FERRITIN PARTICLES IN MACROPHAGES AND IN ASSOCIATED MAST CELLS

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ABSTRACT

In a variety of tissues (lymph node and glandular stroma), mast cells have been found in close and often intimate association with macrophages containing numerous ferritin-like particles in their cytoplasm and within cytoplasmic vacuoles (siderosomes). Phagocytic vacuoles in a given macrophage differed markedly. Some contained abundant Prussian blue–reactive material and others contained periodic acid–Schiff reactive substance at the light microscope level, and ultrastructurally some were filled with ferritin particles and others were not. Ferritin-like particles have also been observed occasionally in the mast cells associated with macrophages and even within the matrix of some of the granules in these mast cells.

In certain tissues a relationship has been noted at the light microscope level between mast cells and iron-laden macrophages (Spicer, 1960, 1963; Hall, 1964) referred to previously as siderophages. The frequency and distribution of mast cells and these siderophages were parallel in many tissues, particularly in aged animals, and the two cell types were often observed in intimate association (Spicer, 1960, 1963). In an effort to characterize further the relationship between these two cell types, additional studies have been undertaken at both the light and electron microscope levels. During the course of these studies, it has been observed that phagocytes in close association with tissue mast cells consistently contain in their cytoplasm and phagosomes numerous electron-opaque particles with the dimensions and characteristics of ferritin particles and that similar particles are present, although in less abundance, in neighboring mast cells.

MATERIALS AND METHODS

Female Sprague-Dawley rats, weighing between 150 and 200 g, were anesthetized with pentobarbital, and specimens of cervical lymph node and salivary glands were promptly fixed for either light or electron microscopy. For light microscopy, tissue was fixed in 10% Formalin buffered with 2% calcium acetate, embedded in paraffin, and sectioned at 5 μ. Deparaffinized sections were stained with Perls' Prussian blue technique (see Lillie, 1965) to demonstrate hemosiderin, followed either by safranin (Spicer, 1960) to demonstrate acid mucosubstances, or by the periodic acid–Schiff (PAS) method commonly used to demonstrate neutral mucosubstances.

Tissue for electron microscopy was fixed with either cacodylate-buffered glutaraldehyde (Sabatini et al., 1963) or paraformaldehyde-glutaraldehyde (Karnovsky, 1965, cacodylate buffered, without $CaCl_2$) and postfixed in collidine-buffered 2% osmium tetroxide (Bennett and Luft, 1959). The tissues were dehydrated, embedded in Epon, and sectioned at about 500 A. Thin sections were examined in the electron microscope either unstained or after staining with lead citrate alone (Reynolds, 1963). The micrographs used as illustrations were taken on an AEI-6B, at an accelerating voltage of 50 kv at relatively low power (5000–15,000 direct magnification) with a well spread beam, and enlarged to the magnification indicated in the figure legends.

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RESULTS

At the light microscope level, the Prussian blue procedure for Fe^{+++} demonstrated many siderophages in lymph nodes although they were rarely evident in the salivary gland stroma. Generally, these cells contained intensely stained inclusions, as well as a faint blue cytoplasmic background. Prussian blue staining followed by safranin indicated that mast cells were frequently associated with siderophages (Fig. 1 a; see also Spicer, 1960, 1965), confirming previous observations, and extending these to younger animals. When stained with a Prussian blue–PAS sequence, the siderophages often contained iron-reactive bodies in addition to discrete PAS-positive inclusions which showed little or no Prussian blue reactivity (Fig. 1 b), suggesting that these phagocytes contained a variety of ingested materials, reflected in variable phagocytic vacuoles.

Electron microscope examination of a large number of mast cells from lymph nodes and salivary gland stroma disclosed mast cells regularly associated with cells containing ferritin-like particles in the cytoplasm and in phagocytic vacuoles (Figs. 2, 3, and 4). The latter macrophages apparently were the ultrastructural counterpart of Prussian blue–positive macrophages observed in close association with mast cells at the light microscope level. Processes from apposing surfaces of the two cell types often interdigitated intimately (Fig. 2).

