# PHAGOCYTOSIS OF MAST CELL GRANULE BY THE EOSINOPHILIC LEUKOCYTE IN THE RAT*

RONALD A. WELSH, M.D., and JACK C. GEER, M.D.
*From the Department of Pathology, Louisiana State University, School of Medicine, New Orleans, La.*

During the course of an electron microscope study of mast cell degranulation in the rat peritoneal cavity, following administration of compound 48/80, eosinophilic leukocytes were discovered containing large ovoid bodies in their cytoplasm, identical in structure to liberated mast cell granules. The present report concerns the identification of these ovoid bodies in the eosinophils as mast cell granules which have undergone phagocytosis, and includes a discussion of the possible implications of this phenomenon.

## MATERIALS AND METHODS

An inbred strain of crossed Sprague-Dawley and Rose-Illinois adult rats was used for study. One milliliter of Tyrode's solution containing 0.1 mg. of compound 48/80 was injected intraperitoneally into each of 12 rats. Compound 48/80, a condensation of p-methoxyphenethylmethylamine with formaldehyde, was graciously supplied by Burroughs Wellcome and Company, Inc., Tuckahoe, New York. At intervals of 5, 10, and 15 minutes, the peritoneal cavity was opened under ether anesthesia and the fluid aspirated gently with a pipette. Twelve control rats were treated in a like manner with Tyrode's solution injected into the peritoneal cavity. A portion of the aspirated fluid was fixed in buffered 1 per cent osmic acid[1] with added sucrose[2] for 30 minutes, and each specimen was then centrifuged at 2,000 r.p.m. for 2 minutes, dehydrated in graded alcohols, and embedded in methacrylate. Ultrathin sections were prepared and examined in an RCA EMU-3c electron microscope. Fresh smears of the aspirated fluid were made on glass slides. These were allowed to dry in air, and were stained with Wright stain.

## RESULTS

### *Electron Microscopy*

The changes in the mast cells were similar to those described in previous work by Bloom, Larsson and Smith,[3] and Smith and Lewis,[4] and will not be elaborated in detail. Figure 1 depicts a normal mast

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cell as observed in our study and Figure 2, a mast cell undergoing degranulation following the administration of compound 48/80.

Extracellular mast cell granules were noted at all time intervals following the injection of compound 48/80. The extracellular granules were similar to those within the mast cells; however, the majority of liberated granules were covered by a thin irregular coating of granular amorphous material (Fig. 3).

Eosinophilic leukocytes were identified by their smaller, often elliptical granules containing a typical dense band or bar. In some cases the central bar in the eosinophilic granule appeared less dense than the remainder of the granule. This change was observed in the control rats with the same frequency as in the cells of animals to which compound 48/80 had been administered. No granules resembling mast cell granules were seen in the cytoplasm of the eosinophilic leukocytes of the controls.

At all time periods following the injection of compound 48/80, occasional eosinophils contained varying numbers of small ovoid bodies in their cytoplasm, lying in a clear space surrounded by a single membrane. The ovoid bodies were identical in size and shape with mast cell granules and often had small bits of granular amorphous material adherent to their surfaces (Fig. 3).

In the controls a rare histiocyte or mesothelial cell was found containing ingested ovoid granules morphologically identical to mast cell granules. Since mesothelial cells may act as phagocytes under certain conditions, exact differentiation from histiocytes was not attempted, and the noncommittal term "phagocyte" was used for the two cells when ingested material was found in their cytoplasm. Phagocytes containing similar ovoid bodies surrounded by a membrane were noted frequently at all time intervals after injection of compound 48/80.

### *Light Microscopy*

Wright-stained, air-dried smears from the control rats showed no evidence of blue granules in over 500 eosinophils examined; however, in smears at all time intervals following the injection of compound 48/80, varying numbers of large blue stained granules were found in an average of 20 per cent of the eosinophilic leukocytes. There were usually 2 or 3 blue granules per cell, but occasional eosinophils contained 12 or more. These granules were similar to those in mast cells and to the large numbers of extracellular mast cell granules encountered. Mast cell granules were seen in the cytoplasm of other phagocytes and eosinophilic leukocytes with approximately the same frequency.

