

Perivascular Mast Cells in Advanced Gastric Adenocarcinomas: An Electron Microscopic Study

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Abstract. Mast cells are frequently found in close proximity to blood vessels and have been implicated in tumour angiogenesis. The aim of the present ultrastructural study was to characterize, in detail, the mutual association between mast cells and microvasculature in 9 cases of advanced gastric carcinoma. Perivascular mast cells were ultrastructurally identified as T mast cells and exhibited piecemeal degranulation, indicative of a slow release of granule-stored contents. In two cases they were adjacent to endothelial cells showing vesiculo-vacuolar organelles, a newly-defined endothelial cell permeability structure. Collagen fibres and dispersed fibrous long-spacing collagen were found near T mast cells in one case, suggesting their role in perivascular collagen degradation and/or turnover. Mast cells were associated with microvascular basal lamina changes including irregular thickness, multiple layers and loose association with endothelial cells and pericytes, reminiscent of degenerating or regenerating blood vessels. In conclusion, our ultrastructural study suggests that mast cells contribute to the remodelling of existing vessels by a slow release of granule-stored contents in advanced gastric adenocarcinomas.

Human mast cells are predominantly found in tissues forming an interface between the host and external environment, such as skin, respiratory and gastrointestinal mucosa. Mast cells are also localized around blood vessels and often close to nerves.

Two types of human mast cells have been identified based on their composition of neutral proteases (1). TC mast cells contain tryptase, chymase, mast cell carboxypeptidase and cathepsin G in their secretory granules and are the predominant type of mast cells in the skin and the

gastrointestinal submucosa. T mast cells contain only tryptase in their granules and are the predominant type of mast cells in gastrointestinal mucosa and alveolar walls of the lung (1,2). Employing ultrastructural techniques, Craig *et al.* (3) have shown that TC mast cell granules more often exhibit crystalline structures of the lamellar, lattice and grated type, whereas in T mast cell granules, the discrete scroll-type morphology predominates. These morphological findings thereby permit T and TC mast cells to be distinguished by ultrastructure alone (3).

Tumour vessels are recognized as dynamic, both in terms of the formation of new vessels by angiogenesis (4,5) and the remodelling of existing vessels (6,7). Mast cells have also been implicated in the angiogenic process because of the temporal relationship between their appearance in the tumour and the in-growth of vessels, as well as the observation that tumour angiogenesis is retarded in mast cell-deficient animals (8,9). Degranulating mast cells accumulate within and around solid human tumours (10-20). Despite the enormous efforts aimed at elucidating the molecular determinants of angiogenesis, little is known about the nature of the vascular bed in human tumours (21). Almost all of the studies that have assessed endothelial cell turnover in tumours were performed in experimental animal models with rapidly growing tumours, the growth kinetics of which are vastly different from the growth kinetics of human tumours (22,23). Methods for studying relationships and interactions between mast cells and other stromal cells have focused mainly on their behaviour in culture media. *In vitro* studies are inadequate experimental systems through which to address major features of tumour invasion and host reaction (24). Electron microscopy provides an accurate method, not only to identify those mast cells that have degranulated (25,26), but also for the investigation of cell interaction in human tumours.

Our previous studies on gastric carcinomas showed that mast cells are often spatially isolated from adenocarcinoma cells and that they are only seldom localized interepithelially (26). In this paper, we further extend our ultrastructural observations to study the mutual association between mast cell and host microvasculature in advanced gastric carcinomas.

