Repopulation and Maturation of Guinea Pig Mesentery Mast Cells:A Light and Electron Microscopic Study

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Abstract

Mast cells are known to play a critical role in asthma, allergy and inflammation. Although, the guinea pig has been widely used as an experimental animal to study mast cell function in these conditions, little is known about repopulation and maturation of guinea pig mast cells *in vivo*. The present study was undertaken to characterize the maturation and repopulation of guinea pig mesenteric mast cells following intraperitoneal injection of distilled water. In uninjected animals, mesenteric mast cells can be found lying adjacent to blood vessels in the fat sheaths and throughout the windows of the mesentery. By 24 hours after distilled water injection, the mesenteric mast cells appeared lysed with many granules lying free in the extracellular matrix. At 3 days after injection, the mesentery was almost devoid of mast cells. A few immature cells could be found associated with the blood vessels in the fat sheaths. By 5 days, mast cells were found not only adjacent to the blood vessels, but also in the mesenteric windows. During this initial stage of repopulation of the mesentery, a very immature mast cell lying along the blood vessels in the fat sheaths was also identified by electron microscopy. On day 7, the appearance of the mast cells in the mesentery was similar to that seen in control animals. The repopulation and maturation of guinea pig mesenteric mast cells *in vivo* was similar to that previously reported for rats, but differed in the time course of disappearance of the guinea pig mast cell granules and the appearance of immature mast cells.

Keywords: Guinea pig, mast cell, maturation, repopulation, mesentery

Introduction

Mast cells play a central role in a variety of allergic and inflammatory processes including asthma [5, 7, 16, 22]. The guinea pig has been widely used in mast cell research since the early 1900's when histamine was recognized as a spasmogen in guinea pig airways. Subsequently, both the rat and the guinea pig have been used extensively to examine mast cell biology in a variety of tissues. One of the more widely used model systems for mast cell maturation is repopulation of the rat peritoneal cavity after distilled water injection. The intraperitoneal injection of distilled water lyses rat peritoneal and mesenteric mast cells [4, 15], and the mast cells disappear from the mesentery and peritoneal cavity within 24 hours after injection [15]. Immature mast cells containing a few metachromatic granules first appear in the mesentery alongside blood vessels present within fat sheaths 5 days after distilled water injection. Later, the mast cells become progressively more mature and are found toward the middle of the mesenteric windows as well as along blood vessels. By the third week, repopulation of the mesentery is complete. Although this process of repopulation has been well characterized in the rat, relatively little is known about it in the guinea pig.

Differences in the response of rat and guinea pig mast cells to various stimuli have been reported [14, 17, 18, 26]. The present study was undertaken to characterize the response of guinea pig mesenteric mast cells to intraperitoneal injection of distilled water and to examine the repopulation of mast cells in the mesentery in order to compare these results to those previously reported for rat mesenteric mast cells.

Materials and Methods

Guinea pigs (male and female, 300 g) were used. Experimental animals were injected intraperitoneally with 30 ml of