## Discussion

The peritoneal cavity of the rat is an ideal locale for this type of study since a rich harvest of mast cells, eosinophils, histiocytes, lymphocytes, and mesothelial cells can be obtained normally from this region. The differential counts of the peritoneal fluid vary somewhat, but mast cells range between 5 and 15 per cent, and eosinophils from 20 to 30 per cent of all cells. Compound 48/80 is one of the least toxic histamine liberators available and appears to show cytologic effect only upon mast cells, producing a rapid discharge of granules to the extracellular environment. Previous reports of mast cell changes seen by electron microscopy following the administration of compound 48/80 and other agents that produce mast cell degranulation, e.g. stilbamidine, protamine, toluidine blue, and x-radiation, have not specifically indicated alterations of eosinophilic leukocytes.[3,4] The mastocytoma of the dog was studied by electron microscopy, but no granules resembling those of the mast cell were described in the cytoplasm of eosinophils.[5]

Several possibilities should be considered to account for the presence of mast cell granules in eosinophils: (1) artifacts of manipulation prior to fixation or during centrifugation; (2) specific alteration of granules in the eosinophilic leukocytes; and (3) phagocytosis of mast cell granules by eosinophils.

The first possibility does not appear to be likely since control specimens did not show similar bodies in the cytoplasm of eosinophils when examined either by electron or light microscopy. It is unlikely that granules could be forced into the fixed cytoplasm of a cell without definite evidence in the electron photomicrographs of mechanical rupture of the cell membrane, and such a rupture has not been found. The possibility that the cytoplasmic bodies in eosinophils were altered eosinophilic granules is feasible if one were to postulate that the rat eosinophil contains granules which possess the properties of mast cell granules. Study of the eosinophils in the controls failed to reveal any granules exhibiting this capacity, and there was no indication that the eosinophil granule underwent any morphologic change following the injection of compound 48/80.

The third possibility, that these are ingested mast cell granules, seems most likely in view of the known phagocytic activity of the eosinophil.[6,7] The ovoid bodies observed are the same size and shape as mast cell granules; they have a coating or rim of granular material attached to their outer surfaces similar to that noted on granules liberated from mast cells, and are surrrounded by a limiting membrane identical to that of mast cell granules ingested by other phagocytes. A

question that immediately comes to mind is why these bodies have not been identified previously in eosinophils with the light microscope under these conditions. One probable answer to this lies in the fact that following administration of compound 48/80, the mast cell granule loses its metachromatic property,[8] thus making it difficult if not impossible to identify it with any degree of certainty. In smears procured after the administration of compound 48/80 and stained with Wright stain, bluish bodies similar to mast cell granules can be identified easily in eosinophils. In this case, however, the possibility of superimposition of extracellular granules on the eosinophils must be considered. Electron microscopy proves that granules identical to those in mast cells are incorporated within the cytoplasm of eosinophils.

To our knowledge, this is the first report of phagocytosis of mast cell granules by the eosinophilic leukocyte. It is possible that this represents a nonspecific action, but other experimental work on these two cells points to interrelationships that lend more significance to the phenomenon. Mast cell granules are known to be a potent source of histamine,[9] and histamine alone produces an increased number of eosinophils in the circulating blood.[10] A chemical substance with antihistaminic properties has been obtained from eosinophils,[11] and it has been postulated that the eosinophilic leukocyte acts as an inactivator of histamine and histamine-like substances.[12] The eosinophil has also been shown to be capable of transporting specific antigen.[13] The fact that this cell engulfs mast cell granules may indicate a common line of defense. Thus, in the event of marked histamine release, the eosinophil could serve as a means of rapid isolation and neutralization of the histamine-laden particles. In the lesion of urticaria pigmentosa, for example, characterized as it is by a dense infiltration of tissue by mast cells and a rich histamine content, stroking of the lesion causes disruption of the mast cells, edema, and an influx of eosinophils.[14] In the mastocytoma of the dog, a tumor of mast cells, eosinophils form a prominent component and are scattered diffusely throughout the lesion.[8] This tumor also contains a high concentration of histamine.[15]

"Basophilic bodies" have been described in the cytoplasm of human eosinophilic leukocytes.[16,17] The exact nature of these has not been determined, although Di Mayorca, Lanzavecchia and Le Coultre[17] have suggested that they are mast cell granules. They differ from those seen in the rat eosinophil in the absence of a surrounding clear space with outer limiting membrane. It is not known whether or not mast cell granules engulfed by rat eosinophils assume a similar appearance after a period of several days; further studies are needed to determine this possibility. Based on the observations in our study, we believe that

the "basophilic bodies" in circulating eosinophils in man should be seriously suspected as representing phagocytosis of mast cell granules.

## SUMMARY

Phagocytosis of mast cell granules by eosinophilic leukocytes is described in the rat following intraperitoneal injection of compound 48/80. The possible significance of this phenomenon is discussed in relation to histamine release and allergic inflammation. It is suggested that the phagocytosis of mast cell granules may be the basis for the "basophilic bodies" seen in circulating eosinophils in human subjects.

