, during phagocytosis, generate the H_2O_2 that mediates lysosomal bactericidal activity.[12] However, in contrast to monocytes of the blood, resident peritoneal macrophages bear no cytochemical evidence of lysosomal peroxidase for antibacterial action in phagosomes.[13,14]

From the Department of Pathology, Medical University of South Carolina, Charleston, South Carolina.

Supported by Grants AM-10956 and AM-11028 from the National Institutes of Health.

Dr. Sannes is NIH Postdoctoral Fellow IF32GM05854-01, Institute of General Medical Sciences. His present address is Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD 21205.

Accepted for publication December 8, 1978.

Address reprint requests to Dr. Mitsuoki Eguchi, Department of Pathology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29403.

0002-9440/79/0510-0281$01.00

Catalase, a peroxisomal enzyme with bactericidal activity in the presence of H_2O_2,[15] has been identified biochemically in the phagocytic vesicle fraction of lysed alveolar macrophages.[16] The present investigation was undertaken to appraise with ultrastructural cytochemical methods the possible role of peroxisomes in phagocytic activity of rat peritoneal macrophages.

Materials and Methods

Peritoneal fluid was collected from 150- to 200-g Sprague-Dawley rats immediately after the intraperitoneal injection of 15 ml of phosphate-buffered saline (PBS). The fluids were promptly centrifuged and decanted, and cells were resuspended in a mixture of 0.7 ml of Eagle's minimum essential medium (F-12, Gibco Co., Grand Island, N.Y.) with 0.02 ml of an aqueous suspension of polystyrene latex microbeads (0.45 μ, Polysciences, Inc., Warrington, Pa.). The peritoneal cells were incubated for 3, 10, and 60 minutes at 37 C, washed in PBS, and fixed 1 hour at 4 C with 2% glutaraldehyde solution buffered with 0.1 M cacodylate (pH 7.4).

A portion of each glutaraldehyde-fixed specimen was divided into two aliquots, one of which was postfixed with osmium tetroxide, dehydrated, and processed into low-viscosity epoxy resin for subsequent ultrastructural morphologic examination. The other part was carried similarly to the epoxy resin embedment without exposure to osmium tetroxide for subsequent cytochemical localization of periodate-reactive complex carbohydrates in thin sections.

Another portion of the glutaraldehyde-fixed cells was rinsed in the cacodylate-buffered, 7.0% sucrose and was minced with a razor blade into small blocks. The blocks were incubated 1 hour at room temperature with catalase substrate medium consisting of 20 mg 3-3′-diaminobenzidine tetra-HCl (DAB), 9.8 ml 0.05 M Tris buffer, pH 9.5, and 0.2 ml freshly prepared 2.5% H_2O_2. The final pH was adjusted to 9.7 with NaOH.[17] For controls, the cells were incubated in the same medium lacking H_2O_2 or were boiled for 5 minutes in 2% glutaraldehyde before incubation in the complete medium. To determine the inhibitory effect of 3-amino-1H-1,2,4-Triazol (AMT), the specimens were maintained in the buffer containing 0.02 M AMT for 30 minutes at 4 C and were subsequently incubated in the appropriate medium containing 0.02 M AMT. The DAB medium of Graham and Karnovsky was employed at neutral pH on another portion of the glutaraldehyde-fixed cells to localize peroxidase.[18] For acid phosphatase demonstration, some of the glutaraldehyde-fixed cells were incubated in the Barka-Anderson substrate medium as previously detailed.[19] The several enzyme cytochemical preparations were postfixed 1 hour in aqueous 1% osmium tetroxide at room temperature, dehydrated through graded ethanol, and embeded in low-viscosity epoxy resin.[20] Propylene oxide or acetone was avoided in all embedments because they dissolve the latex microbeads. Ultrathin sections of the glutaraldehyde-fixed cells that were not postfixed with osmium tetroxide were stained with the peroidate-thiocarbohydrazide-silver proteinate (PA-TCH-SP) procedure of Thiery [21] for localizing periodate-reactive, vicinal glycols of hexoses in complex carbohydrates. Thin sections were examined at 50 kV in an Hitachi HS-8 electron microscope with and without heavy-metal counterstaining.

Results

Morphology

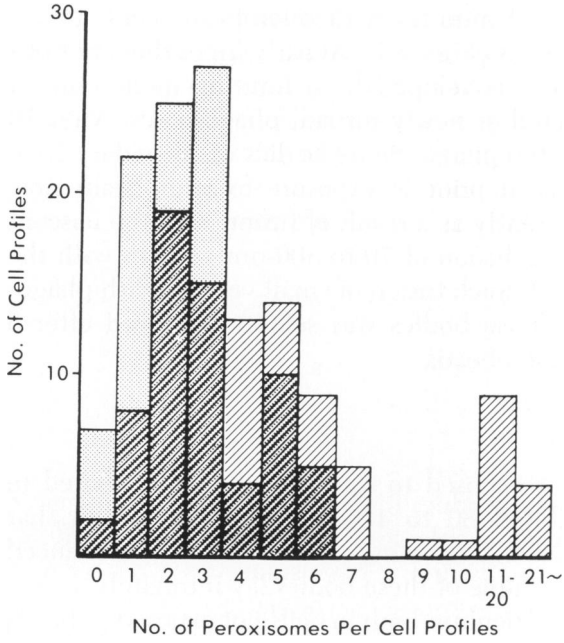
Ultrastructural features of the peritoneal macrophages harvested immediately after injection of the PBS resembled those previously reported.[22]

Macrophages incubated 3 to 60 minutes with microbeads contained a variable number of ingested beads (Figure 1). At early times these peripherally located beads lay closely enveloped by a limiting membrane in vacuoles which were interpreted as newly formed phagosomes. After 10 minutes of incubation, the heterophagic dense bodies or secondary lysosomes which presumably existed prior to exposure to microbeads now contained these spheres, apparently as a result of fusion with the nascent phagosomes. Profiles indicating fusion of 70 to 300-nm vesicles with the phagosomes were also observed. Such fusion of small vesicles with phagosomes or of the latter with dense bodies was seldom observed after 3 minutes of incubation with microbeads.