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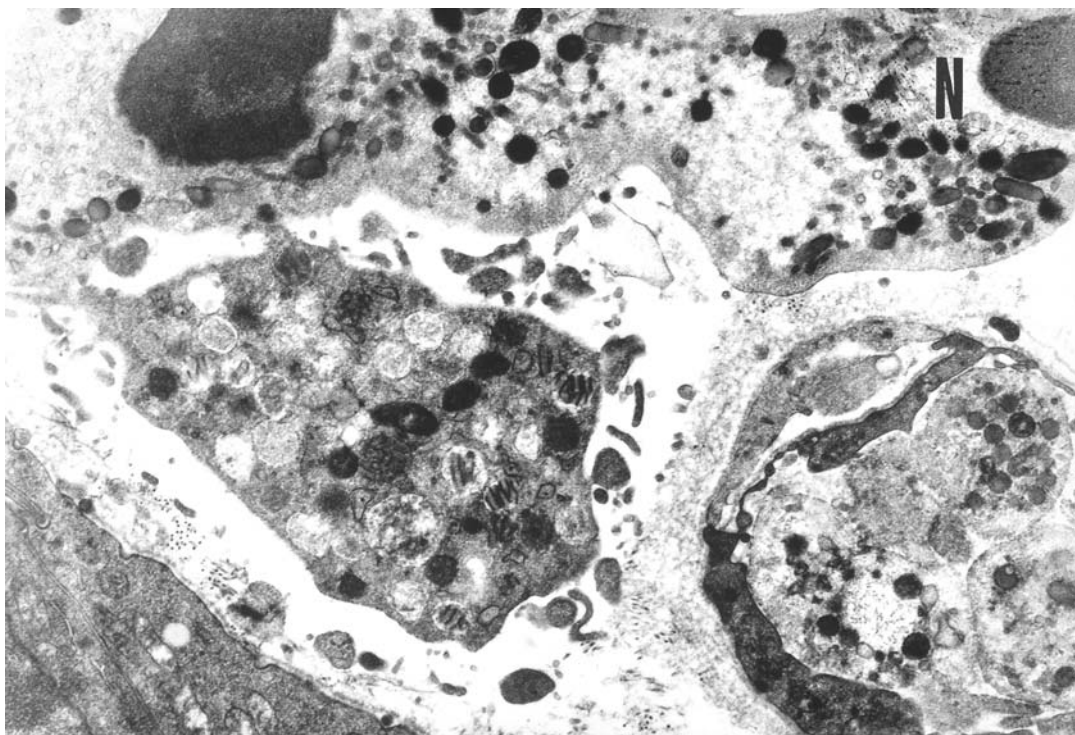


Figure 1. Perivascular mast cell showing piecemeal degranulation, characterized by a marked decrease in cytoplasmic granule electron density and the formation of intracytoplasmic vacuoles. Platelet aggregates are present in the lumen of a small capillary, which is surrounded by multilayered basal lamina. An adjacent neutrophil (N) shows early apoptotic changes including condensed nuclear chromatin, rounded nuclear profiles, glycogen granules depletion, cytoplasmic vacuoles and preservation of the cytoplasmic granules. x 12,000

Materials and Methods

Surgically resected specimens were obtained from 9 patients with advanced gastric carcinoma.

For light microscopy, the specimens were fixed in 10% formalin for 24 hours at room temperature and embedded in paraffin. Haematoxylin and eosin staining was used for general evaluation of tissue morphology.

The 9 cases were processed for transmission electron microscopy examination. Briefly, in each case, small pieces of the fresh tumour tissue were immediately fixed in 3% phosphate-buffered glutaraldehyde pH 7.4 and post-fixed in 1% osmium tetroxide. Semi-thin araldite-embedded sections were made from 4 to 6 blocks prepared from each tissue specimen and were stained with Giemsa's reagent. Thin sections were double-stained with uranyl acetate and lead citrate; they were then examined and photographed in a Zeiss EM 109 electron microscope (Carl Zeiss, Oberkochen, Germany).

Results

The 7 men and 2 women with advanced gastric carcinomas ranged in age from 60 to 75 years. Histologically they were classified into intestinal (6 cases) and diffuse type (3 cases), according to the Laurèn (27) classification. All the cases showed invasive growth beyond the muscularis mucosae and metastases in the regional lymph nodes.