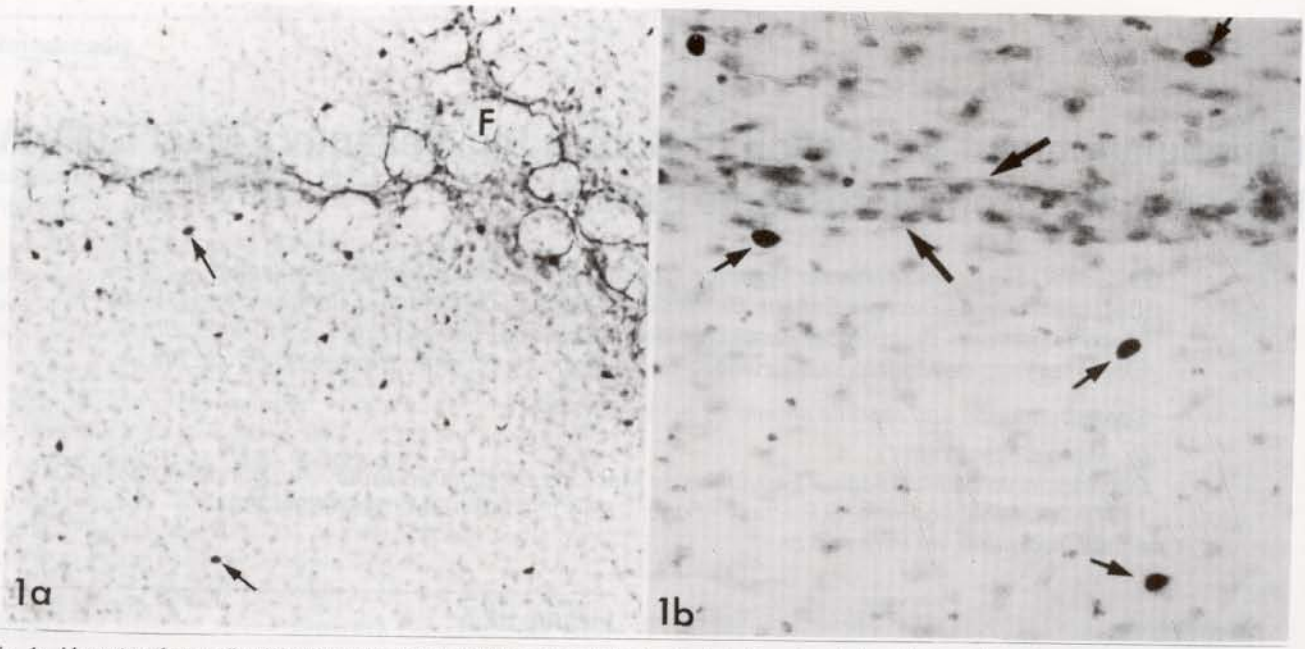

Fig. 1: *Mesentery from saline injected guinea pigs. Toluidine blue stained -* **1A:** *Intact mast cells (small arrows) are found adjacent to blood vessels in fat sheaths (F) and spread throughout the mesentery window. x100 .* **1B:** *Mature mast cells (small arrows) with their cytoplasm filled with metachromatic granules are associated with a blood vessel (large arrows). x250.*

warm (37°C) distilled water and control animals were either uninjected or received 30 ml of warm saline (0.9% NaCl). At various time intervals after injection, the animals were decapitated and the mesentery of the small intestine was removed.

For light microscopy, mesenteries were collected 1, 3, 5 and 7 days after distilled water injection. They were then washed twice in saline, fixed in 2% lead subacetate [18] for 20 min, washed in distilled water, spread on glass slides and stained with 0.1% toluidine blue in water plus 1% acetic acid, pH 3.5.

For electron microscopy, mesenteries were collected 5 days after distilled water injection and rinsed in saline. In order to study the immature mast cells associated with the blood vessels, the tissue had to be processed and embedded flat. The mesentery fragments were stretched on the bottom of a glass petri dish and a second smaller petri dish was placed on top of the fragments to hold them in place. The tissue was rinsed in 0.2M cacodylate buffer, pH 7.4 and fixed in 2% glutaraldehyde (Ladd Research Industries, Burlington, VT) and 2% formaldehyde (Ladd) containing 0.05% $CaCl_2$ in cacodylate buffer for 1 hr at room temperature, post-fixed in 1% OsO_4 for 1 hr, dehydrated through a graded series of ethanol and infiltrated with Spurr's resin [23]. Selected areas of the mesentery fragments were then cut into 2 x 2 cm pieces and placed on glass coverslips. Beem capsules containing partially polymerized resin were inverted over the tissue and the coverslips placed in a 70°C oven until polymerization was complete. The samples were removed from the glass coverslips by plunging them into liquid nitrogen. Thin sections were cut with a diamond knife, and stained with uranyl acetate and lead citrate [20].

