PARKE-DAVIS
LECTURE

MECHANISMS OF EXOCYTOSIS IN PHAGOCYTIC INFLAMMATORY CELLS

Mechanisms of Exocytosis in Phagocytic Inflammatory Cells

Parke-Davis Award Lecture

Peter M. Henson, PhD

THE INFLUX OF NEUTROPHILS and mononuclear phagocytes into tissues may be seen as the hallmark of inflammation and significantly contributes to both the injury and the subsequent repair seen in the normal, circumscribed, inflammatory reaction. Since the time of Metchnikoff, considerable emphasis has devolved on the phagocytic properties of these cells. Their ability to engulf, kill, digest, and remove offending organisms and particles clearly represents one of the critical factors in protection of the body against the outside world. Because of the importance of these functions, in which the action occurs intracellularly within a phagocytic vacuole or phagosome, the concept that these cells secrete enzymes not only into phagosomes but also to the outside milieu has received less attention. When free lysosomal hydrolases have been detected in inflammatory lesions in the past, they have been considered a consequence of inflammatory cell death and lytic release of constituents. However, while cell lysis undoubtedly occurs, there is now considerable evidence for noncytolytic extracellular release of constituents from inflammatory cells, both *in vitro* and *in vivo*, and for its contribution to the inflammatory process itself.

In this discussion I will focus on one type of release of enzymes from phagocytes, discuss some questions and speculations as to the mechanisms of extracellular discharge involved, and present some thoughts as to its possible involvement in the inflammatory process. The importance of the release of enzymes from phagocytes in the natural history of inflammation presupposes the ability of acid hydrolases and proteases to escape the inhibitory action of the buffered environment or specific inhibitors they encounter in the extracellular milieu. Evidence for a role for such enzymes

---

From the Department of Pediatrics, National Jewish Hospital and Research Center, and the Department of Pathology, University of Colorado Medical School, Denver, Colorado.

Supported by NIH Grants GM-24834 and HL-21565.

Presented at the Annual Meeting of the American Association of Pathologists (FASEB), April 15, 1980, Anaheim, California.

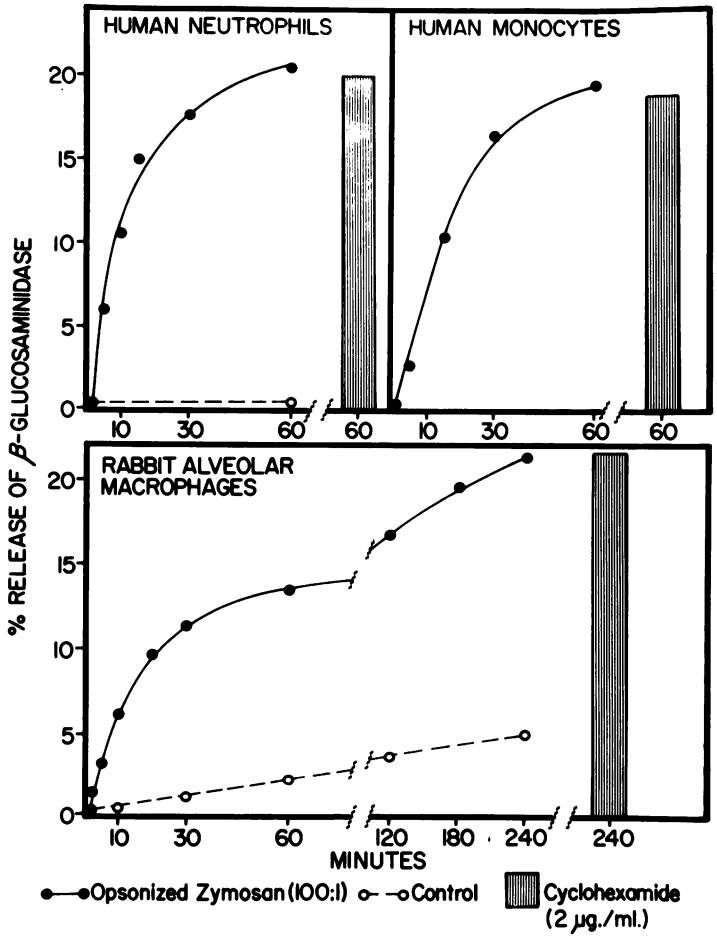
Address reprint requests to Peter M. Henson, PhD, Department of Pediatrics, National Jewish Hospital and Research Center, Denver, CO 80206.

0002-9440/80/1209-0494$01.00

in inflammatory injury is beginning to accumulate, and it is therefore appropriate to speculate briefly below on some of the possible escape mechanisms. The context in which this examination of phagocyte exocytosis is presented is my belief that inflammation is a nonspecific response of tissues to injury and that it is inherently good for you. The basic components of inflammation have been conserved and expanded in evolution until in mammals one finds a highly interactive dynamic and redundant system in which elimination of one particular component seldom leads to abrogation of the response because of extensive collateral mechanisms. This makes the overall process both fascinating and extremely difficult to examine. I would also like to express an additional bias towards a belief in the conservative nature of biology (or evolution). In the context of phagocyte exocytosis this leads to raising first the suggestion that exocytosis of preformed, granule-associated constituents from the different cells (neutrophils, monocytes, macrophages, and perhaps also basophils and mast cells) is accomplished by a similar mechanism. An attempt therefore will be made to examine this possibility in the case of the human neutrophil and monocyte and the rabbit alveolar macrophage. Human macrophages, derived from monocytes *in vitro* [1,2] behave similarly in most respects to the rabbit alveolar cells but for purposes of simplicity are not described separately. This brief description of ongoing studies of such systems will intentionally exclude experiments employing the fungal metabolite cytochalasin B (or A, C, or D). The effects of this drug on exocytosis are dramatic and hence interesting but are also quite different for the different cell types. Unfortunately, however, because we do not yet thoroughly understand the various modes of action of the cytochalasins, the results of their use are extremely difficult to interpret in a physiologic context.

The Immediate "Release Reaction" of Phagocytic Inflammatory Cells and Its Possible Phlogistic Potential

During phagocytosis, neutrophils, monocytes, and macrophages release to the outside of the cells a significant amount of lysosomal hydrolases. As depicted in Text-figure 1, release of β-glucosaminidase occurred rapidly, and the kinetics of release for the three cell types employed were remarkably similar. Without stimulus, the enzyme was not released during the 1 or 4 hours of the assay. Although not depicted, other lysosomal hydrolases were released with similar kinetics. In order to control for cell lysis, the presence in the supernatant of the cytoplasmic enzyme lactic dehydrogenase (LDH) was also measured. In no case did significant LDH release occur, and the release of β-glucosaminidase must be considered selective in nature, presumably the result of a secretory event.



TEXT-FIGURE 1—Exocytosis from three phagocytic inflammatory cells. Cells were prepared as described previously [1,7,17] and stimulated by zymosan opsonized with isologous serum.[7] Neutrophils, 4×10^6/ml; monocytes and macrophages, 1×10^6/ml. Phagocytosis was checked by microscopy, and the percent release of β-glucosaminidase calculated from the enzyme activity in the supernate and in detergent-lysed cell pellets. Cyclohexamide was shown in each case to prevent incorporation of ^{3}H-leucine into protein. Lactic dehydrogenase was not released in these experiments, indicating the absence of cell lysis.