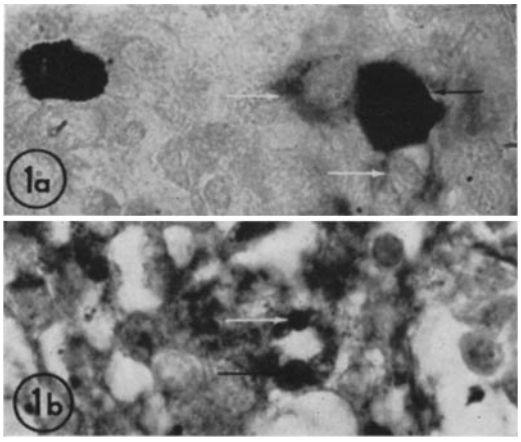

FIGURE 1 Rat lymph node. Fig. 1 a, Stained with Prussian blue–safranin. Mast cell (black arrow) in association with two siderophages (white arrows) which show faint Prussian blue cytoplasmic reaction. × 800. Fig. 1 b, Stained with Prussian blue–PAS. A siderophage containing both Prussian blue–positive (white arrow) and PAS-positive (black arrow) inclusions in its cytoplasm. × 800.

The fine, electron-opaque particles in both cytoplasm and vacuoles of these phagocytes could be distinguished easily in unstained tissue or in tissue stained with lead citrate alone, but were obscured by uranyl acetate–lead citrate staining. Photographic enlargement of these particles revealed a dense core, about 60–80 A in diameter, surrounded by a light halo about 150 A in diameter (Fig. 3, inset). The size of the internal dense core corresponds well with the size of the iron micelles in ferritin as reported by Farrant (1954) and by Richter (1957), although configurations resembling a tetragonal structure were observed only rarely (Fig. 3, inset). Despite their profusion in the cytoplasm, the ferritin-like particles were absent from mitochondria and were rare in or even absent from some of the phagocytic vacuoles of the same cells (Fig. 2). These latter vacuoles probably correspond to the PAS-positive, Prussian blue–negative inclusions seen with the light microscope.

Although mast cells and adjacent ferritin-loaded cells often had extensive interdigitations, the processes of the two cell types could be distinguished readily due to the paucity of ferritin-like particles in the mast cell cytoplasm compared with the macrophage cytoplasm. However, ferritin-like particles were occasionally observed within mast-cell cytoplasm, especially at the periphery of occasional granules (Figs. 4, 5, 6). In fortuitous sections, when the inherent electron-opacity of the granules was not too great, ferritin-like particles could be discerned within the translucent rim and dense center of some mast-cell granules themselves (Fig. 6). The granules containing such particles usually had a moderately dense core and differed from those which had a core with a filamentous appearance and which were presumably less mature (Combs, 1966). The latter disclosed ferritin particles only in rare instances.

DISCUSSION

Although the association of mast cells and siderophages has been noted previously (Spicer, 1960, 1963; Hall, 1964, and, although there have been reports suggesting erythrophagocytosis by mast cells (Ultmann et al., 1964; Greene and Spicer, 1969), the mast cell has rarely been associated with iron metabolism. Iron is not detectable by the

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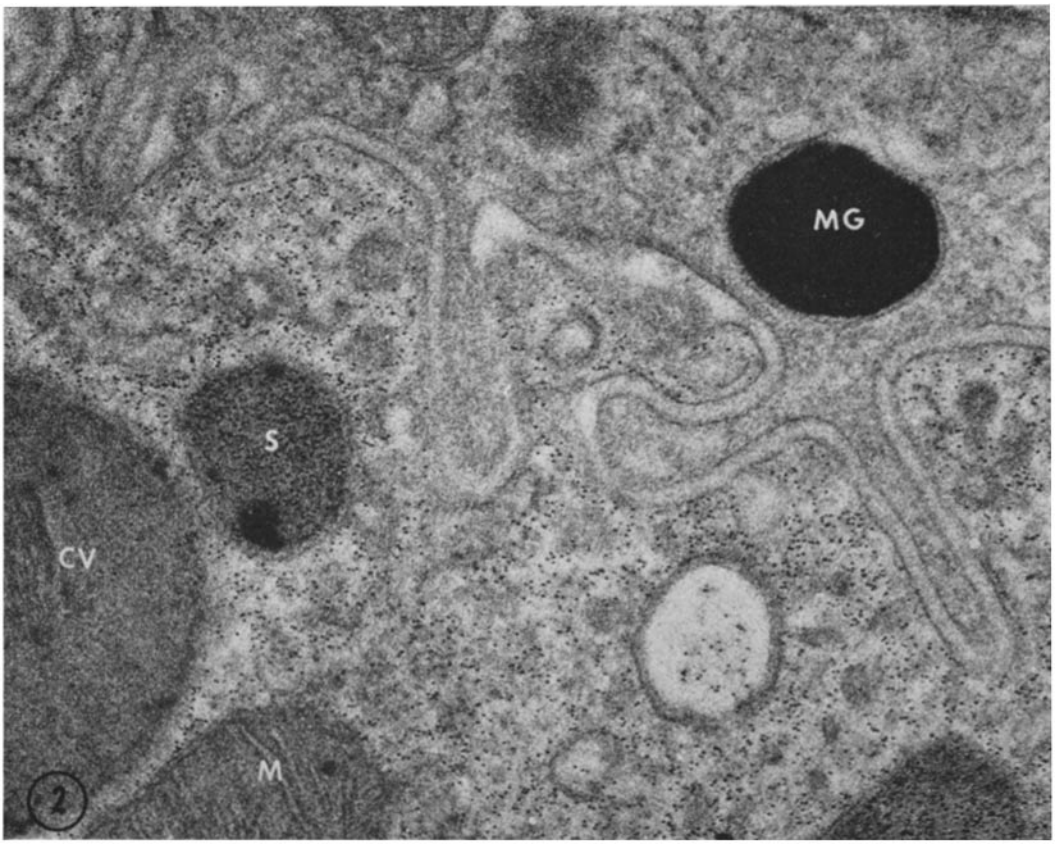