Cytochemistry

Peritoneal macrophages not exposed to microbeads but incubated in alkaline DAB medium exhibited 80 to 400-nm diameter bodies that appeared to have been densified by the incubation and thus evidenced content of catalase (Figure 2). Some of these bodies lay intimately associated with rough endoplasmic reticulum both in cells not exposed to beads and in phagocytosing cells (Figures 2 and 3). These catalase-containing bodies, ie, peroxisomes, disclosed no evidence of fusion with the phagosomes after 3 minutes of incubation with microbeads. However, after a 10- to 60-minute incubation with microbeads, macrophage profiles often contained peroxisomes closely associated with newly formed phagosomes. Peroxisomes closely bordered the phagosomes in configurations indicative of fusion of the peroxisomes with the phagosomes (Figure 3) and were also observed bordering phagocytized mast cell granules (Figure 4). Densification indicative of catalase was observed in the periphery of some bead-laden phagosomes, further suggesting fusion of peroxisomes with phagosomes. The Golgi cisternae and associated vesicles lacked densification indicative of catalase (Figure 5).

The mean number of peroxisomes per cell profile appeared increased after 30 minutes of incubation with beads. Thus, the mean number of peroxisomes equalled 2.5 in profiles of macrophages not exposed to beads compared with 2.9 at 10 minutes, 5.9 at 30 minutes, and 3.6 at 60 minutes of incubation with microbeads. The number of peroxisomes varied considerably among cells at 30 minutes of incubation, but the proportion with few peroxisomes decreased and the proportion with more abundant peroxisomes increased (Text-figure 1, Figure 5). Although many profiles of phagocytosing cells lacked evidence of increased peroxisomes, approximately 20% contained a few to many more than any profile of a control cell (Text-figure 1). Two peaks were apparent in the histogram of the



TEXT-FIGURE 1—Histogram of peroxisome prevalence in control macrophages and in macrophages phagocytosing microbeads. Peroxisomes were counted in 100 thin-section profiles of control macrophages (*dotted columns*) and of cells incubated 30 minutes with microbeads (*cross-hatched columns*). Approximately 20% of the phagocytosing cells contained a few to many more peroxisomes than any cell not exposed to microbeads.

prevalent peroxisome in macrophages. On the other hand, the size of the peroxisomes decreased after 30 minutes of incubation with beads. In macrophages not incubated with beads, the peroxisomes measured 2.00 ± 80 nm in greatest dimension (mean ± standard deviation), whereas they measured 130 ± 40 nm in macrophages expose 30 minutes to the beads ($P < 0.01$).

Macrophages incubated in control media lacking H_2O_2 or containing AMT and macrophages exposed to heat before incubation showed no dense foci except for the intrinsically dense droplets presumably composed of lipid in the heterophagic dense bodies (Figure 6). Incubation of the fixed cell in neutral DAB medium for peroxidase did not show any densification indicative of this enzyme (Figure 7).

Acid phosphatase reaction product was observed in the Golgi cisternae, in Golgi-associated vesicles (primary lysosomes), and in the heterophagic dense bodies representing secondary lysosomes (Figure 8). Small vesicles separated from the region of the Golgi apparatus lacked acid phosphatase reactivity as did similar vesicles observed in contact and apparent incipient fusion with phagosomes containing microbeads (Figure 10).

The PA-TCH-SP method stained variably the heterophagic bodies (secondary lysosomes), Golgi cisternae, small vesicles, and plasma membrane (Figure 9) of peritoneal macrophages as reported previously.[23]

Numerous small vesicles or granules in the cytoplasm were weakly to strongly stained with the PA-TCH-SP procedure. The lightly reactive structures outnumbered the more heavily stained ones; at least some of the more reactive small granules bordered and appeared to fuse with nascent phagosomes in the cell periphery. The latter phagosomes appeared from their peripheral staining and proximity to stained small vesicles to have gained their PA-TCH-SP reactive content through fusion with the vesicles (Figure 11).

Discussion

Small catalase-reactive bodies interpreted as peroxisomes were observed in resident peritoneal macrophages, in agreement with previous reports.[10,11] These bodies could only be identified in cytochemical preparations, wherein they were recognized by their size and, more precisely, their content of dense reaction product indicative of catalase. In macrophages processed for routine morphologic examination, peroxisomes appeared indistinguishable, fine structurally, from small Golgi-related vesicles, presumed to be primary lysosomes. The latter, however, were identified by their reaction product in specimens processed for acid phosphatase demonstration and were presumed to be catalase-negative in that peroxisomes rarely occurred in the Golgi region (Figures 6 and 9). Peroxisomes were usually located in close association with the endoplasmic reticulum or dispersed throughout the cell periphery. This distribution is consistent with the view that peroxisomes do not arise from Golgi elements, as is the lack of cytochemically demonstrable catalase in Golgi cisternae.