Alveolar macrophages engulf particulate material, which they may encounter in the lower airways. This encounter is often associated with an inflammatory response, but whether as cause or effect is not always clear. However, it is not unreasonable to suppose that the products of the secretory reaction described above might actively induce or contribute to the inflammatory reaction. In the experiment depicted in Figure 1, the phlogistic potential of the materials released from rabbit alveolar macro-

phages during phagocytosis (in the experiment described in Text-figure 1) was examined. Supernatants collected 4 hours following a phagocytic or control challenge of rabbit alveolar macrophages *in vitro* were instilled into normal rabbit lungs. The histologic appearance of the lungs following sacrifice of the animals 24 hours later is depicted. The macrophages that encountered and ingested the particulate stimulus *in vitro* released materials with significant phlogistic activity, inducing accumulation over 24 hours of neutrophils, and even more strikingly, mononuclear phagocytes. Alveolar edema and fibrin deposition were evident, and the cellular response was in both the alveolar interstitium and air space. Supernates from nonstimulated macrophages (ie, those that did not undergo the secretory reaction) did not induce pulmonary alteration (Figure 1A).

The data suggest that the noncytolytic secretory products of alveolar macrophages can by themselves induce pulmonary inflammation. Further experiments have shown that neutrophil-dependent pulmonary inflammation induced after 4–6 hours by other stimuli instilled directly into the lungs (C5a des Arg,[3] immune complexes[4]) results in a lesion in which some evidence for noncytolytic release reactions from neutrophils can also be obtained. Thus bronchoalveolar lavage fluids contain high concentrations of lysosomal hydrolases, with disproportionate levels of cytoplasmic enzymes, suggesting discharge of lysosomes. Moreover, neutrophils observed morphologically in such lesions are apparently intact, and can be isolated by lavage and shown to be chemotactically responsive (Henson et al, unpublished observations), surely an indication of viability.

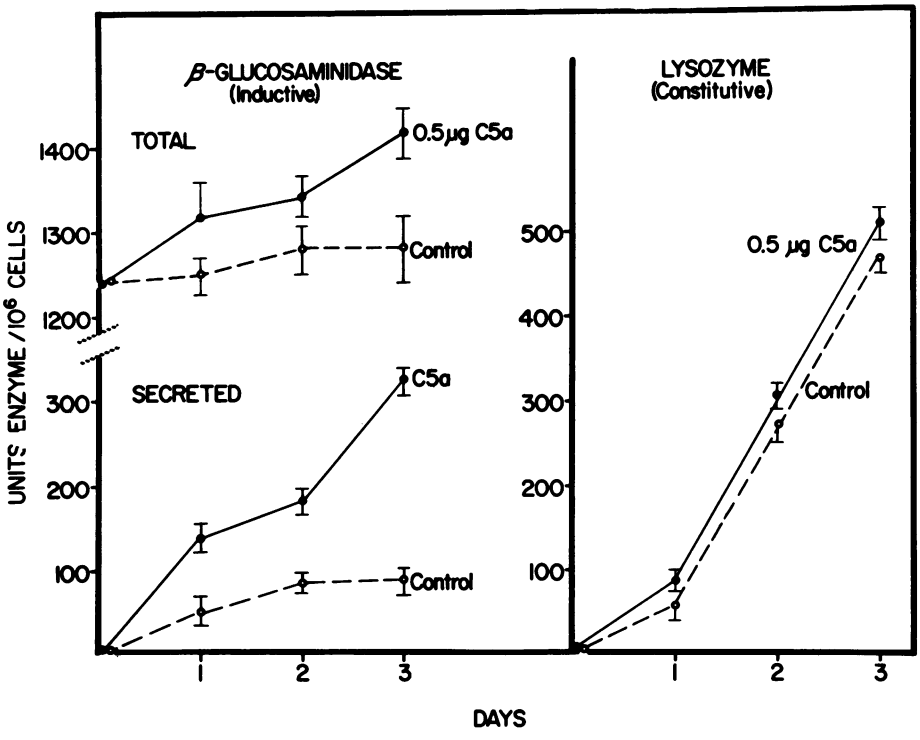
While none of the above-mentioned observations are conclusive evidence for the contribution of exocytosis to inflammation, they do suggest that an understanding of the mechanisms by which neutrophils and mononuclear phagocytes undergo this rapid (within 4 hours) release reaction would be important.

Three Modes of Secretion From Mononuclear Phagocytes

Release of enzymes from mononuclear phagocytes to the extracellular environment can be considered to follow at least three different patterns. Two of these, depicted for rabbit alveolar macrophages in Text-figure 2, are dependent upon protein synthesis.[2]

Constitutive Secretion

Constitutive secretion, of lysozyme,[1,2,5,6] implies a continuous synthesis and secretion that are relatively independent of external stimulus to the cell (in this case by C5a, but equally true for phagocytic or other stimuli)



TEXT-FIGURE 2—Synthesis dependent secretion in rabbit alveolar macrophages. Macrophages were stimulated with purified C5a as described.[7] Total enzyme in the culture was calculated as the sum of that in the supernate (secreted) and in the cell lysates.

(Text-figure 2, right panel). Little enzyme is stored intracellularly in this case.

Inductive Synthesis-Dependent Secretion

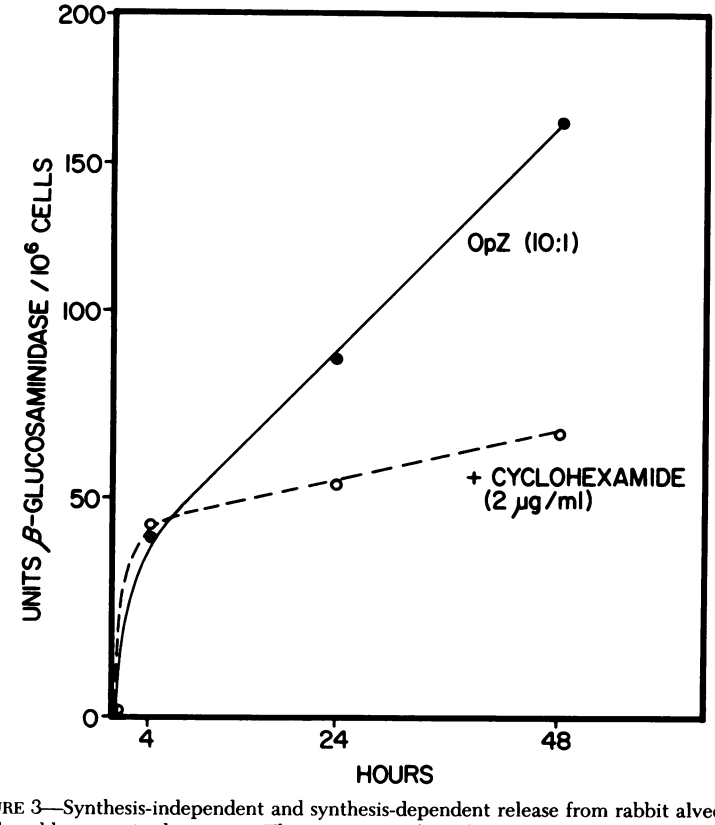
Inductive synthesis-dependent secretion involves the synthesis of new enzyme as well as of secretion extracellularly as a result of cell stimulation. As shown in Text-figure 2, C5a induces the release of the lysosomal hydrolase β-glucosaminidase from alveolar macrophages.[7] This is a slow release, dependent on protein synthesis, and accompanied by a net increase in total enzyme in the culture. A similar process has been shown for mouse peritoneal cells with phagocytic stimuli,[8,9] and this process is also presumably related to the well-known ability of "digestible" materials, when ingested by macrophages, to induce lysosomal hydrolase synthesis.[6,10,11] At this point, we have no proof that the enzymes that are secreted are the newly synthesized molecules or that they are packaged differently from those destined for lysosomes. However, in the case of neutral proteases

(plasminogen activator, collagenase, and in some cases elastase), the inductive secretory reaction does not involve intracellular storage; so a direct sequence of synthesis, packaging, and secretion is implied.