Actively degranulating mast cells in semi-thin sections were identified by the presence of fewer peripherally distributed metachromatic granules.

Electron microscopy showed hypogranular mast cells containing few unaltered granules including particulate elements and discrete scrolls (Figure 1). There was no evidence of cytoplasmic or nuclear degeneration in these mast cells, suggesting that their state of degranulation was the result of previous granule discharge and not due to degenerative alterations. Moreover, there were mast cells exhibiting the ultrastructural morphology of piecemeal degranulation, that was characterized by a marked decrease in cytoplasmic granule electron density and retention of the granule containers. These degranulating mast cells were surrounded by edema (Figures 1,2).

There was a considerable degree of heterogeneity of blood vessel maturation within each tumour. Mitoses and cytoplasmic sprouting of endothelial cells were not seen. In all cases we studied, mast cells were associated with basal lamina changes including irregular thickness, multiple layers and loose association with endothelial cells and pericytes (Figures 1,3). In two cases, mast cells were associated with endothelial cells characterized by vesiculo-vacuolar organelles (Figures 3,4). Other mast cells were adjacent to

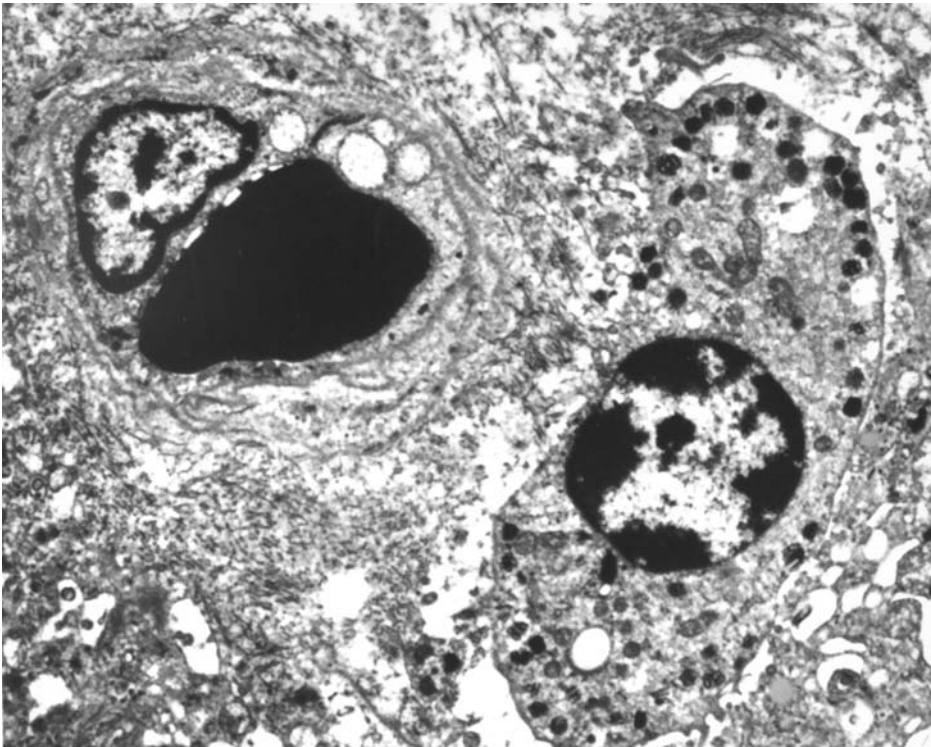


Figure 2. Mast cell partially surrounded by edema. Collagen fibres are seen in between mast cell and tumour vessel. x 8,000