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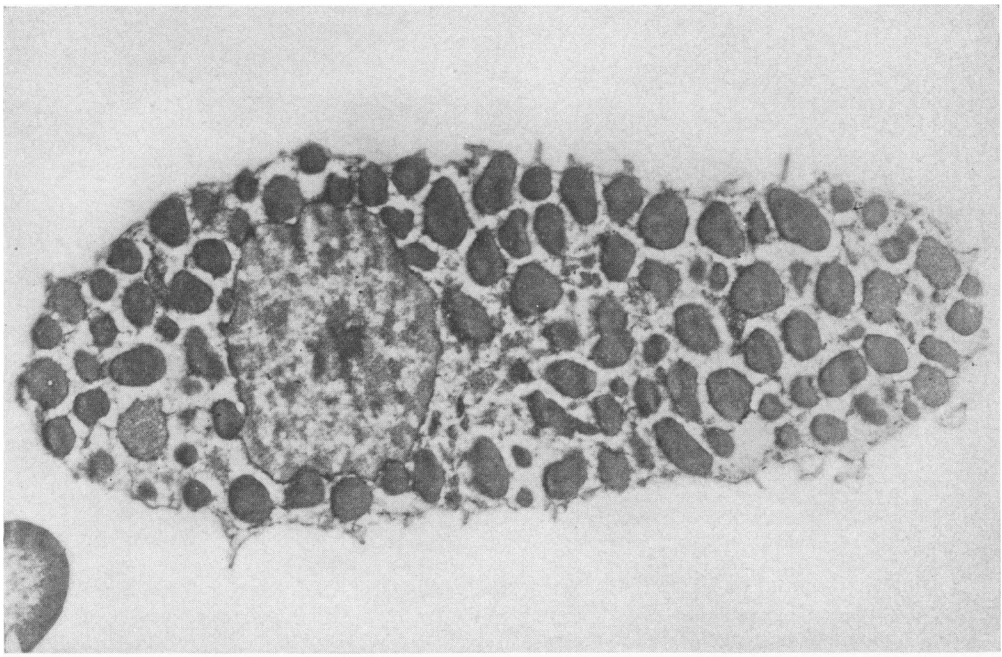
## LEGENDS FOR FIGURES

FIG. 1. Normal mast cell from peritoneal cavity of control rat. × 8,350.

FIG. 2. Mast cell five minutes after administration of compound 48/80. The granules lie in a clear space and can be seen escaping extracellularly. Note the granular amorphous material that surrounds the granules. × 8,350.



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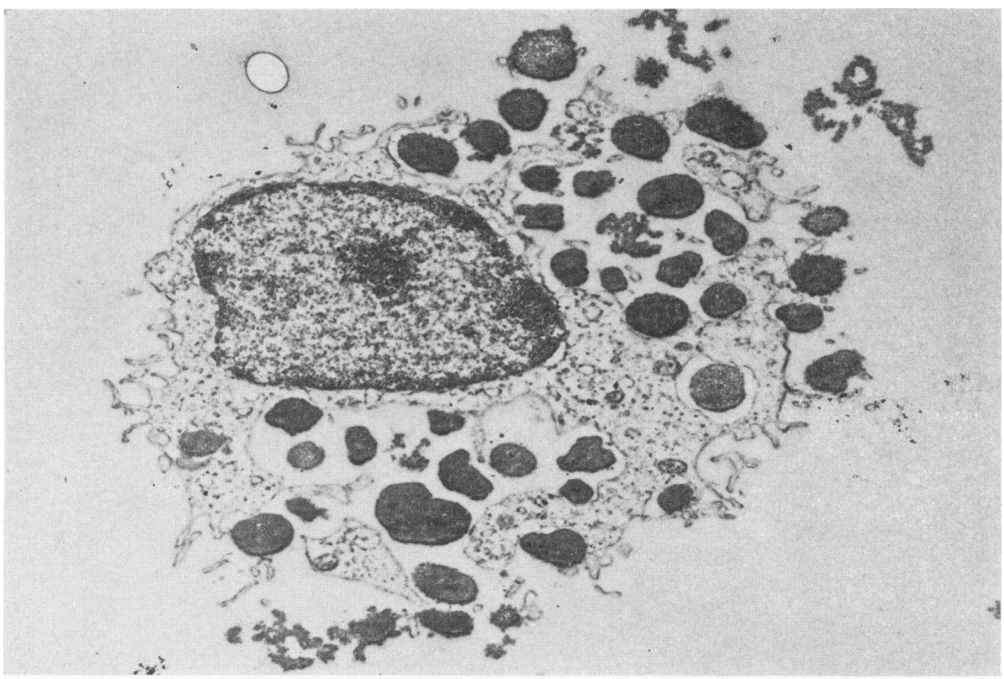