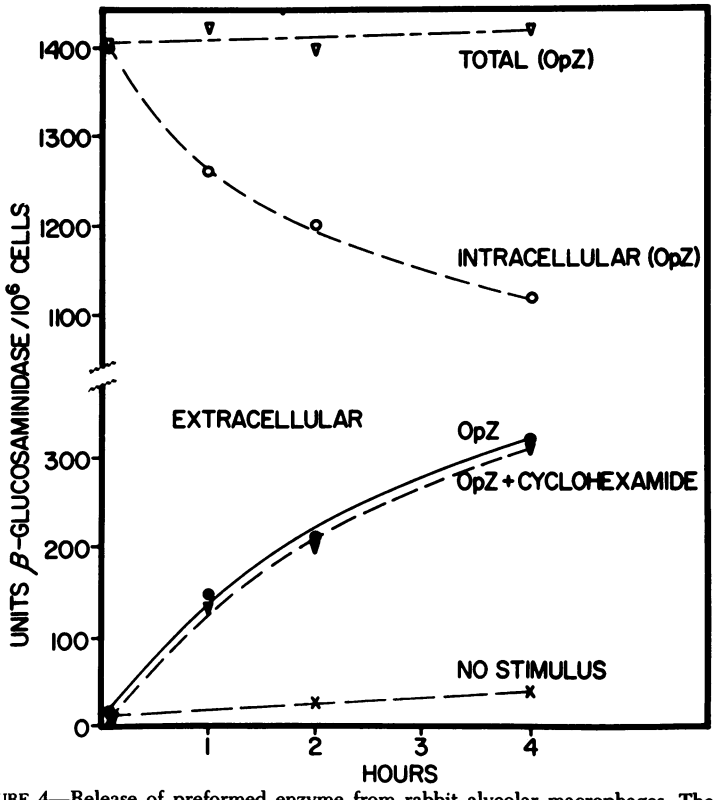
As shown in Text-figure 3, particulate, phagocytosable stimuli also induce an inductive secretion of lysosomal hydrolases over a period of 24–48 hours, which is inhibited by the protein synthesis inhibitor cyclohexamide.[8,9] By contrast, the release of β-glucosaminidase during the first 4 hours is entirely resistant to the effects of cyclohexamide, ie, protein-synthesis–independent (Text-figure 3).

Synthesis-Independent Release

Synthesis-independent release (the immediate release of preformed constituents) was shown earlier in Text-figure 1 and is depicted in Text-figure 4 in detail. Five points can be made. 1) The extracellular release was



TEXT-FIGURE 3—Synthesis-independent and synthesis-dependent release from rabbit alveolar macrophages induced by opsonized zymosan. The experimental conditions were the same as those outlined in Text-figures 1 and 2. Lactic dehydrogenase was not liberated. Up to 4 hours the release was independent of protein synthesis.



TEXT-FIGURE 4—Release of preformed enzyme from rabbit alveolar macrophages. The conditions were those described for Text-figures 1 and 2. Intracellular enzymes (and therefore total enzyme) was assayed in cell lysates from wells containing the opsonized zymosan stimulus. Lactic dehydrogenase was not released.

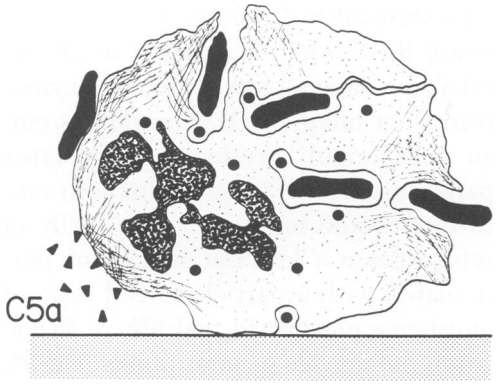
dependent on the presence of the stimulus, in this case the phagocytosable particle opsonized zymosan. 2) The release occurred in the presence of cyclohexamide. (Although not shown in the figure, the incorporation of radiolabeled amino acids into protein was inhibited by cyclohexamide in the experiments.) 3) There was a concomitant drop in intracellular enzyme, suggesting that intracellular stores of performed enzymes were being discharged. 4) The total enzyme activity in the culture remained unchanged indicating that the enzyme was not being destroyed or inactivated. 5) The release was noncytotoxic, since lactic dehydrogenase was not released (data not included).

Taken together, the data show synthesis-independent extracellular release of preformed constituents. As indicated in Text-figure 1, the phenomenon can be demonstrated in human neutrophils and monocytes as well as rabbit alveolar macrophages and also occurs in human monocyte-derived macrophages.[2] The question that now must be addressed is, What

is the mechanism of this response to stimulation? However, before this mechanism is discussed in more detail, three additional points need emphasis. 1) The experiments described above have all used opsonized zymosan as a stimulus. That the exocytosis is not unique to this particle is emphasized by its occurrence when cells engulf erythrocytes or latex (Henson et al, manuscript in preparation). 2) The experiments also employed higher particle-to-cell ratios than would be expected normally *in vivo*. Nevertheless, human monocytes release a low but significant percentage of their enzymes when stimulated with a particle-to-cell ratio of only 1:1, which is certainly a circumstance possible in real life. 3) When low levels of enzyme are released from monocytes or neutrophils (eg, 5% of total β-glucosaminidase or myeloperoxidase), histochemical staining has shown that this is not the result of 5% of the cells releasing all their enzyme—a much larger proportion of the cells is involved (Henson et al, manuscript in preparation). This then sets the stage for considering by what mechanism the lysosomal constituents are discharged from the cell. In this discussion I will focus on the physical mechanisms by which lysosomes (granules) gain access to the extracellular milieu. This is not to discount the importance of the biochemical steps, from receptor–ligand interaction to calcium mobilization to membrane fusion and perhaps enzyme—matrix dissociation. The many and varied biochemical steps in these processes are of great interest and are being studied intensely in many laboratories, including our own. However, in thinking about these processes, it became apparent that we did not in fact have a sure indication of the physical events involved, let alone the biochemical ones. Hence the current emphasis.

Release From Neutrophils and the Problems With "Accidental" Fusion of Lysosomes With the Plasma Membrane or Incompletely Sealed Phagosome

Over the last 10 years we and others have suggested mechanisms of granule discharge from neutrophils, depicted in Text-figure 5. Each of these is closely related and represents a conceptual bias mentioned earlier, namely that the "purpose" of the cell is to discharge its granules into phagosomes and that extracellular release is accidental. Examples would include premature fusion of granules with developing phagosomes; incomplete closure of phagosomes leaving channels to the outside; later opening of phagosomes during incorporation of a new particle. These could be considered examples of what Weissmann has called "regurgitation while feeding."[12] In addition, studies by ourselves [13] and Hawkins [14] showed discharge of lysosomal enzymes when neutrophils interacted with stimuli on surfaces too large to engulf. We felt that the mechanisms in-



TEXT-FIGURE 5—Diagrammatic representation of some mechanisms by which neutrophils might release granule contents.