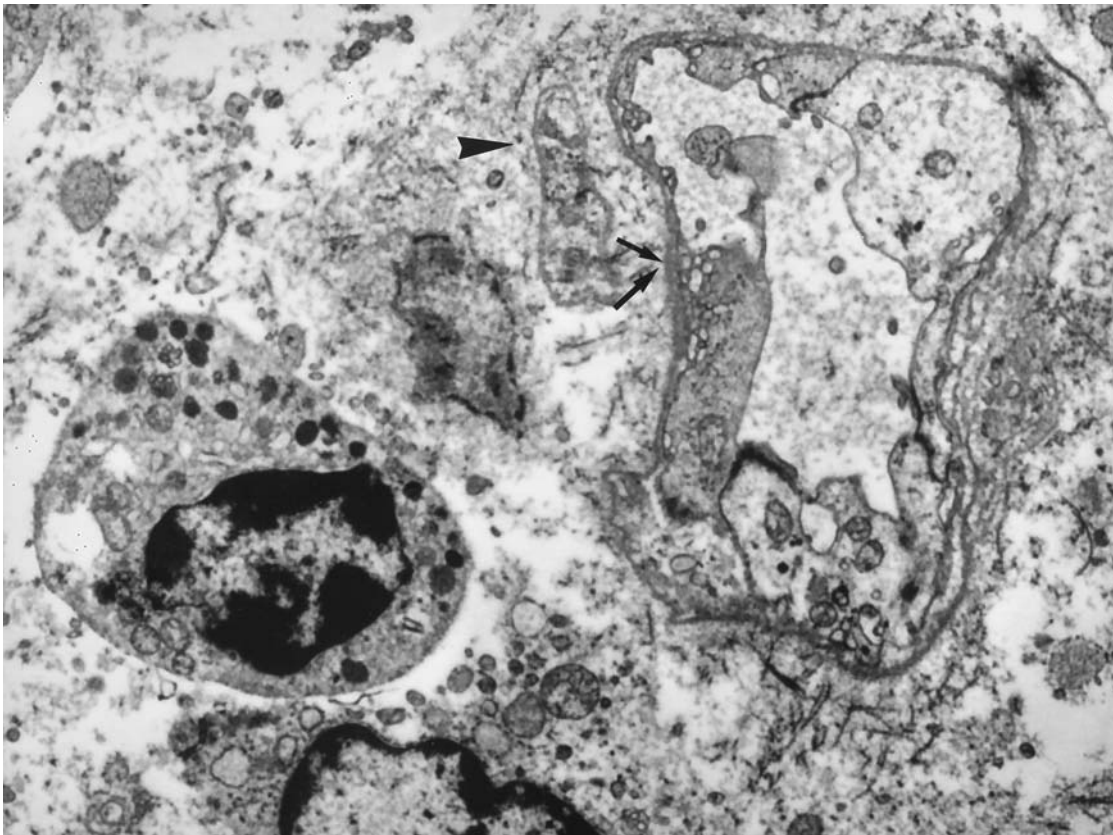


Figure 3. Mast cell is adjacent to mural cell (arrowhead) loosely associated with endothelium of tumour vessel. The endothelial cell, partially surrounded by multilayered basal lamina, shows abundant clear cytoplasm containing vesiculo-vacuolar organelles (arrows). x 12,000