Results

In uninjected guinea pigs or in saline injected controls, the distribution of mast cells in the mesentery was similar to that seen in other species. Mast cells were found dispersed throughout the mesentery window and concentrated in the fat sheaths along the blood vessels (Fig. 1a and b). The cells were round or elongated and the cytoplasm was filled with metachromatic granules. By electron microscopy (Fig. 2), the granules were electron dense and were polymorphic in shape. Many of the granules also contained a crystalline matrix characteristic of guinea pig mast cell granules [1, 25]. At 24 hours after intraperitoneal injection of distilled water, the majority of the mast cells present in the mesentery had been lysed and were extensively degranulated with many metachromatic granules scattered outside the cells (Fig. 3). By day 3, only a few mast cells could be identified (Fig.4a). These cells were found lying adjacent to the blood vessels in the fat sheaths and the mesenteric windows were devoid of mast cells. Most of these cells appeared to be immature and not yet fully granulated (Fig. 4a, inset). By day 5, mast cells not only were found adjacent to the blood vessels, but also were beginning to repopulate the mesenteric windows (Fig. 4b). Many of these mast cells were similar in appearance to the immature cells seen on day 3. By day 7, mast cells were present in the mesentery both adjacent to the blood vessels and throughout the mesenteric windows (Fig. 4c). Their appearance and distribution was similar to that seen in the control animals. By electron microscopy, during the initial phases, of repopulation of the mesentery very immature mast cells were also identified lying