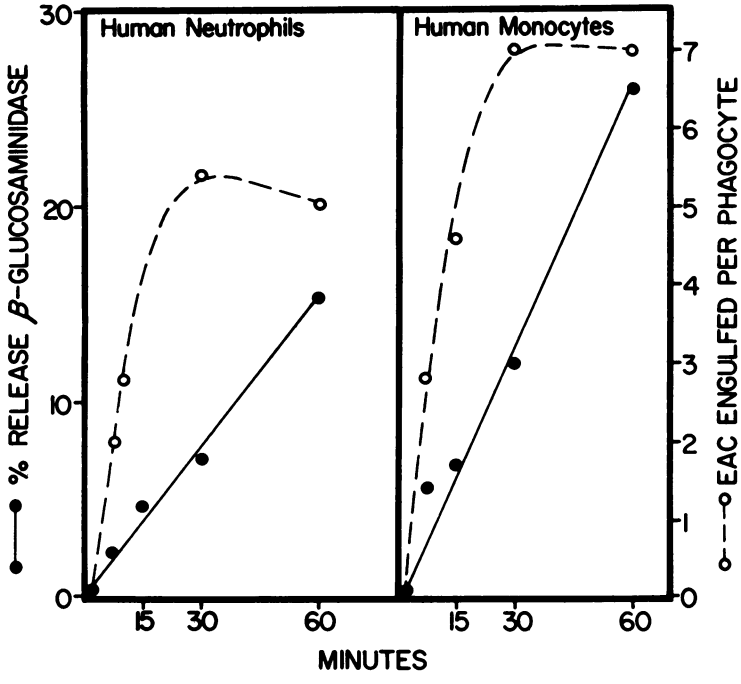
volved here were similar to those pertaining to phagosome–lysosome fusion, the difference being the lack of an enclosed phagosome. The processes we colloquially called "frustrated phagocytosis."[15] Soluble stimuli, in this context, were inefficient stimuli for the exocytotic process as described, unless they were presented on a particle or surface [16,17] (Henson and Keeling, unpublished observations on monocytes).

Examples of all the above-mentioned mechanisms have been observed in studies *in vitro* [15,18] with human and rabbit neutrophils, and there seems little doubt that they are real mechanisms of release that contribute to the reactions *in vivo*.

Nevertheless, a number of unanswered questions, and a change in my concept of these processes toward a hypothesis that the extracellular release reaction represents a physiologic response of the cells, has led to a reconsideration of what other mechanisms might be operative.

The first problem has been the difficulty in finding numerous morphologic examples of the above-described processes, *in vivo*. While pictures of premature fusion and extracellular discharge against surfaces have been presented,[eg,19] the frequency with which they are found is low even in lesions for which alternative evidence of exocytosis can be obtained. We have suggested that part of the problem may reflect the time required for fixative penetration in tissue samples, allowing discharged granules to dissolve before they can be preserved. Immediate fixation of cells in the *in vitro* experiments would have increased the frequency of observation of these transient events. Nevertheless, it may be unrealistic to explain phenomena *in vivo* in this way.

A second difficulty is depicted in Text-figure 6. A necessary consequence of the exocytotic mechanisms indicated above (ie, in Text-figure 5), is that release should correlate temporally with engulfment. However,



TEXT-FIGURE 6—Comparison of exocytosis of β-glucosaminidase and uptake to stimulating particles. Opsonized sheep erythrocytes (EACs) labeled with ^{51}Cr were prepared with IgG antibody and complement components through C3 as described.[42] Uptake was assessed by determination of neutrophil- or monocyte-associated ^{51}Cr after lysis of extracellular EACs with NH_4Cl. Particle(EAC)-to-phagocyte ratios were 100:1. Other conditions were as for Text-figures 1 and 2. The experiments suggest that uptake does not correlate with enzyme secretion.

using opsonized erythrocytes, one can see that for both neutrophils and monocytes, the release reaction continues when ingestion has ceased.

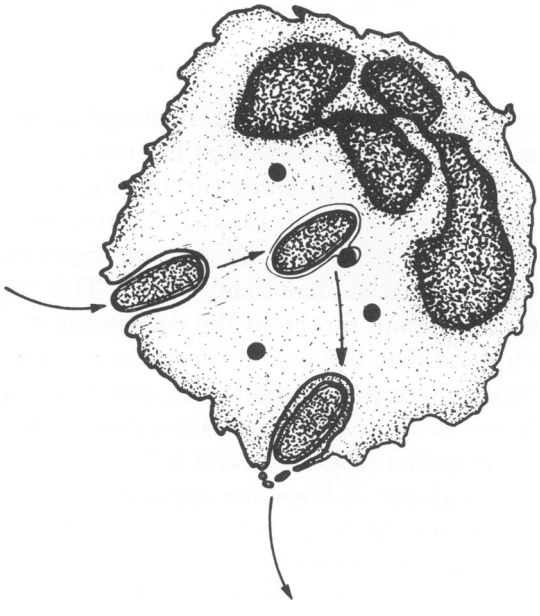
Finally, when macrophages are examined ultrastructurally (Figures 2 and 3), an additional difficulty must be considered. During the process of engulfment of opsonized zymosan (Figure 2), a broad area of organelle clearance is seen at the point of particle attachment and uptake. This has been shown by a number of investigators to represent the contractile elements of the cell.[20,21] It can be argued that this would represent a significant barrier to lysosome fusion with developing phagosomes, and a similar point was made earlier by Oliver et al concerning monocytes.[22] Only when the particle is completely engulfed (Figure 3) and the contractile proteins have been removed can evidence of lysosome approach to, and fusion with, the phagosome be obtained. In fact, we have been unable to obtain any examples of "premature" lysosome fusion in macrophages (although one such example was suggested by Ackerman [23]). Admittedly, it

could be argued that macrophages and neutrophils discharge their contents by different mechanisms. Nevertheless, the similarities in cell response (Text-figure 1), the increasing evidence of common secretory mechanisms among different cells,[24] and my own bias toward examining the simple suggestion first have prompted a search for a mechanism that might occur in both cell types.

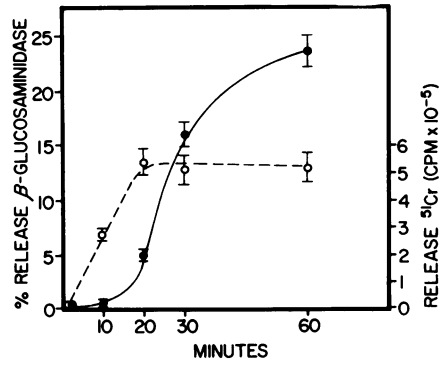
Lack of Evidence for Release During an Excretion Phase

Berlin et al [25] have shown that ingestion of paraffin oil particles by neutrophils is followed by their subsequent discharge from the cell and suggest that this involves a mechanism resembling reversal of the initial phagocytic event. The process is depicted schematically in Text-figure 7. If phagosome–lysosome fusion occurs during the sojourn of the particle within the phagosome, subsequent discharge of lysosomal (granule) contents to the exterior would be expected.

In order to test this possibility, the experiments depicted in Text-figure 8 were performed, using ^{51}Cr-labeled opsonized erythrocytes as particles and human monocytes as phagocytic cells. The monocytes were preloaded with ^{51}Cr-EACs for 30 minutes at 37 C, unbound ^{51}Cr-EACs were removed by washing and externally adherent ^{51}Cr-EAC by ammonium chloride lysis. The cells were then incubated for 60 minutes and the kinetics of lysosomal enzyme release compared with that of erythrocyte (chro-



TEXT-FIGURE 7—Diagrammatic representation of possible enzyme release accompanying expulsion of previously ingested particles from neutrophils.[25]



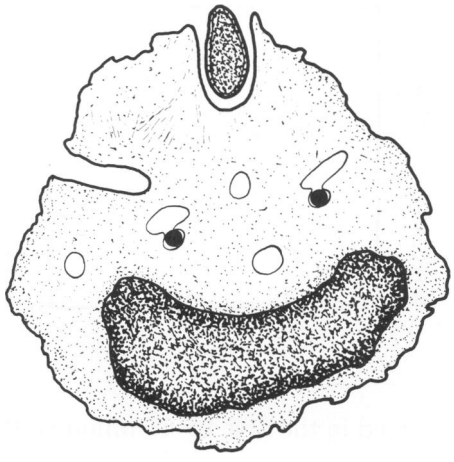
TEXT-FIGURE 8—Release of β-glucosaminidase from monocytes preloaded with opsonized erythrocytes. Monocytes were incubated with ^{51}Cr-EACs (see Text Figure 6) for 30 minutes at 37 C. Extracellular EACs were removed by washing and NH_4Cl lysis. Monocytes (with engulfed EACs) were incubated for 60 minutes and the extracellular release of β-glucosaminidase and ^{51}Cr assessed. The data suggest that enzyme release is not correlated with expulsion of previously ingested particles.

mium-51) discharge. Release of ^{51}Cr occurred in the first 10–15 minutes. It is not clear at this point whether this represents excretion of erythrocytes from inside the monocyte (as occurred for paraffin oil particles in Berlin's experiments), or whether this is release of ^{51}Cr-labeled membrane fragments from the monocyte surface. In either case, the release of ^{51}Cr (erythrocytes) was complete by 20 minutes; yet the secretion of β-glucosaminidase extended from 20 to 60 minutes. Identical results were obtained with neutrophils. This data is not easily compatible with the release of lysosomal enzymes accompanying the excretion of preingested particles.