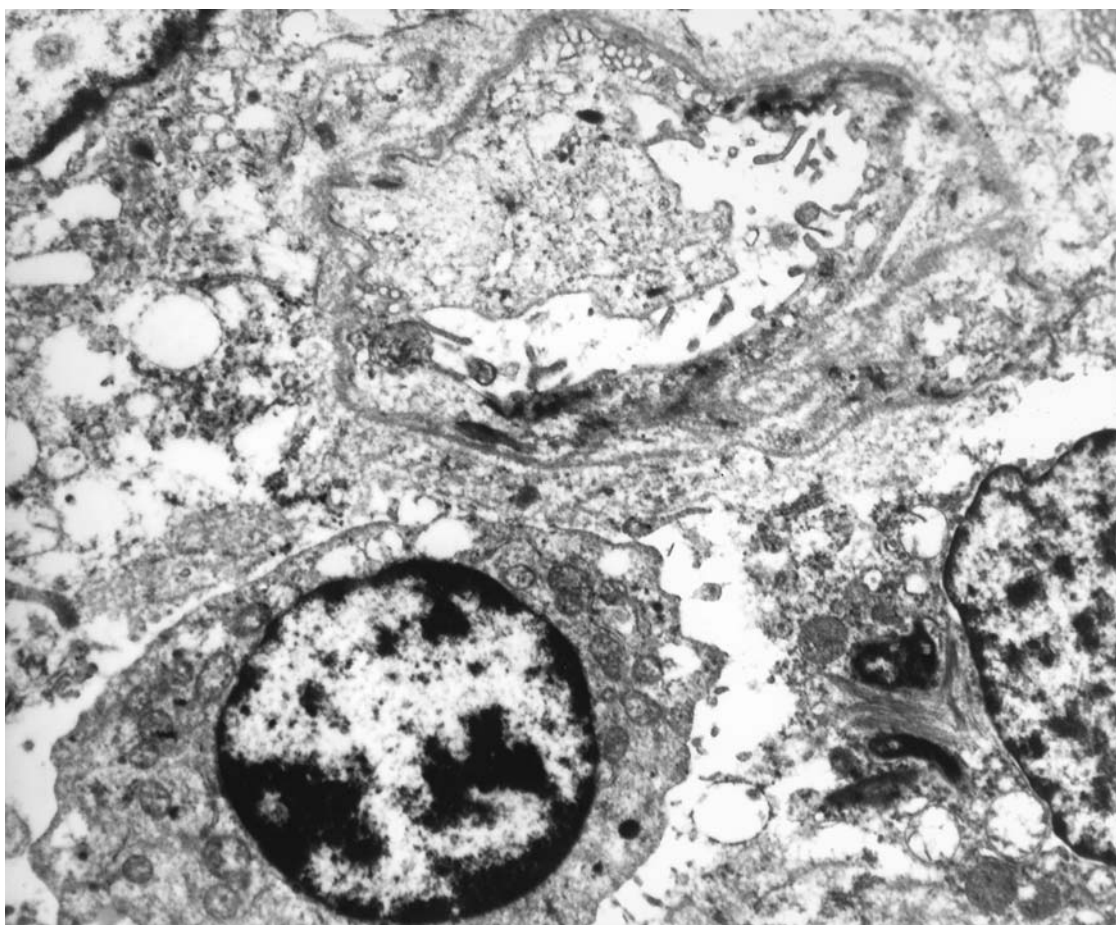


Figure 4. Endothelial cell showing vesiculo-vacuolar organelles. $\times 12,000$

activated endothelial cells showing clear cytoplasm and forming slit-like lumina (Figure 5). In one case, dispersed fibrous long-spacing collagen was observed in the subendothelial space (Figure 6). A series of contact points were seen between collagen fibres and the cell membrane of mast cells (Figure 7). Multilayering basal lamina and collagen fibres intermingled with dispersed fibrous long-spacing collagen were also noticed (Figure 7).

Discussion

Our ultrastructural study showed T mast cells by virtue of the presence of granules composed of discrete scrolls (3). Some mast cells were hypogranular, reflecting immaturity and/or granule reduction from prior secretory events (28). Other mast cells exhibited ultrastructural signs of piecemeal degranulation such as slightly enlarged, non-fused, partially empty granule containers (28). According to experimental data (28,29), piecemeal degranulation suggests a slow mechanism of mast cell secretion.