In macrophages exposed to microbeads, peroxisomes evidenced fusion with the newly formed phagosomes by their close association with the bead-containing vacuoles and the presence of catalase in the latter structures. Acid phosphatase preparations provided additional evidence for peroxisome fusion with phagosomes in evidencing fusion of the latter with small vesicles that appeared to be peroxisomes from their size and lack of acid phosphatase activity. The peroxisomes possibly also corresponded with structures which were similar to peroxisomes in size, shape, and cytoplasmic distribution and exhibited strong PA-TCH-SP reactivity. The PA-TCH-SP reactivity of these presumed peroxisomes could not be attributed to catalase, since this reactivity was negative in peroxisomes of proximal renal tubules of the kidney,[24] although peroxisomes of some blood cells appeared positive. The complex carbohydrate thus appeared to vary independently of catalase content and to reflect the presence of a substance other than catalase. However, the microperoxisomes of macro-

phages apparently play some role in phagocytosis, delivering to phagosomes complex carbohydrate which is important in phagocytosis.[23] The structures which greatly outnumbered the more heavily stained ones appeared too numerous to be peroxisomes, however. Further evidence for the role of peroxisomes in phagocytosis is suggested by the increase in the number of peroxisomes per cell profile and by the decrease in the size of the peroxisomes in macrophages ingesting beads.

Although catalase constitutes the "marker" enzyme for peroxisomes, the enzyme composition of these structures apparently varies depending on their biologic source and stage of development.[9] Peroxisomes appear to play a role in lipid metabolism [3-7] and detoxification of H_2O_2 [8] in many sites, but it is possible that the organelle functions differently in different cell types. Catalase has been demonstrated in several types of macrophages both biochemically [16,25] and cytochemically.[10,11] Its role in phagocytosis has not been clearly delineated. This enzyme has been shown to exhibit peroxidase-like activity at relatively low pH levels in the presence of H_2O_2.[15,26,27] Since resident peritoneal macrophages apparently lack peroxidase activity,[22,28,29] except for that which is localized in rough endoplasmic reticulum [13,14,29-33] and presumably is not transferable to phagosomes,[13] perhaps catalase assumes a peroxidase-like function during phagocytic activity. Phagosomes have been shown to have an acidic pH range,[34] and phagocytizing macrophages generate considerable H_2O_2.[12] Under these conditions, catalase discharged from peroxisomes into phagosomes as indicated here could act like peroxidase, a proved antimicrobial agent.[35]

Catalase activity in isolated phagosomes of alveolar macrophages has been demonstrated biochemically by Stossel et al,[16] who found that catalase, as well as acid phosphatase and β-glucuronidase, transferred from the sedimenting fraction to the phagocytic vacuole fraction during phagocytosis. Macrophage peroxisomes were thought from these observations to resemble primary lysosomes in discharging their content into phagocytic vacuoles. On the other hand, Fahimi et al [11] obtained no evidence for fusion of the limiting membrane of peroxisomes with that of phagosomes in Kupffer cells. The present results agree with those on alveolar macrophages [16] but are at variance with those on Kupffer cells,[11] possibly as a reflection of different catabolic activity in these cells.

Biochemical assay has provided evidence for an increase in macrophage catalase during phagocytosis.[25] In the present study, it appeared that the number of peroxisomes increased in some cell profiles after 30 minutes of incubation with microbeads. Since catalase synthesis occurs very rapidly,[36] this increase of peroxisomes could be interpreted as a prompt response to activity.

The latex microbeads used in this study retained their morphologic features after incubation and lacked nonspecific reactivities for incubation medium, whereas other kinds of phagocytizable objects, eg, bacteria or red blood cells, were deformed during incubation for phagocytosis and had nonspecific reactivity for enzyme incubation medium. Since the resident peritoneal macrophages frequently had large secondary lysosomes or residual bodies, the phagocytized bacteria were sometimes hard to differentiate from these organelles.[37] Bacteria were not useful in assessing the relation of phagocytosis and peroxisomes histochemically.