Possible Fusion of Lysosomes With Invagination of the Plasma Membrane

During the course of particle uptake by alveolar macrophages, as indicated above, we could find no evidence of lysosome fusion with the developing phagosome. On the other hand, as shown in Figure 4, lysosome discharge into what appeared to be vacuoles was not infrequently observed. Evidence for this phenomenon was strengthened by our preloading the macrophages with horseradish peroxidase (HRP) (which localizes in the secondary lysosomes) and observing discharge of HRP into apparent vacuoles, not into developing phagosomes (Henson and Henson, manuscript in preparation).

The possibility was raised, therefore, that these "vacuoles" represented invagination of the plasma membrane (see Text-figure 9) that were open to the outside and resembled the deep invaginations seen in neutrophils [26] and monocytes (Henson and Henson, unpublished observations) after treatment with a stimulus in the presence of cytochalasin B. The process might involve imbalance of opposing contractile elements [26] and might provide a common link between physiologic secretory processes and the massive enhancing effect of the cytochalasins. This is an attractive possibility, and was supported by similar morphologic observations of dis-



TEXT-FIGURE 9—Diagrammatic representation of possible discharge into membrane invaginations.

charge into "vacuoles" (though with less frequency) in monocytes and neutrophils (Figure 5). However, attempts to date to demonstrate that these "vacuoles" were open to the outside by penetration of cationized ferritin or ruthenium red following fixation have been unsuccessful (Henson and Henson, manuscript in preparation). Admittedly, more complicated explanations may exist; eg, the external opening of the vacuoles may be transient. This idea is being examined. Nevertheless, the data suggest that it might be more fruitful at this point to seek alternative possibilities.

Piecemeal Granule "Dissolution" and Vesicular Transport Mechanisms

Careful examination of neutrophils undergoing a release reaction *in vitro* revealed (Figure 5) a phenomenon of apparent granule dissolution *in situ* as well as the discharge into "vacuoles." Effectively, what this means is that the electron density of the stained contents of the granules is altered, a process which may indicate physiologic changes in the granule contents associated with secretion. The observation is similar to pictures obtained earlier in our laboratory of rabbit basophils undergoing degranulation [27] and more recently to basophil degranulation in other species.[28] In the latter context, Dvorak et al [29] have suggested a vesicle cycling between plasma membrane and granule, which can bring material to the granule, and more importantly for secretion, carry them back to the plasma membrane. Caulfield et al [30] have recently shown pictures of apparent granule dissolution in human pulmonary mast cells, and we showed that a not dissimilar effect can be seen on occasions in rat mast cells undergoing a relatively prolonged release reaction.[31] These data add

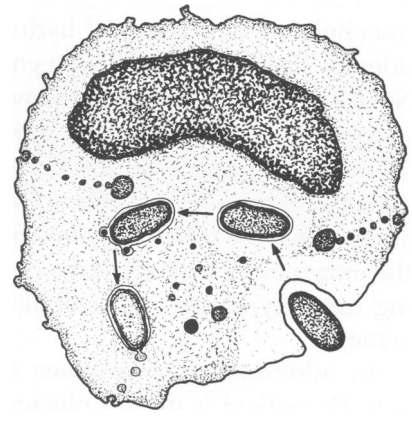
further credence to the commonality of the observation and thus perhaps to an underlying mechanism of discharge of granule constituents.

Finally, when we went back and reexamined neutrophils in inflammatory processes *in vivo*, we found striking examples of granule "dissolution" in these cells and in infiltrating monocytes (see Figure 6) [32] (Henson and Henson, manuscript in preparation). At this point it would be fruitless to discuss possible mechanisms in any detail, because the phenomenon itself must be placed upon a firm footing. Nevertheless, some intriguing implications and possibilities relating to vesicle transport and membrane recycling can be raised, with a view to suggesting further lines of investigation.

The data and calculations of Steinman [33] suggest the need for membrane recycling in phagocytic cells to replenish internalized membrane, and there is currently a great deal of interest in, and some preliminary confirmatory data on, this phenomenon.[34–36] One of our early suggestions to explain synthesis-independent discharge of preformed constituents was related to membrane recycling. Thus, when membrane was recycled from phagosomes with which lysosomes had fused (perhaps by vesicular transport processes), some of the contents of these phagolysosomes, including the hydrolytic enzymes, would reach the outside of the cell.

A problem with this simple concept is that enzyme release occurs immediately, before phagosome-lysosome fusion is evident to any degree. (It should be noted, however, that Segal et al [37] have provided evidence for extremely early fusion in neutrophils.) An extension of the above-mentioned suggestion that takes this problem into account is depicted in Textfigure 10, and described below.

Hypothesis. Triggering of membrane receptors, especially via particle-



TEXT-FIGURE 10—Diagrammatic representation of possible enzyme release accompanying vesicle transport from lysosomes and phagolysosomes to the plasma membrane.

bound or surface-bound ligands [38] induces a variety of intracellular events. Among these is an immediate transport of lysosomal membrane, or phagolysosomal membrane, to the outside of the cell. The transport is via vesicles, and some of the contents of the lysosomes or phagolysosomes (or granules) are carried along and therefore discharged. This would explain the stimulus-induced expression of new receptors derived from specific granules on the neutrophil membrane described by Fletcher and Gallin.[39] It would provide an immediate supply of membrane for the anticipated engulfment process. It would also provide an immediate extracellular supply of lysosomal enzymes, which, we argue, is important to the physiologic function of the cell (see below). At this point, for lack of evidence, no distinction is made between the ability of primary or secondary lysosomes, phagolysosomes, or nonlysosomal granules to undergo this hypothetical dissolution. Nor is there any evidence to support this concept—the necessary experiments as yet are lacking.

A Possible Physiologic Role for Immediate Secretion of Preformed Constituents

Phagocytic inflammatory cells have been shown to exhibit a rapid discharge of preformed hydrolytic enzymes upon reaction with particular stimuli. In the context of their involvement in inflammatory lesions, it is reasonable to suppose that their role involves the killing of microorganisms or abnormal cells and removal of the debris, both of endogenous (eg, dead and dying cells, disordered and altered connective tissue) as well as of exogenous origin. Much of this unwanted material is likely to be in a form that cannot initially be engulfed by the phagocyte (too big or attached to surfaces). A mechanism for discharge of hydrolases, leading to extracellular digestion, would be of immense value in such a process. As mentioned in the introduction, the question of effective action in this extracellular environment of hydrolases with low pH optima must be considered. Unfortunately, such a consideration at present is only speculative. Nevertheless, we know that low pH values are achieved in the phagolysosomes,[40] and vesicle transport (or other ion transport processes) may therefore result in extracellular discharge of H^+ ions as well. Despite the effective extracellular buffers, local pH at the sites of phagocyte substrate contact may be transiently lowered to values that allow transient action of the enzymes. A very effective control mechanism (rising pH due to buffering capacity of the extracellular fluid) would limit the action of the enzymes.