Degranulating mast cells were found associated with conspicuous abnormalities in the vascular basal lamina. These included irregular thickness, multiple layers and loose association with endothelial cells and pericytes, reminiscent of degenerating or regenerating blood vessels (30,31). For a number of reasons, we hypothesize that mast cells and their granular products, specifically histamine, might play a pivotal role in the remodelling of tumour microvasculature. We observe that mast cells were surrounded by edema, a typical histamine-mediated response (32). It has been demonstrated that partially and completely empty cytoplasmic granules, characteristic of piecemeal degranulation of mast cells, had diminished and absent histamine stores, whereas electron-dense granules in the same cells retained their histamine (32). Histamine induces the formation of vesiculo-vacuolar organelles, a newly-defined endothelial cell permeability structure (33). This organelle provides the major route for extravasation of macromolecules at sites of increased vascular permeability such as in the microvasculature that accompanies tumours, in venules associated with allergic inflammation and

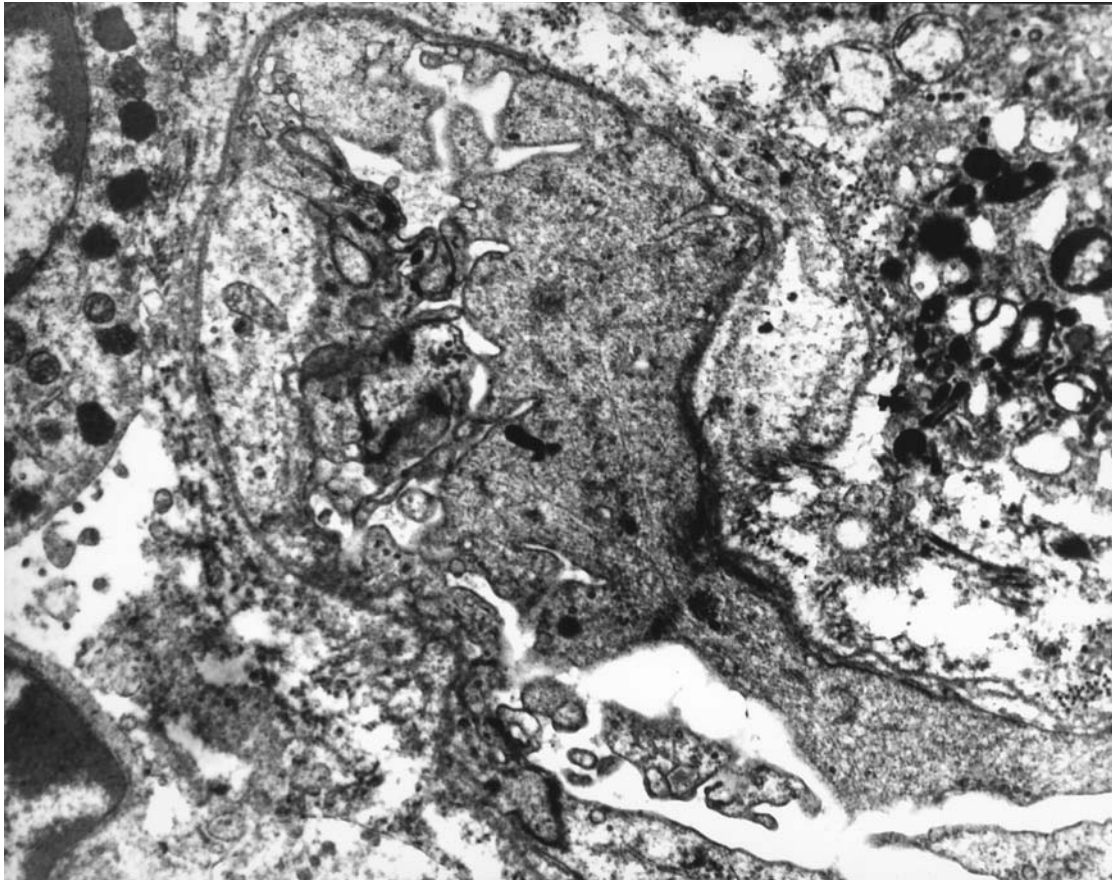


Figure 5. Mast cell is adjacent to a vessel wall with large endothelial cells forming slit-like lumina. $\times 12,000$

in the endothelia of normal venules (34,35). Accordingly, the presence of vesiculo-vacuolar organelles in two cases of advanced gastric carcinomas which we studied is probably related to secretion of histamine by mast cells.