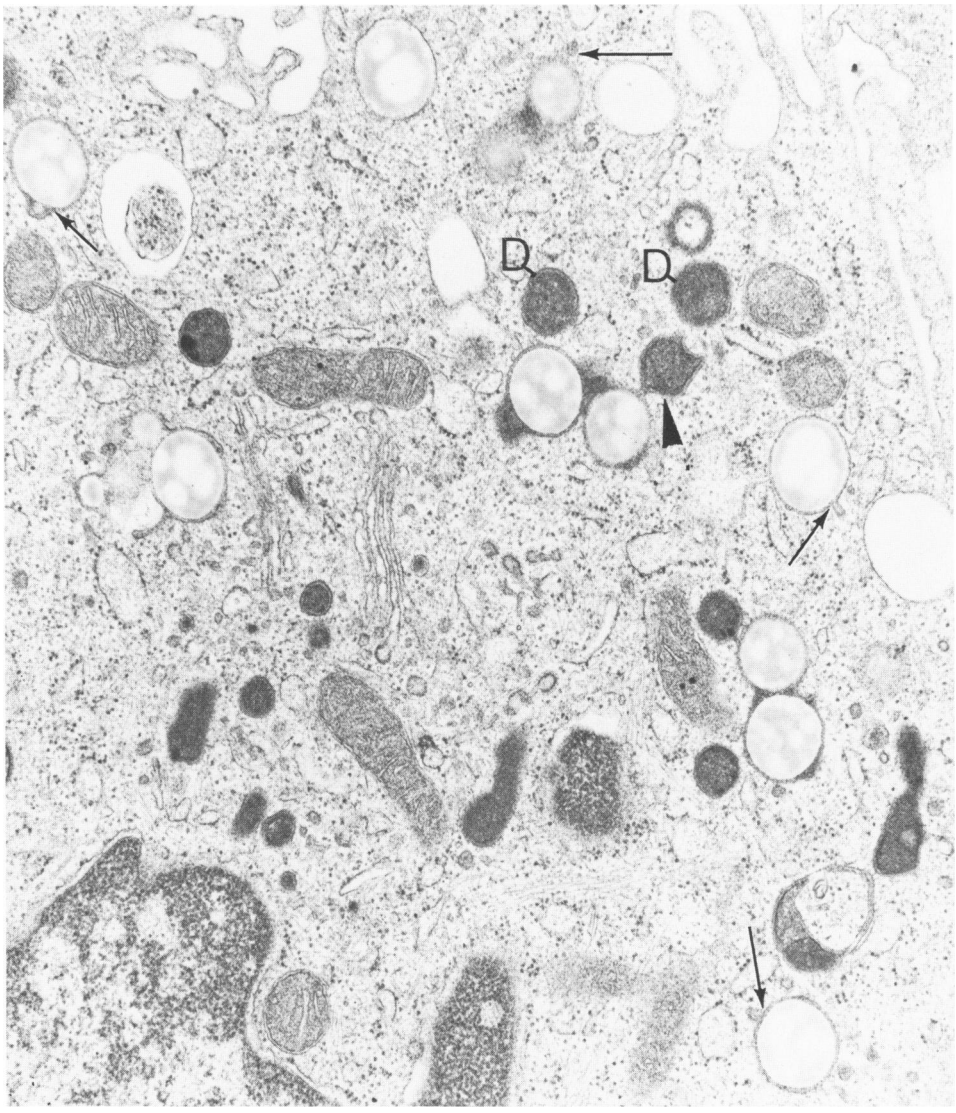
References

1. Hruban Z, Vigil EL, Slesers A, Hopkins E: Microbodies: Constituent organelles of animal cells. Lab Invest 27:184–191, 1972
2. Novikoff AB, Novikoff PM, Davis C, Quintana N: Studies on microperoxisomes. V. Are microperoxisomes obiquitous in mammalian cells? J Histochem Cytochem 21:737–755, 1973
3. Goldfischer S, Roheim PS, Edelstein D: Hypolipidemia in a mutant strain of "acetalasemic" mice. Science 173:65–66, 1973
4. Masters CJ, Holmes RS: The metabolic roles of peroxisomes in mammalian tissues. Int J Biochem 8:549–553, 1977
5. Novikoff AB, Novikoff PM: Microperoxisomes. J Histochem Cytochem 21:963–966, 1973
6. Stäubli W, Schweizer W, Suter J, Weibel ER: The proliferative response of hepatic peroxisomes of neonatal rats to treatment with SU-13 437 (Nafenopin). J Cell Biol 74:665–689, 1977
7. Svoboda D, Reddy J: Microbodies in experimentally altered cells. IX. The fate of microbodies. Am J Pathol 67:541–554, 1972
8. Reddy JK: Possible properties of microbodies (peroxisomes): Microbody proliferation and hypolipidemic drugs. J Histochem Cytochem 21:967–971, 1973
9. McGroarty E, Tolbert NE: Enzymes in peroxisomes. J Histochem Cytochem 21:949–954, 1973
10. Breton-Gorius J, Guichard J: Fine structural and cytochemical identification of microperoxisomes in developing human erythrocytic cells. Am J Pathol 79:523–536, 1975
11. Fahimi HD, Gray BA, Herzog VK: Cytochemical localization of catalase and peroxidase in sinusoidal cells of rat liver. Lab Invest 34:192–201, 1976
12. Paul BB, Strauss RR, Jacobs AA, Sbarra AJ: Function of H_2O_2, myeloperoxidase and hexose monophosphate shunt enzymes in phagocytizing cells from different species. Infect Immun 1:338–344, 1970
13. Cotran RS: Peroxidase ultrastructural cytochemistry monocytes and macrophages of guinea pigs. J Cell Biol 55:51a, 1972
14. Daems WT, Poelmann RE, Brederoo P: Peroxidatic activity in resident peritoneal macrophages and exudate monocytes of the guinea pig after ingestion of latex particles. J Histochem Cytochem 21:93–95, 1973
15. Klebanoff SJ: Antimicrobial activity of catalase at the acid pH. Proc Soc Exp Biol Med 132:571–574, 1969
16. Stossel TP, Mason RJ, Pollard TD, Vaughan M: Isolation and properties of phagocytic vesicles. II. Alveolar macrophages. J Clin Invest 51:604–614, 1972
17. Novikoff AB, Novikoff PM, Davis C, Quintana N: Studies on microperoxisomes. II. A cytochemical method for light and electron microscopy. J Histochem Cytochem 20:1006–1023, 1972

18. Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. J Histochem Cytochem 14:291–302, 1966
19. Komiyama A, Spicer SS, Bank H, Farrington J: Induction of autophagic vacuoles in peritoneal cells. J Retoculoendothel Soc 17:146–161, 1975
20. Spurr AR: A low-viscosity epoxy embedding medium for electron microscopy. J Ultrastruct Res 26:31–43, 1969
21. Thiery J-P: Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J Microscop 6:987–1018, 1967
22. Hirsch J, Fedorko ME: Morphology of mouse mononuclear phagocytes. Mononuclear phagocytes. Edited by R van Furth. Philadelphia, F. A. Davis Company, 1970, pp 7–28
23. Eguchi M, Sannes PL, Spicer SS: Alterations in ultrastructural cytochemistry of complex carbohydrates of rat peritoneal macrophages during phagocytosis. (Submitted)
24. Eguchi M, Sannes PL, Spicer SS: Unpublished data
25. Gee JBL, Vassallo CL, Bell P, Kaskin J, Basford RE, Field JB: Catalase-dependent peroxidative metabolism in the alveolar macrophage during phagocytosis. J Clin Invest 49:1280–1287, 1970
26. Deisseroth A, Dounce AL: Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. Physiol Rev 50:319–375, 1970
27. Feinstein RN, Savol R, Howard JB: Conversion of catalatic to peroxidatic activity in livers of normal and acetalasemic mice. Enzymologia 43:345–358, 1971
28. Cline MJ, Sumner MA: Bone marrow macrophage precursors. I. Some functional characteristics of early cells of the mouse macrophage series. Blood 40:62–69, 1972
29. van Furth R, Hirsch JG, Fedorko ME: Morphology and peroxidase cytochemistry of mouse promonocytes, monocytes, and macrophages. J Exp Med 132:794–812, 1970
30. Bodel PT, Nichols BA, Bainton DF: Appearance of peroxidase reactivity within the rough endoplasmic reticulum of blood monocytes after surface adherence. J Exp Med 145:264–274, 1977
31. Daems WT, Brederoo P: The fine structure and peroxidase activity of resident and exudate periotoneal macrophages in the guinea pig. Advances in Experimental Medicine and Biology. Vol 15: The Reticuloendothelial System and Immune Phenomena. Edited by NR Di Luzio, K Flemming. New York, Plenum Press, 1971, pp 19–31
32. Robbins D, Fahimi HD, Cotran RS: Fine structural cytochemical localization of peroxidase activity in rat peritoneal cells: Mononuclear cells, eosinophils and mast cells. J Histochem Cytochem 19:571–575, 1971
33. Wisse E: Observations on the fine structure and peroxidase cytochemistry of normal rat liver Kupffer cells. J Ultrastruct Res 46:393–426, 1974
34. Jensen MS, Bainton DF: Temporal changes in pH within the phagocytic vacuole of the polymorphonuclear neutrophilic leukocyte. J Cell Biol 56:379–388, 1973
35. Klebanoff SJ, Clem WH, Leubke RG: The peroxidase-thiocyanate-hydrogen peroxide antimicrobial system. Biochim Biophys Acta 117:63–72, 1966
36. Lazarow PB, deDuve C: The synthesis and turnover of rat liver peroxisomes. IV. Biochemical pathway of catalase synthesis. J Cell Biol 59:491–506, 1973
37. Eguchi M, Sannes PL, Spicer SS: Unpublished data

Acknowledgments

The authors would like to express their appreciation to Ms. Betty Hall, Dana Miller, and Kathleen Bowles for their technical assistance and to Ms. Dot Smith for her typing expertise.



Figures 1 through 11 show ultrastructural profiles of unexposed control macrophages or macrophages that were exposed to microbeads for varying lengths of time. They were fixed and processed for morphologic observation or catalase or carbohydrate PA-TCH-SP cytochemistry as described in *Materials and Methods*. The thin sections of cytochemical preparations were not counterstained with metal salts. **Figure 1**—This macrophage encloses a number of microbeads in membrane-limited vacuoles. Some of the pleomorphic heterophagic dense bodies (*D*) appear to have fused with the bead-laden phagosomes (*arrowhead*). Small vesicles also evidence fusion with the phagosomes (*arrows*) after 10-minute exposure to microbeads. (Morphologic preparation, uranyl acetate, lead citrate, ×24,000)

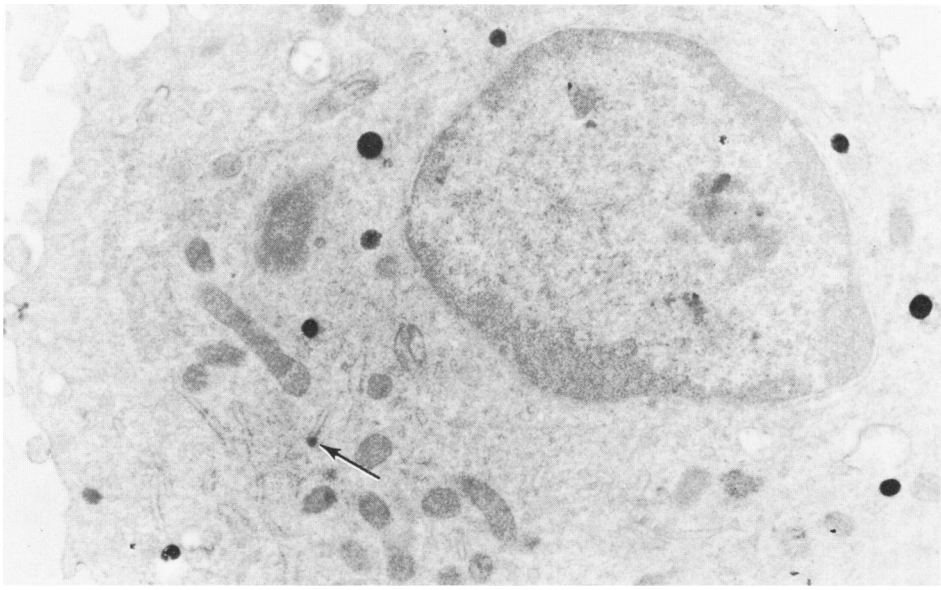


Figure 2—Small bodies with DAB reactivity indicative of peroxisomes are scattered in the cytoplasm. The peroxisomes often lie in association with endoplasmic reticulum (*arrow*). No exposure to microbeads. Incubated in alkaline DAB medium for catalase. (×15,000)