FIGURE 2 Mast cell and siderophage showing interdigitating cytoplasmic processes. The siderophage contains numerous fine, ferritin-like particles in its cytoplasm, as well as in a siderosome (*S*). Note that particles are absent from mitochondria (*M*), and are rare in some cytoplasmic vacuoles (*CV*). The mast cell may be identified by its typical cytoplasmic granule (*MG*). Rat lymph node. Unstained thin section. × 50,000.

Prussian blue method in mast-cell granules, although the presence of a reddish ash upon microincineration of mast cells led Padawer (1963) to suggest the possibility of its presence.

The identification of the fine particles in the cytoplasm and vacuoles of macrophages (Figs. 3, 4, 5) as ferritin is based on morphological criteria. The electron-opaque core of 60–80 A corresponds in size to the iron micelle of ferritin, and the surrounding clear halo resembles that described by Richter (1957) and presumed to represent the protein apoferritin. Although the diameter of the clear halo (about 150 A) is larger than that reported by Farrant (1954) for shadow-cast ferritin molecules, the ferritin molecules on which he did his classical measurements were dried before examination and thus were probably smaller than native ferritin. The particles reported in the present study lie within the range that might be expected for hydrated ferritin on the basis of X-ray crystallographic measurements (Hodgkin, 1949). The scarcity of tetragonal configurations in the fine particles observed is not inconsistent with their identification as ferritin (see Muir, 1960, and Haydon, 1969 for a discussion of the fine structural morphology of ferritin). In addition to the fact that the particles have the appropriate size and density for ferritin, the conviction that the dense particles reported herein are, in fact, ferritin rests largely upon the observation (Figs. 3–5) that they are usually located within morphologically identifiable siderophages (Richter, 1958).

The basis of the inherent electron opacity of many mast-cell granules is not understood, al-

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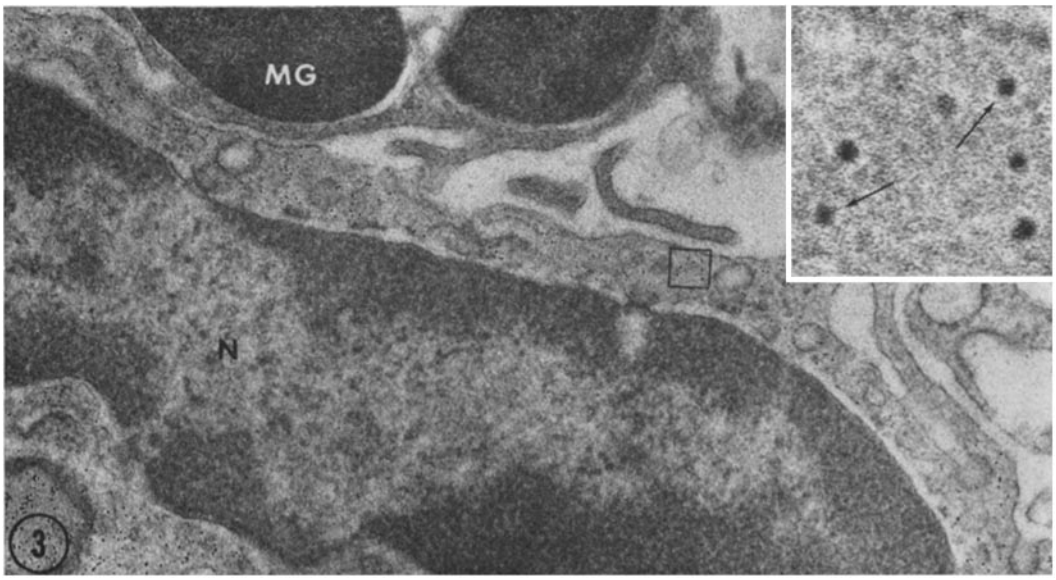