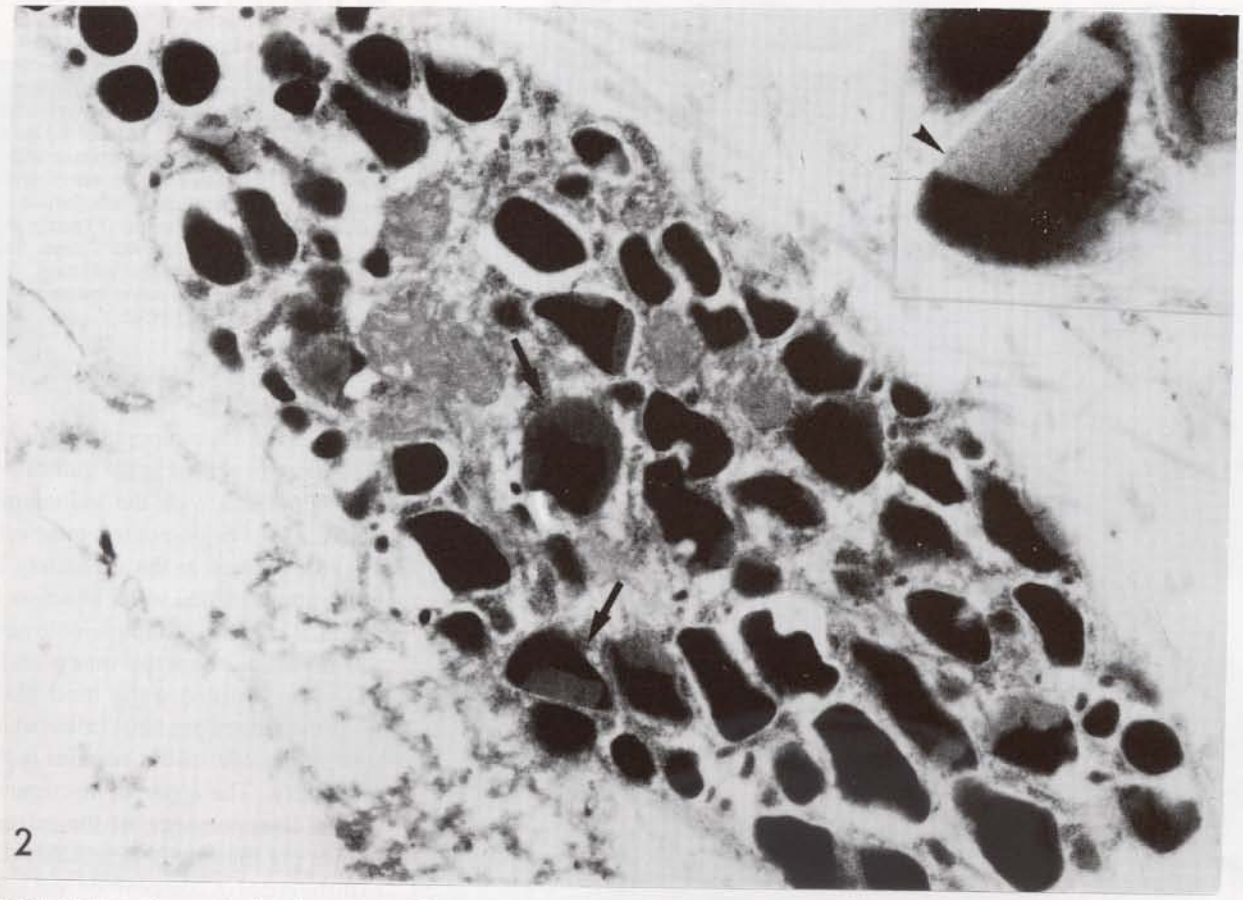

Fig. 2: *Electron micrograph of mature mast cell from saline injected guinea pig. The cytoplasm is filled with electron dense polymorphic granules (arrows), many of which contain a characteristic crystalline matrix (inset, arrowhead). x25,000; inset x61,500.*

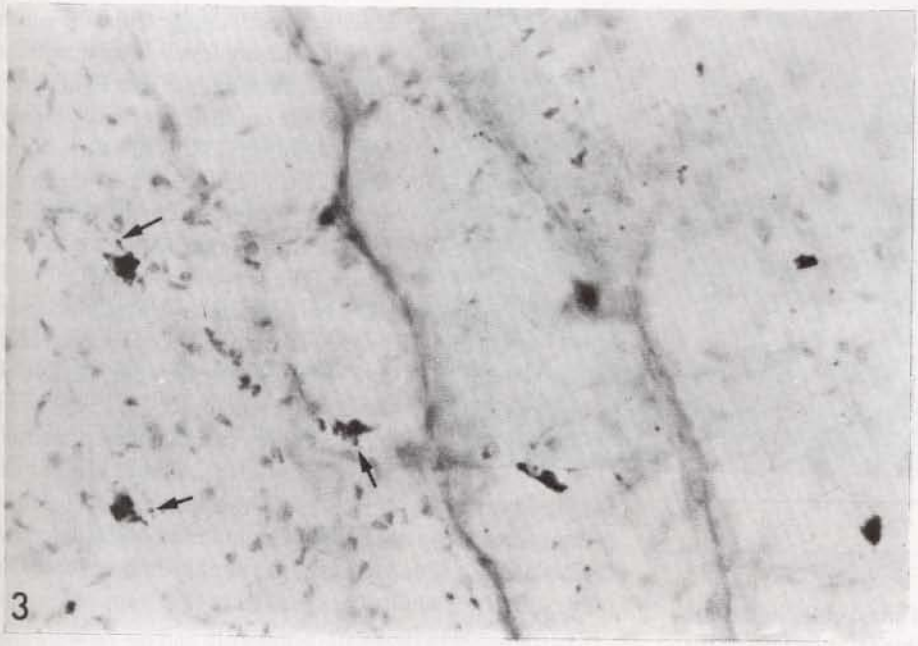

Fig. 3: *Mesentery from guinea pig 24 hours after intraperitoneal injection of distilled water. Toluidine blue stained. The cells are lysed with many metachromatic granules (arrows) present outside the cells in the extracellular matrix. x300.*

along the blood vessels in the mesenteric fat sheaths (Fig. 5b). These cells could not be recognized as mast cells by light microscopy because they contained only a few cytoplasmic granules (5b). However, although these granules were less electron dense than those seen in mature mast cells, they did contain the crystalline matrix characteristic of guinea pig mast cell granules (Fig. 5b, inset). The granules were generally clustered at one end of the cell. The cells themselves were elongated with the nucleus occupying most of the cell.