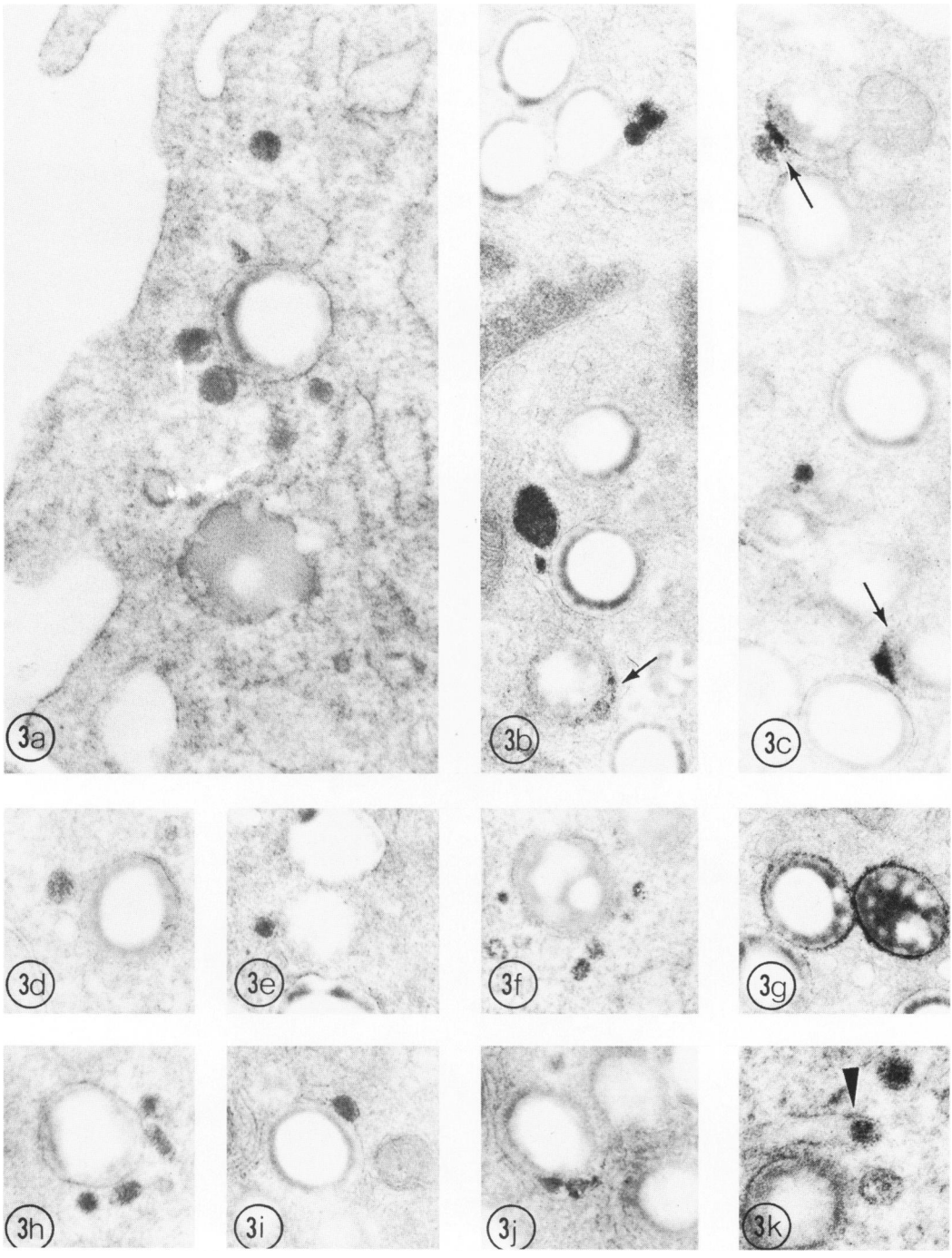
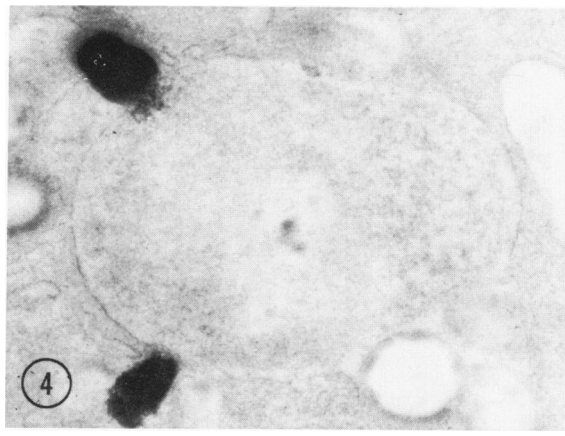


Figure 3—Catalase-reactive peroxisomes lie in close proximity to bead-laden phagosomes (**a–f, h,i**). The phagosomes reveal peripheral densification demonstrative of catalase as evidence of their fusion with peroxisomes (**b,c,g,j**) (*arrows*). A peroxisome is often also associated with endoplasmic reticulum (**k**) (*arrowhead*). Incubated for catalase. **a**—Thirty-minute exposure to microbeads. **b,c**—Sixty-minute exposure to beads. **d–g**—Ten-minute exposure to microbeads. **h–k**—Thirty-minute exposure to microbeads. (**a,** ×34,000; **b,c,** ×33,000; **d–k,** ×30,000)