FIGURE 3 Mast cell and siderophage from stroma of rat salivary gland. *MG*, mast-cell granule; *N*, nucleus of siderophage. Unstained. × 37,500. Inset, high-power magnification of region outlined. Note dense core surrounded by lighter halo in ferritin-like particles. Two dense cores (arrows) show tetragonal structure. × 300,000.

though the presence of zinc has been reported by Amann (1962), and the possibility of iron, calcium, and magnesium in mast cells was suggested by Padawer (1963). Selye et al. (1963, 1964) have demonstrated the local precipitation of various cations (including iron and lead) on discharged granules of tissue mast cells. Evidence presented by Padawer (1969) suggests that extracellular fluid may percolate over mast-cell granules. Strongly anionic (heparin; Jorpes, 1947) or cationic (basic protein; Spicer, 1963) macromolecules in the granules could serve to extract and bind ions from the surrounding fluid, as mast cells are apparently able to selectively extract and bind injected radioiodide (Jones et al., 1964). Thus, the inherent electron opacity of mast-cell granules may be due in part to the presence of metallic cations strongly bound to the highly anionic heparin in the granules.

The observation of ferritin-like particles in occasional mast-cell granules suggests that ferritin may be taken up as such by the granules and then transformed into another form which is no longer recognizable as ferritin. The peptidases demonstrated histochemically in mast cells (Lagunoff and Benditt, 1963) afford a possible biochemical mechanism for such a transformation. The low concentration of ferritin particles observed in occasional mast-cell granules in the present study would be below the limit of detection by the Prussian blue method at the light microscope level. This observation exemplifies the greater sensitivity of the electron microscope compared with the light microscope for detecting small quantities of material. That mast cells are, in fact, capable of endocytosing exogenously administered substances has been amply demonstrated by Padawer (1968, 1969, 1971). The results of the present study suggest that the mast cell performs this function with at least certain endogenous materials as well.

We were impressed by the consistency with which cell processes containing ferritin-like particles were found in association with mast cells in bone marrow and lymph nodes, and even in the stroma of the salivary glands, where few or no Prussian blue–reactive cells could be observed at the light microscope level. In a recent report on the development of mast cells, Combs (1971) noted a consistent relationship between mast cells and macrophages, both in vivo and in vitro. Although these micrographs of stained sections at relatively low magnification did not disclose ferritin particles in the cytoplasm, numerous characteristics reported were similar to those seen in the sidero-