At this same time, fibroblasts in the mesentery that contained mast cell granule remnants were observed. (Fig 6a and b). These remnants were metachromatic with toluidine blue and were polymorphic in shape. They also lacked the crystalline matrix characteristic of mast cells and portions of them were very electron dense (Figure 6b, inset).

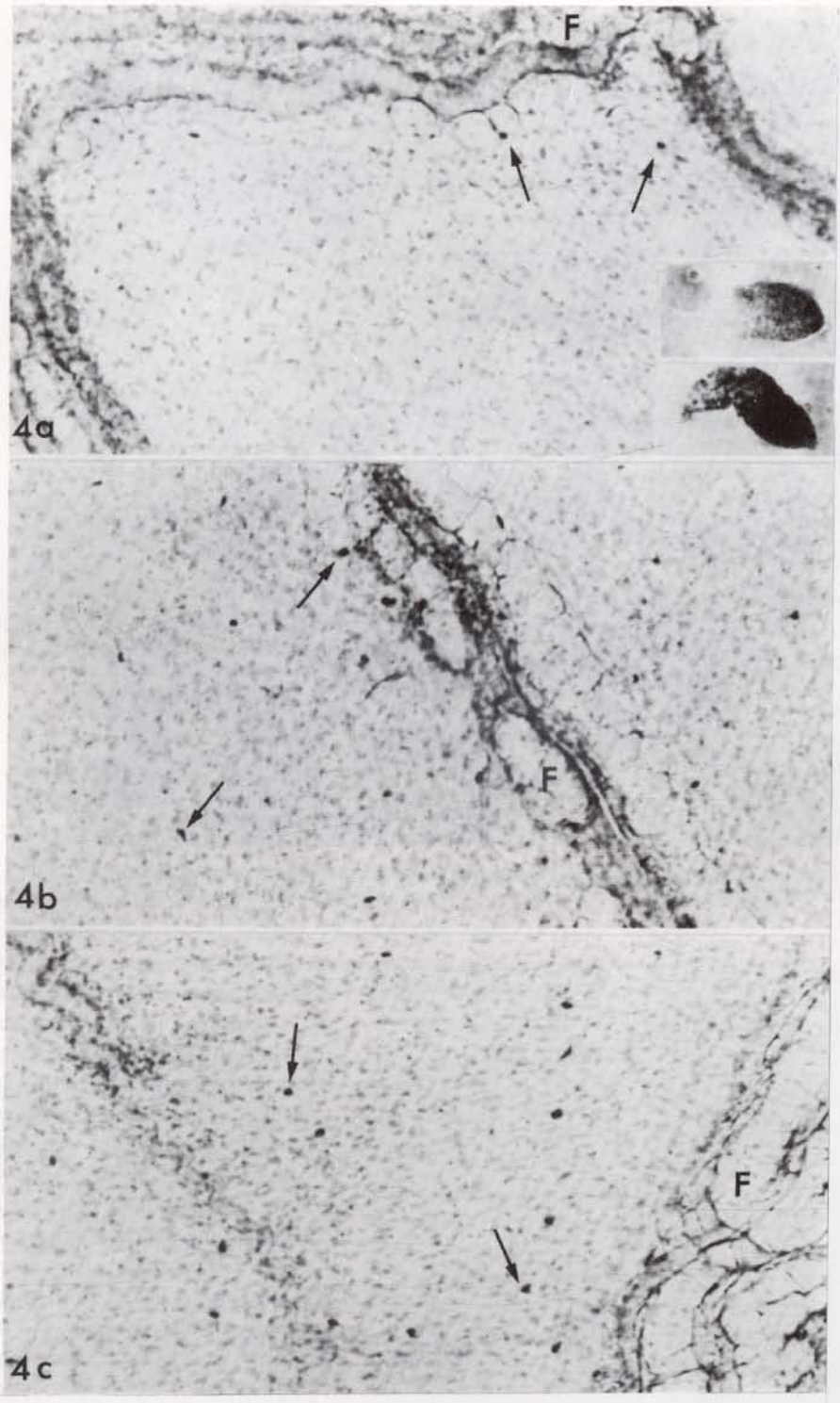

Fig. 4: *Mesentery from guinea pigs after intraperitoneal injection of distilled water. Toluidine blue stained.* **4A:** *3 days. Mast cells are almost absent form the mesentery. Immature mast cells (arrows) can be seen associated with the blood vessels in the fat sheaths (F). x100. Inset. Most of the mast cells present at 3 days are immature and not yet fully granulated. x1000.* **4B:** *5 days. Increased numbers of mast cells (arrows) are present in the fat sheaths (F) and in the mesentery windows. x100.* **4C:** *7 days. The distribution of mast cells (arrows) has returned to that seen in saline injected animals. (F, fat sheath) x100.*

Discussion

Although the overall response of mast cells in the mesentery of the guinea pig to intraperitoneal injection of distilled water was similar to that previously reported for rats, significant differences in the guinea pig response were found. Both the time course of the disappearance of the mast cells as well as in their repopulation in the mesentery is altered in the guinea pig. In comparison with the guinea pig, where many degranulated mast cells are still present in the mesentery 24 hours after distilled water injection, in the rat, it is virtually impossible to identify any mast cells at this time point [4, 15]. The distilled water most likely lyses the guinea pig mast cells, but insoluble granule matrix remains in the mesentery. The apparent discrepancy in the disappearance of the rat and guinea pig mast cells is probably due to differences in composition and solubility of the granule contents. The heterogeneity of mast cell granule contents is well documented [2, 16, 21, 22, 24], and these results suggest that the guinea pig granules are less soluble than those from rat mast cells. This is in agreement with published reports on granule solubility [17]. Due to this presumed insolubility of the guinea pig granules, they may remain identifiable in the extracellular matrix for over 24 hours. The presence of mast cell granule matrix in the extracellular space following mast cell activation has been reported for the guinea pig [18] as well as for other species [6, 11, 13]. The fate of the guinea pig granules present in the extracellular matrix is also similar to that reported for rat [19]. By 3-4 hours after stimulation of rat mesentery mast cells by Compound 48/80, approximately 10% of the cytoplasm in fibroblasts is occupied by phagocytosed granules.

Differences between the guinea pig and the rat were also noted in the kinetics of repopulation of the mesentery. In the guinea pig, by light microscopy, immature mast cells were first identifiable in the mesentery at 3 days after injec-