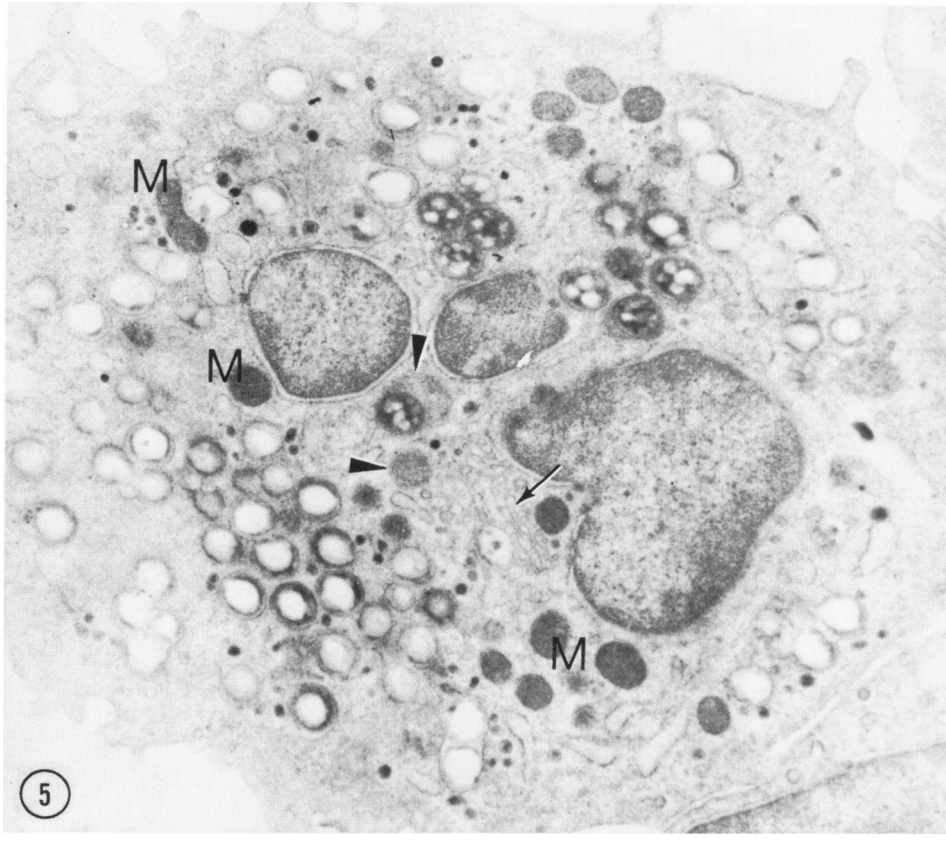
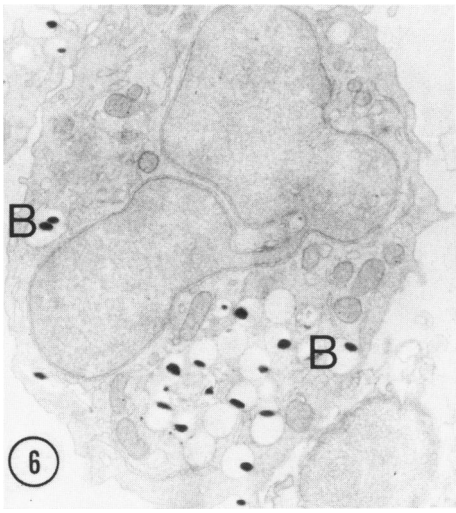
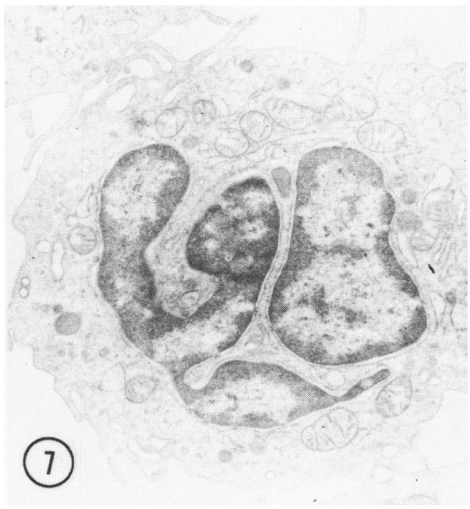


Figure 4—Peroxisomes border a phagocytized mast cell granule. Catalase method. (×38,000) **Figure 5**—Compared with cells not exposed to microbeads, this macrophage contains an increased number of peroxisomes which, in general, appear decreased in size. Golgi cisternae and associated vesicles (*arrow*) as well as the heterophagic dense bodies (*arrowheads*) lack reactivity. *M,* mitochondria. Thirty-minute exposure to beads. Catalase incubation. (×14,000)