An additional circumstance in which extracellular enzyme discharge may be critical is during phagocyte migration into inflammatory lesions.

Emigration from the bloodstream and movement through tissues must involve crossing basement membranes and other connective tissue barriers. The mechanisms by which this is achieved are not yet understood, but early data from our own group (Spears, Henson, in preparation) and from Muller and Schiffman [41] suggest that neutrophils at least may release enzymes capable of digesting extracellular matrix proteins (including collagen) when stimulated with chemotactic factors. A localized digestive process may therefore allow inflammatory cell accumulation in tissues and may reflect the secretory events discussed herein.

References

1. Musson RA, Shafran H, Henson PM: Release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages. Correlation with changes in 5′-nucleotidase. J Reticuloendothel Soc (In press)
2. McCarthy K, Musson RA, Henson PM: Two mechanisms for macrophage enzyme release (Manuscript in preparation)
3. Henson PM, McCarthy K, Larsen GL, Webster RO, Giclas PC, Dreisin RB, King TE, Shaw JO: Complement fragments, alveolar macrophages, and alveolitis. Am J Pathol 1979, 97:93–10
4. Larsen GL, Mitchell B, Henson PM: The pulmonary response of C5 sufficient and deficient mice to immune complexes (Manuscript in preparation)
5. Gordon S: The secretion of lysozyme and a plasminogen activator by mononuclear phagocytes, Mononuclear Phagocyte in Immunity, Infection, and Pathology. Edited by R van Furth. Blackwell Scientific Publications, Oxford, UK, 1975, pp 463–473
6. Davies P, Bonney RJ: Secretory products of mononuclear phagocytes: A brief review. J Reticuloendothel Soc 1979, 26:37–47
7. McCarthy K, Henson PM: Induction of lysosomal enzyme secretion by alveolar macrophages in response to the purified complement fragments C5a and C5a desarg. J Immunol 1979, 123:2511–2517
8. Schnyder J, Baggiolini M: Secretion of lysosomal hydrolases by stimulated and nonstimulated macrophages. J Exp Med 1978, 148:435–450
9. Schnyder J, Baggiolini M: Role of phagocytosis in the activation of macrophages. J Exp Med 1978, 148:1449–1457
10. Axline SG, Cohn ZA: In vitro induction of lysosomal enzymes by phagocytosis. J Exp Med 1970, 131:1239–1260
11. Pesanti EL, Axline SG: Colchicine effects on lysosomal enzyme induction and intracellular degradation in the cultivated macrophage. J Ex Med 1975, 141:1030–1046
12. Weissmann G, Zurier RB, Spieler PJ, Goldstein IM: Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. J Exp Med 1971, 134:149s–165s
13. Henson PM: The immunologic release of constituents from neutrophil leukocytes: I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. J Immunol 1971, 107:1535–1546
14. Hawkins D: Biopolymer membrane: A model system for the study of the neutrophilic leukocyte response to immune complexes. J Immunol 1971, 107:344–352
15. Henson PM: The immunologic release of constituents from neutrophil leukocytes: II. Mechanisms of release during phagocytosis, and adherence to nonphagocytosable surfaces. J Immunol 1971, 107:1547–1557
16. Becker EL, Showell H, Henson PM, Hsu LS: The ability of chemotactic factors to induce lysosomal enzyme release: I. The characteristics of the release, the impor-

tance of surfaces and the relation of enzyme release to chemotactic responsiveness. J Immunol 1974, 112:2047–2054
17. Henson PM, Oades ZG: Stimulation of human neutrophils by soluble and insoluble immunoglobulin aggregates: Secretion of granule constituents and increased oxidation of glucose. J Clin Invest 1975, 56:1053–1061
18. Henson PM: Mechanisms of release of granule enzymes from human neutrophils phagocytosing aggregated immunoglobulin: An electron microscopic study. Arthritis Rheum 1973, 16:207–216
19. Henson PM: Mechanisms of activation and secretion by platelets and neutrophils, Progress in Immunology II. Vol 2. Edited by L Brent, J Holborow. New York, Elsevier North-Holland Publishing, 1974, pp 95–105
20. Stossel TP: Contractile proteins in phagocytosis: An example of cell surface-to-cytoplasm communication. Fed Proc 1977, 36:2181–2184
21. Painter RG, McIntosh AJ: The regional association of actin and myosin with sites of particle phagocytosis. J Supramol Struct 1979, 12:369–384
22. Berlin RD, Oliver JM: Analogous ultrastructure and surface properties during capping and phagocytosis in leukocytes. J Cell Biol 1978, 77:789–804
23. Ackerman NR, Beebe JR: Release of lysosomal enzymes by alveolar mononuclear cells. Nature 1974, 247:475–477
24. Henson PM, Ginsberg MH, Morrison DC: Mechanisms of mediator release by inflammatory cells, Membrane Fusion. Edited by G Poste, GL Nicolson. Elsevier-North-Holland Biomedical Press, 1978, pp 407–508
25. Berlin RD, Fera JP, Pfeiffer JR: Reversible phagocytosis in rabbit polymorphonuclear leukocytes. J Clin Invest 1979, 63:1137–1144
26. Hoffstein S, Weissmann G: Microfilaments and microtubules in calcium ionophore-induced secretion of lysosomal enzymes from human polymorphonuclear leukocytes. J Cell Biol 1978, 78:769–781
27. Benveniste J, Henson PM, Cochran CG: Leukocyte-dependent histamine release from rabbit platelets: The role of IgE, basophils and a platelet-activating factor. J Exp Med 1972, 136:1356–1377
28. Dvorak AM, Mihm Jr MC, Dvorak HF: Degranulation of basophilic leukocytes in allergic contact Dermatitis reactions in man. J Immunol 1976, 116:687–695
29. Dvorak, AM, Hammond ME, Morgan E, Orenstein NS, Galli SJ, Dvorak HF: Evidence for a vesicular transport mechanism in guinea pig basophilic leukocytes. Lab Invest 1980, 42:263–276
30. Caulfield JP, Lewis RA, Hein A, Austen KF: Secretion in dissociated human pulmonary mast cells: Evidence for solubilization of granule contents before discharge. J Cell Biol 1980, 85:299–312
31. Morrison DC, Roser JF, Curry BJ, Henson PM, Ulevitch RJ: Two distinct mechanisms for the initiation of mast cell degranulation: I. A specific inhibition of amine release by serum proteins. Inflammation 1978, 3:7–15
32. Shaw JO, Henson PM, Henson JE, Webster RO: Lung inflammation induced by complement derived chemotactic fragments in the alveolus. Lab Invest 1980, 42:547–558
33. Steinman RM, Brodie SE, Cohn ZA: Membrane flow during pinocytosis: A stereologic analysis. J Cell Biol 1976, 68:665–687
34. Muller WA, Steinman RM, Cohn ZA: Recycling of phagolysosome membrane proteins in cultured macrophages (abstr). J Cell Biol 1979, 83:258A
35. Schneider Y-J, Tulkens P, de Duve C, Trouet A: Fate of plasma membrane during endocytosis: II. Evidence for recycling (shuttle) of plasma membrane constituents. J Cell Biol 1979, 82:466–474
36. Farquhar MG: Recovery of surface membrane in anterior pituitary cells: Variations in traffic detected with anionic and cationic ferritin. J Cell Biol 1978, 77:R35–R42