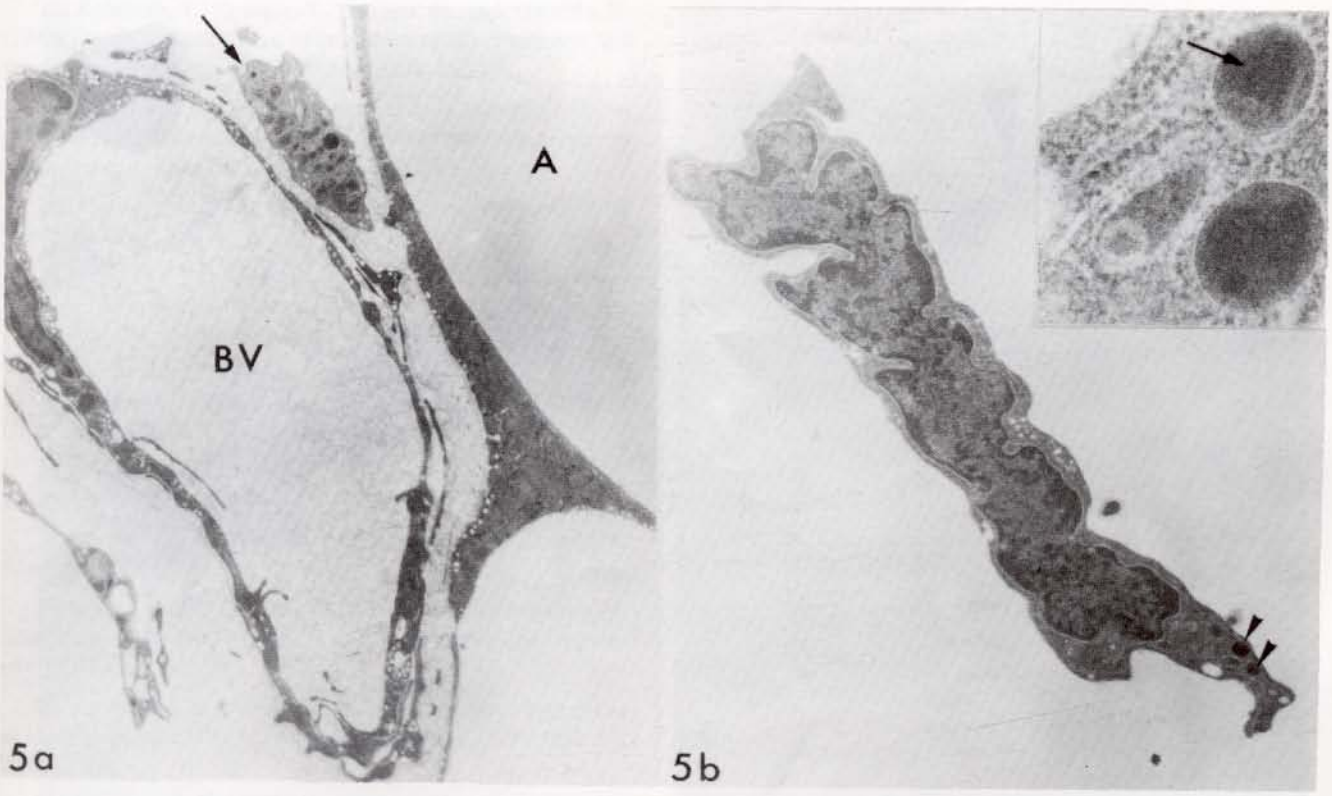

Fig. 5: *Electron micrographs of very immature mesenteric mast cells 5 days after intraperitoneal injection of distilled water.* **5A:** *A very immature mast cell (arrow) is localized between a blood vessel (BV) and an adipocyte (A). x9,000.* **5B:** *These very immature mast cells are elongated with a large nucleus and sparse cytoplasm. A few characteristic cytoplasmic granules (arrowheads) are present at one pole of the cell. x11,000. Inset. The crystalline matrix characteristic of guinea pig mast cells can be seen in the cytoplasmic granules (arrow). x75,000.*

tion of distilled water. These cells were localized to the fat sheaths and contained variable numbers of secretory granules. In contrast in the rat, immature mast cells containing a few cytoplasmic granules first appear near blood vessels in the mesenteric fat sheaths 5 days after distilled water injection [15]. Currently, mast cells precursors are thought to migrate from the bone marrow through the circulation to peripheral sites where they complete their maturation [8, 12, 27]. The appearance of very immature mast cells adjacent to blood vessels in the guinea pig mesentery supports the hypothesis that at least a portion of the mast cells repopulating the mesentery may come from the peripheral circulation. In the guinea pig, these very immature cells may reach the mesentery sooner than the immature cells in the rat, or their maturation in the mesentery may be more rapid than that seen in the rat.

The very immature mast cells seen in the guinea pig mesentery are morphologically similar to very immature rat bone marrow derived and peritoneal mast cells. In the rat, because the mast cell granules lack the characteristic crystalline matrix seen in guinea pig, these very immature cells were identified using a panel of mast cell specific antibodies [9]. They were first seen in rat bone marrow [9], and later identified during repopulation of the rat peritoneal cavity following distilled water injection [3]. Subsequently, these very immature mast cells have been immunomagnetically isolated from rat bone marrow and peritoneal cavity [10].