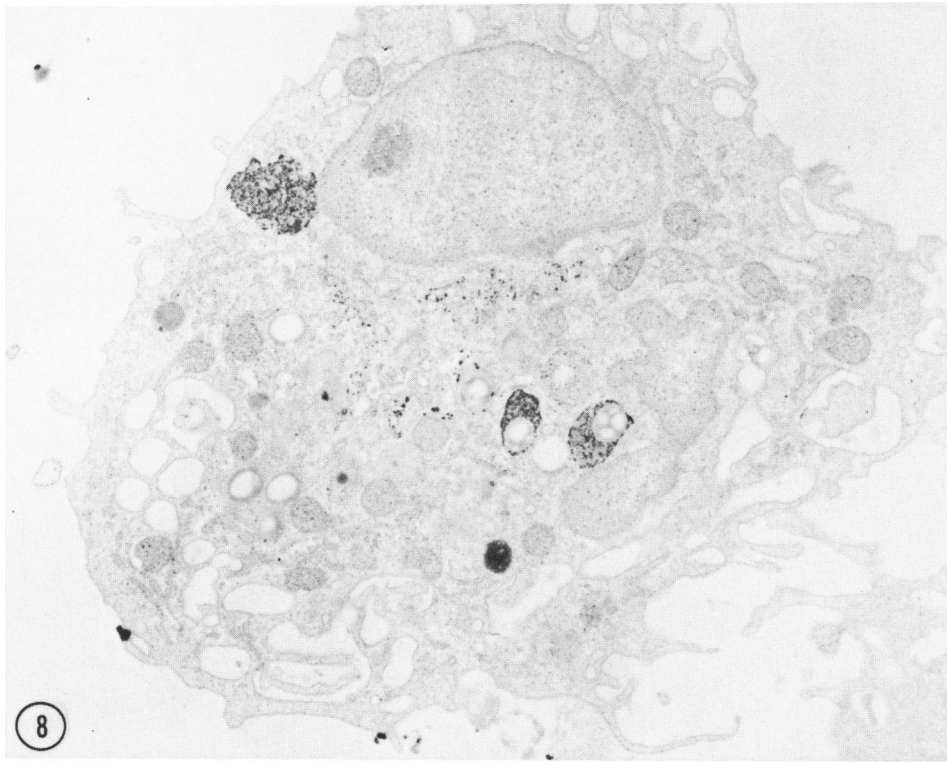
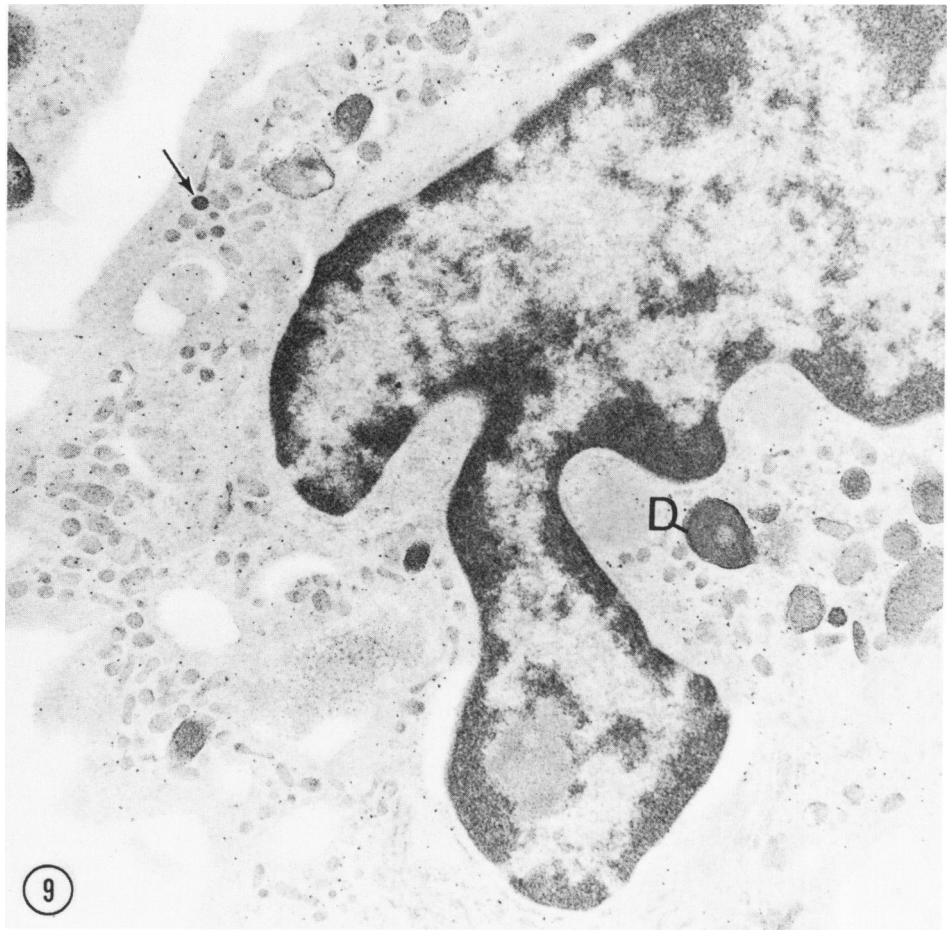
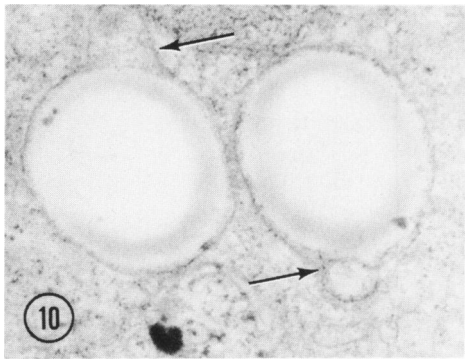


Figure 6—No focal densification is evident in any cell organelles. *B*, part of microbeads. Ten-minute exposure to beads. Cytochemical control for catalase incubated with Aminotriazol (AMT). (×7500) **Figure 7**—A macrophage fixed 60 minutes with glutaraldehyde processed for catalase localization lacks evidence of peroxidase activity. Incubated in neutral DAB medium for peroxidase. (×7000) **Figure 8**—Acid phosphatase reactivity is evident in Golgi cisterns and associated vesicles and in beads containing heterophagic dense bodies. The distribution of this enzyme differs strikingly from that of catalase. Sixty-minute exposure to microbeads. Acid phosphatase preparation. (×12,000)





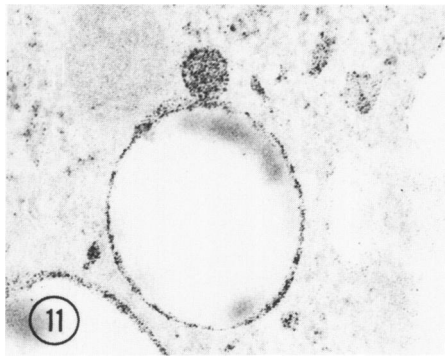


Figure 9—Heterophagic dense bodies (*D*) show strong staining demonstrative of glycoprotein with periodate-reactive hexoses. A moderate number of smaller granules also stain heavily (*arrow*). In addition, the cell contains population of abundant small bodies with moderate reactivity. Not exposed to beads. (Periodate-thiocarbohydrazide-silver proteinate [PA-TCH-SP] stain, ×22,000) **Figure 10**—Acid-phosphatase-negative small vesicles (*arrows*) fuse with bead-laden phagosomes. Ten minute exposure to microbeads. Acid phosphatase preparation. (×46,000) **Figure 11**—A small strongly stained vesicle appears to be fusing with a bead-laden phagosome which exhibited a thin rim of strong staining in evidence of prior fusion with such vesicles. Ten-minute exposure to microbeads. (PA-TCH-SP stain, ×46,000)