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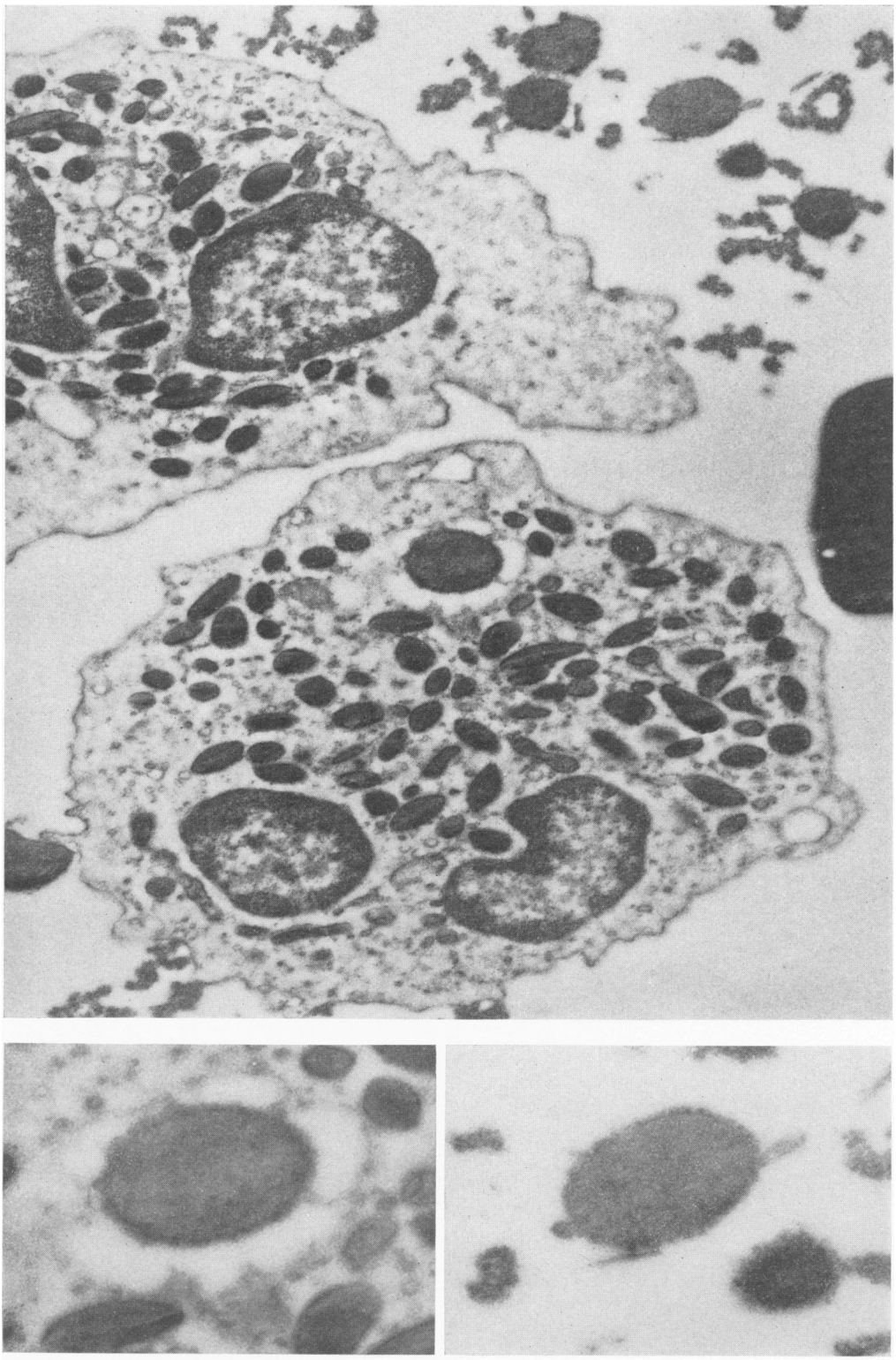
FIG. 3. Upper. Two eosinophilic leukocytes are present. The lower one contains a mast cell granule in its cytoplasm. In the upper right corner are several extracellular mast cell granules. × 13,440.
Insert, bottom left. Higher magnification of mast cell granule seen in an eosinophil. × 31,275. Insert, bottom right. Higher magnification of extracellular mast cell granule. × 31,275.



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