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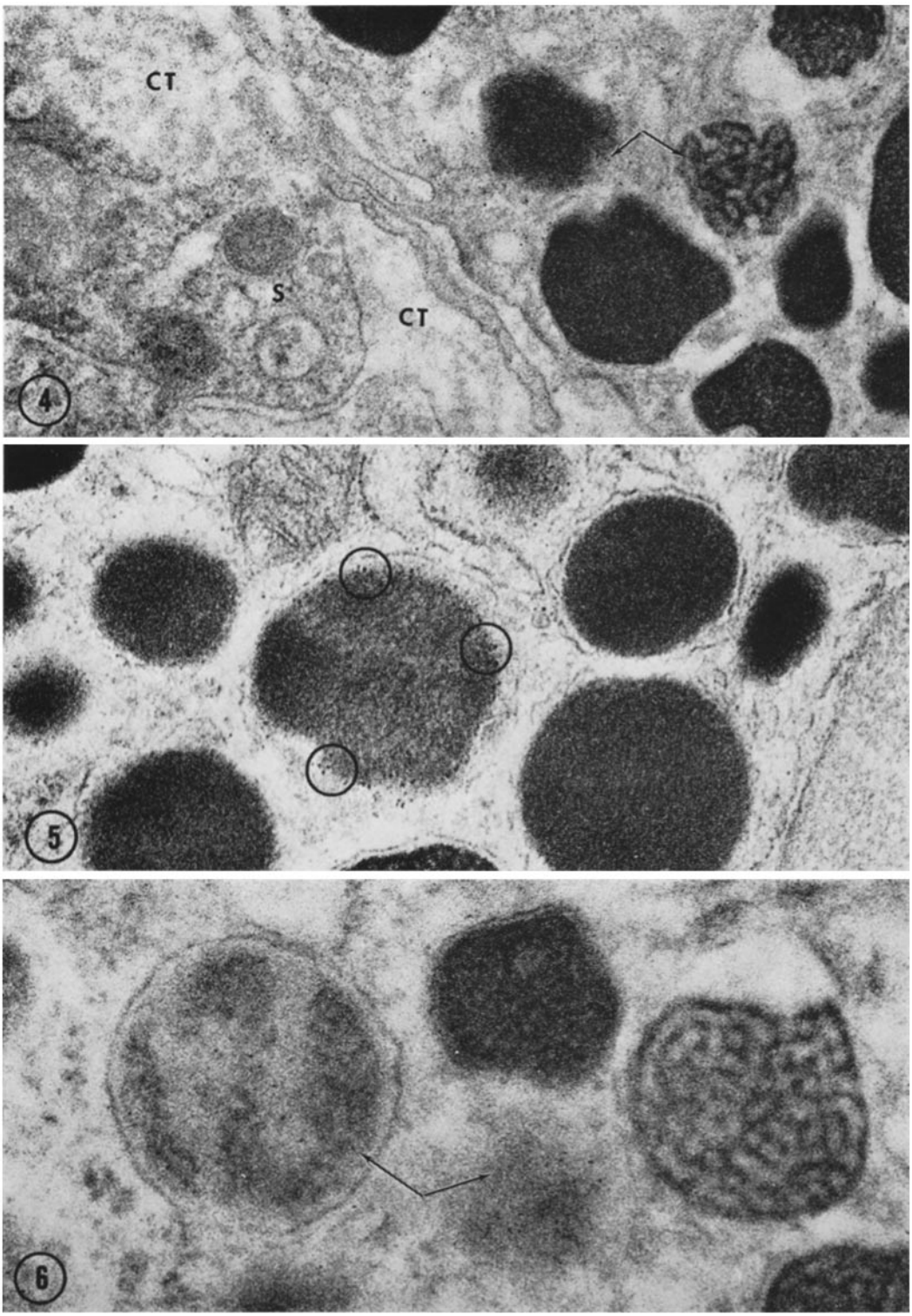

FIGURE 4 Mast cell (on right) and siderophage (*S*) from rat lymph node. Ferritin-like particles are apparent in the mast cell cytoplasm and are associated with some of the mast-cell granules (arrows). Note that no ferritin particles are seen in the extracellular spaces (*CT*). The cell membranes are rather indistinct in this unstained section, particularly where sectioned tangentially, as at the upper border of the siderophage. × 37,500.

FIGURE 5 Mast cell from lymph node of rat. Ferritin particles (encircled) appear to be aggregated around the periphery of the granule in the center of the field. Unstained. × 52,500.

FIGURE 6 Mast-cell granules. Two of the granules (arrows) show ferritin-like particles within them. The larger one shows a distinct limiting membrane. Rat lymph node. Unstained. × 75,000.

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phages of the present study, including smooth and "bristle-coated" vesicles in the cytoplasm immediately adjacent to mast cells, and cytoplasmic processes which interdigitated with those of mast cells.

The consistently observed juxtaposition of these two cell types suggests a functional relationship involving exchange between the cells. Two obvious possibilities present themselves: (*a*) substances released from mast-cell granules may aid in phagocytosis by the macrophage, or (*b*) small molecules and/or ions liberated by the macrophage during the processing of phagocytized material may become bound to macromolecules in mast-cell granules, for storage, for metabolic processing, or to reduce the effective concentration of these substances in the extracellular fluid.

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