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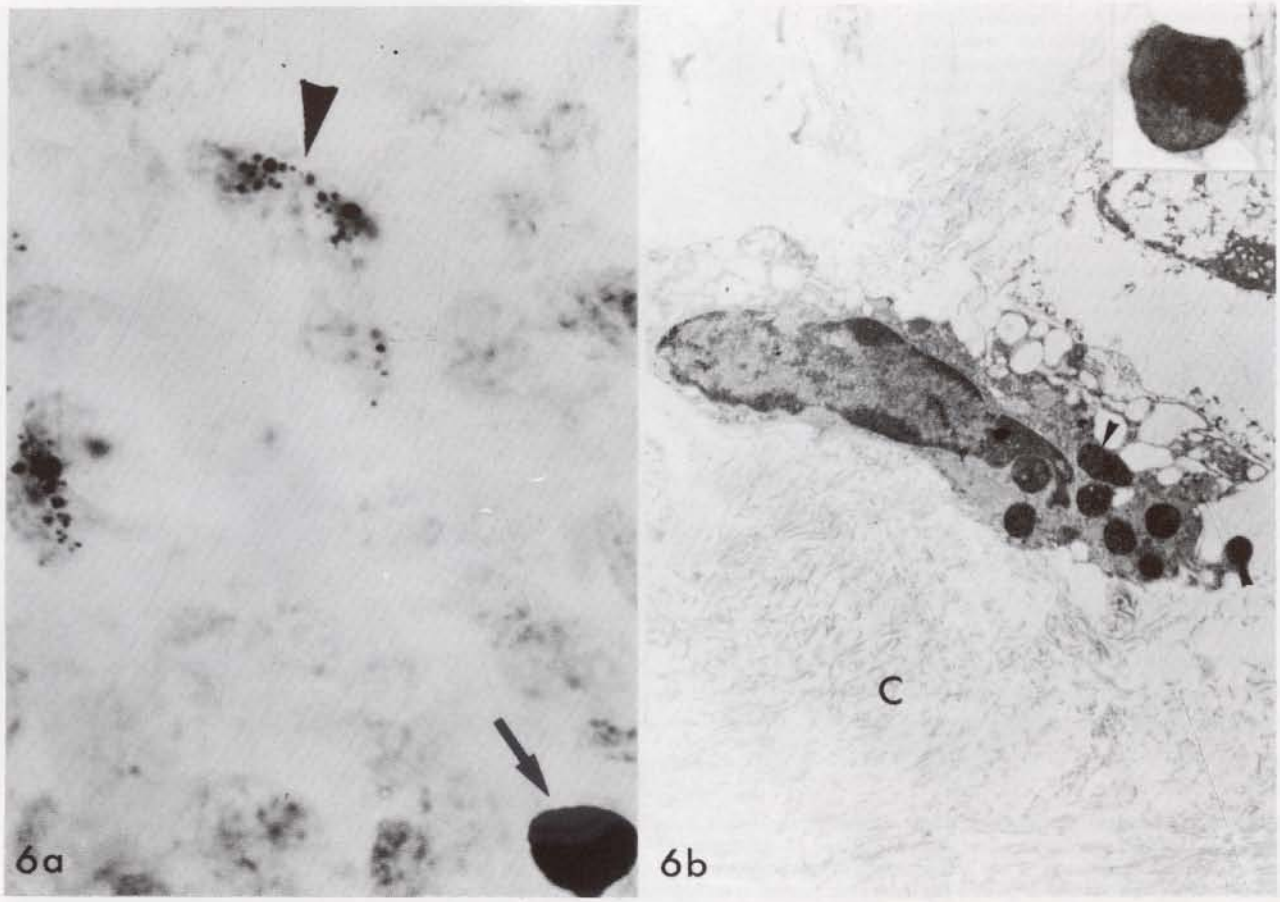

Fig. 6: *Guinea pig mesentery 5 days after intraperitoneal injection of distilled water.* **6A:** *Fibroblasts contain phagocytosed remnants (arrowhead) of mast cell granules. The remnants are metachromatic with toluidine blue staining. A mature mast cell (arrow) is also present. x1,300.* **6B:** *By electron microscopy, the phagocytosed granule remnants (arrowheads) inside a fibroblast appear polymorphic in shape and are frequently very electron dense (inset). The crystalline matrix characteristic of intact guinea pig mast cell granules is never observed. (C, collagen fibers) x3,600. Inset x16,000.*

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