37. Segal AW, Dorling J, Coade S: Kinetics of fusion of the cytoplasmic granules with phagocytic vacuoles in human polymorphonuclear leukocytes: Biochemical and morphological studies. J Cell Biol 1980, 85:42–59
38. Henson PM, Hollister JR, Musson RA, Webster RO, Spears P, Henson JE, McCarthy K: Inflammation as a surface phenomenon: Initiation of inflammatory processes by surface-bound immunologic components. Adv Inflam Res 1979, 1:341–352
39. Flectcher MP, Gallin JI: Degranulating stimuli increase the availability of receptors on human neutrophils for the chemoattractant f-Met-Leu-Phe. J Immunol 1980, 124:1585–1588
40. Jensen MS, Bainton DF: Temporal changes in pH within phagocytic vacuole of the polymorphonuclear neutrophilic leukocyte. J Cell Biol 1973, 56:379–388
41. Muller V, Schiffman E: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 1980, 284:67–68
42. Newman SL, Musson RA, Henson PM: Development of functional complement receptors during *in vitro* maturation of human monocytes into macrophages. J Immunol (In press)
43. Larsen GL, McCarthy K, Webster RO, Henson PM: A differential effect of C5a and C5a des Arg in the induction of pulmonary inflammation. Am J Pathol 1980, 100:179–192
44. Kaplan RL, Schocket AL, King TE, Maulitz RM, Good Jr JT, Sahn SA: A model of immune complex mediated pleuropulmonary injury: Evidence of deposition of circulating immune complexes in the lung. Am J Pathol 1980, 100:115–130

Acknowledgments

I am greatly indebted to a large number of people who, over the last ten years and more, helped, stimulated, and guided me through experiments, concepts, frustrations, and excitement. Particularly, I would like to mention David Garley, Peter Lachmann, Charley Cochrane, and Elmer Becker. Most important, however, is my debt to my wife Jan, whose support has been essential and has extended from the personal to the professional. Her electron-microscopic observations have set the stage for questions raised herein and the forthcoming experiments to explore the hypotheses presented.

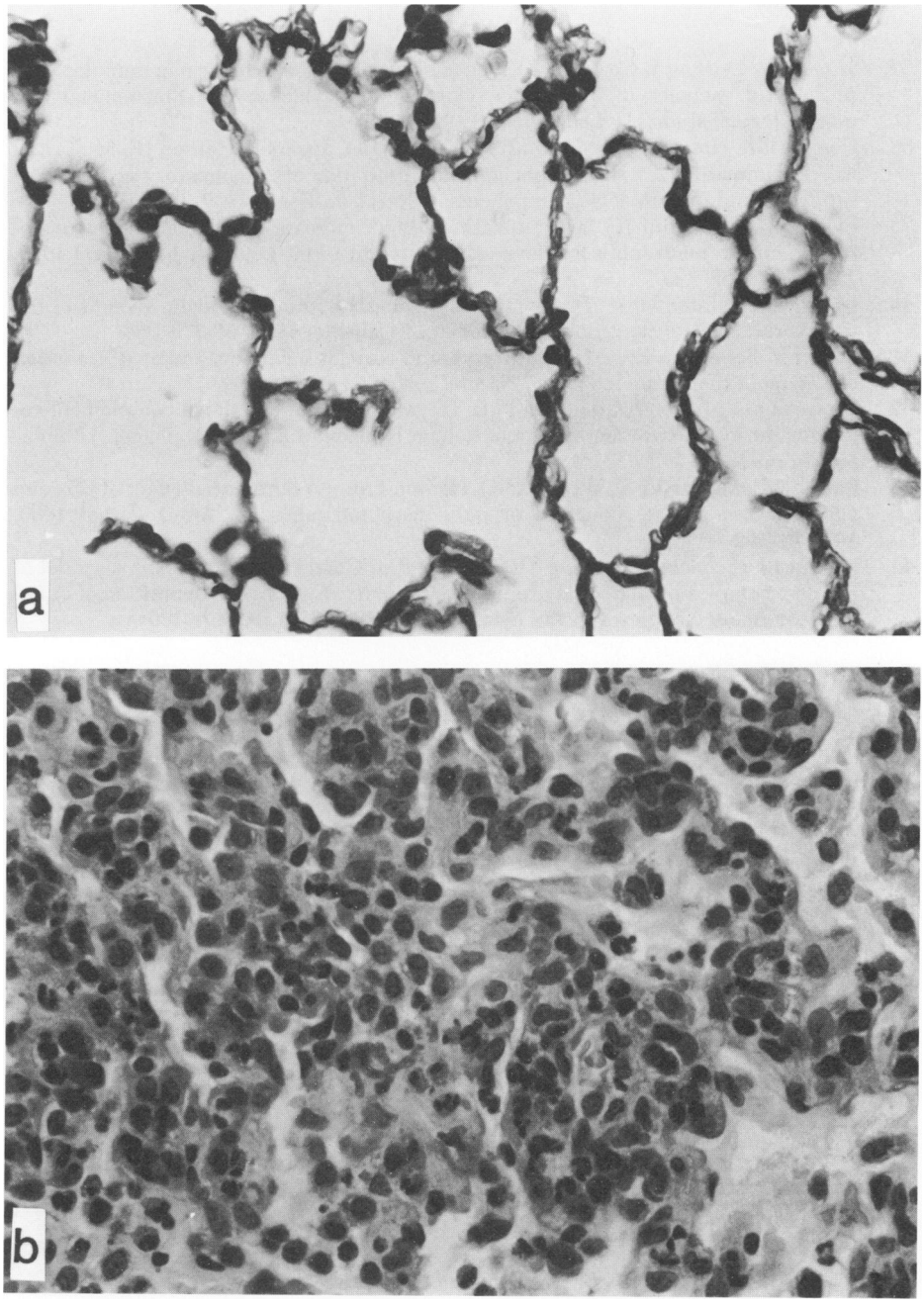
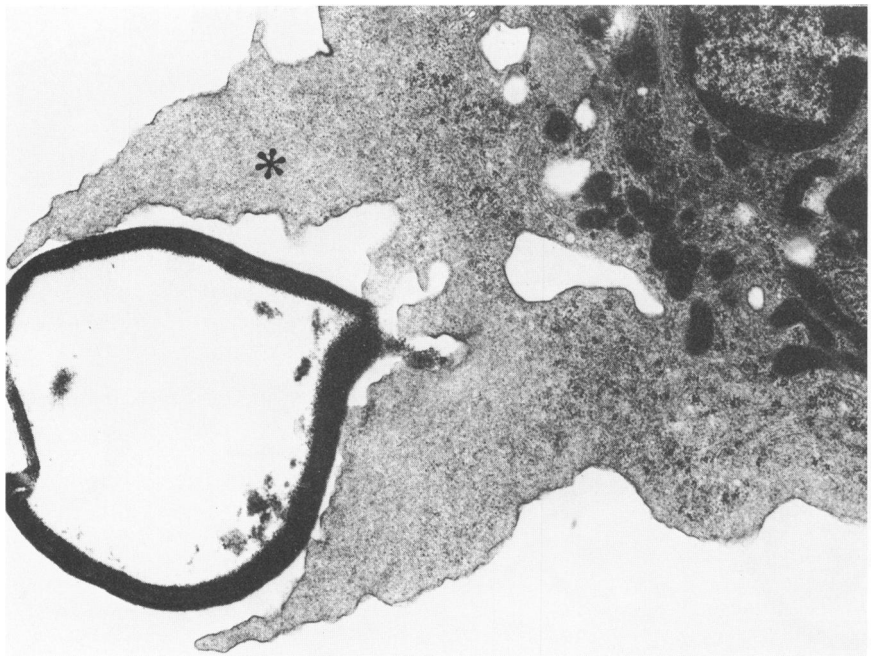


Figure 1—Pulmonary inflammation induced by instillation of the secreted products of alveolar macrophages stimulated *in vitro*. The supernatant fluids from rabbit alveolar macrophages harvested and stimulated as described in Reference 7 and Text-figure 1 were instilled into the lungs of normal rabbits as described.[42] The animals were sacrificed after 24 hours. **A**—Supernatant from control nonstimulated macrophages. **B**—Supernatant from macrophages phagocytosing opsonized zymosan particles. (×300)



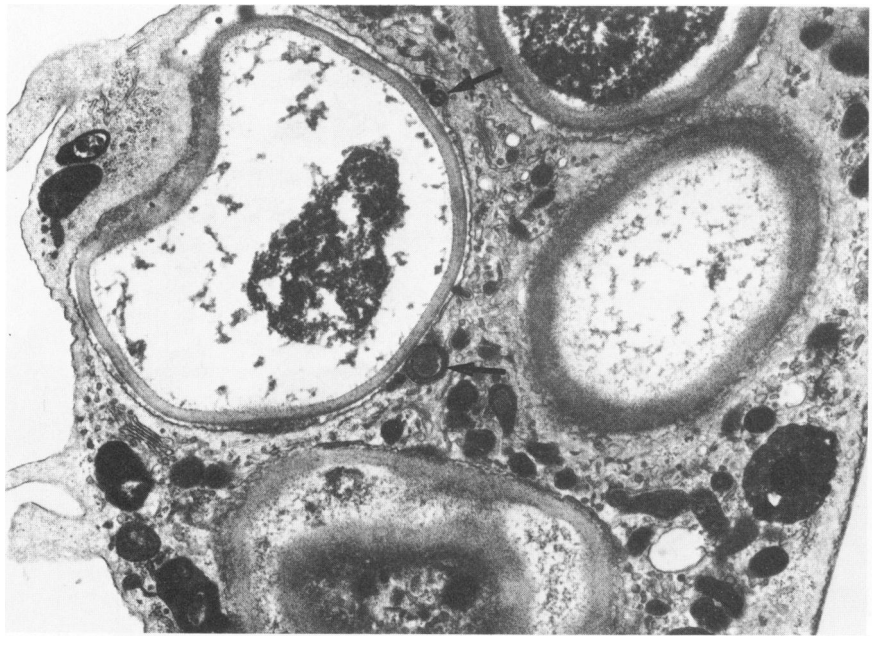
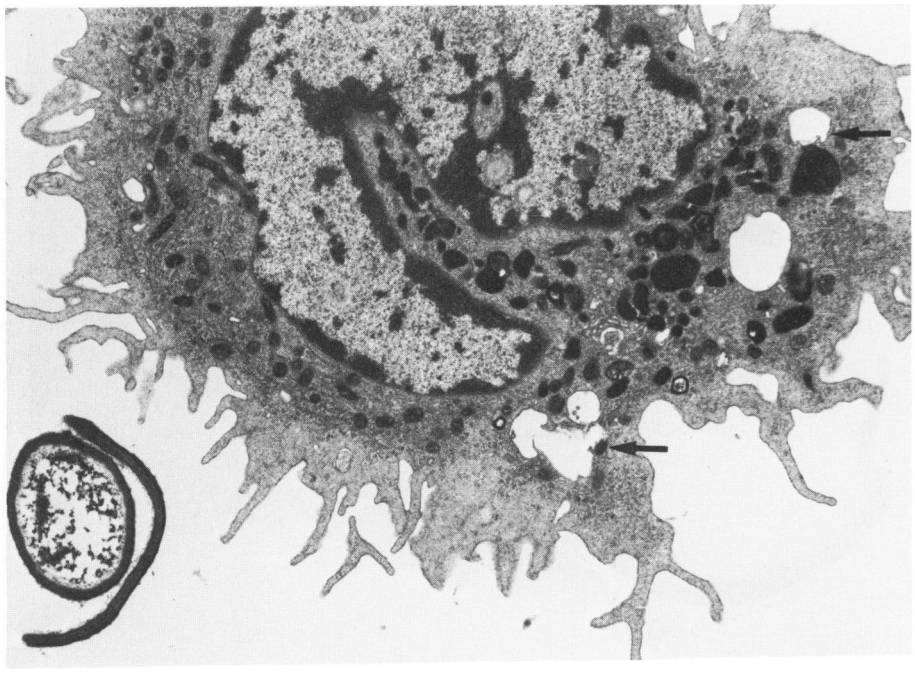


Figure 2—Rabbit alveolar macrophage five minutes after addition of opsonized zymosan. The conditions were those described in Text-figure 1. A zymosan particle is apparently in the process of being engulfed, and extensive organelle clearance (*asterisk*) can be seen in the enveloping pseudopods. (×15,000) **Figure 3**—Rabbit alveolar macrophage 30 minutes after the addition of opsonized zymosan. The particles are now completely enclosed, the areas of organelle clearance have dissipated, and phagosome-lysosome fusion is occurring (*arrows*). (×15,000)



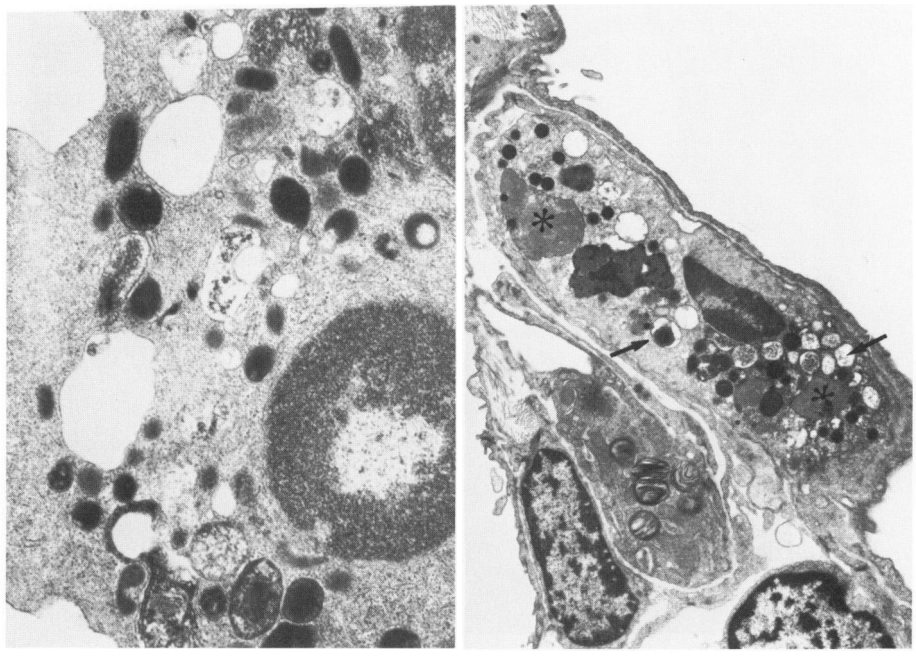


Figure 4—Rabbit alveolar macrophage 30 minutes after addition of opsonized zymosan. Apparent discharge of lysosomes into "vacuoles" is indicated by the *arrows*. (×8000) **Figure 5**—Human neutrophil undergoing a release reaction *in vitro*. Apparent *in situ* dissolution of granules, as well as possible discharge into "vacuoles" may be seen. (×20,000) **Figure 6**—Rabbit neutrophil in an alveolar capillary in a model of immune-complex–induced pulmonary inflammation. The experimental system is that described by Kaplan et al,[44] and the photomicrograph is courtesy of Kaplan and Henson. The neutrophil contains ingested immune complexes (*asterisks*) and exhibits numerous examples of granule "dissolution." (×5300)