Two types of fibrous long-spacing collagen have been distinguished: a compact form that contains proteoglycans and a more dispersed form that does not (36). The compact type of fibrous long-spacing collagen was found especially in Schwannomas and other neurogenic tumours (36). The dispersed fibrous long-spacing collagen was found under circumstances in which there was high collagen breakdown and/or turnover (37) and, to our knowledge, it has rarely been reported in human carcinomas (36,38).

Although rare and usually found in small amounts, the association of a dispersed fibrous long-spacing collagen with degranulating mast cells suggests perivascular collagen degradation, probably related to remodelling of existing vessels. Taken together, these data suggest that mast cell piecemeal degranulation is associated with microvascular changes compatible with a remodelling of existing vasculature in the advanced gastric carcinomas we studied. Further study of this system at the molecular, cellular and tissue levels will

probably provide us with a better understanding of the observed involvement of mast cells in the remodelling of tumour microvasculature. Moreover, by learning how to modulate these interactions, we will probably be able to gain better control over neoplastic disease.

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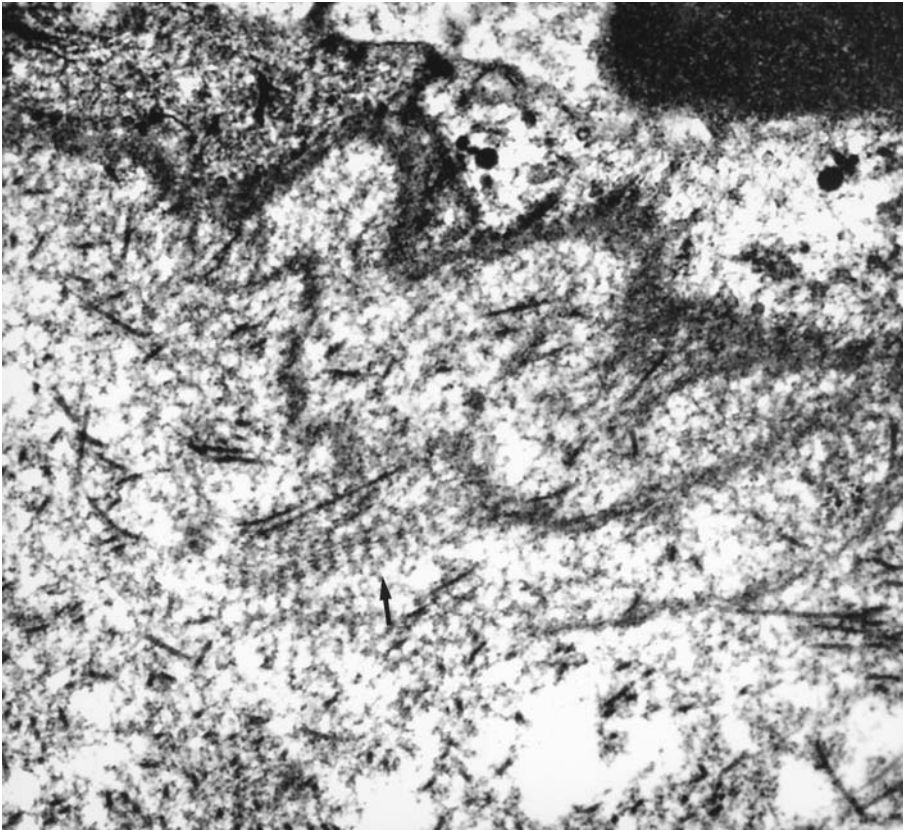


Figure 6. Dispersed long-spacing collagen in the subendothelium (arrow). x 20,000

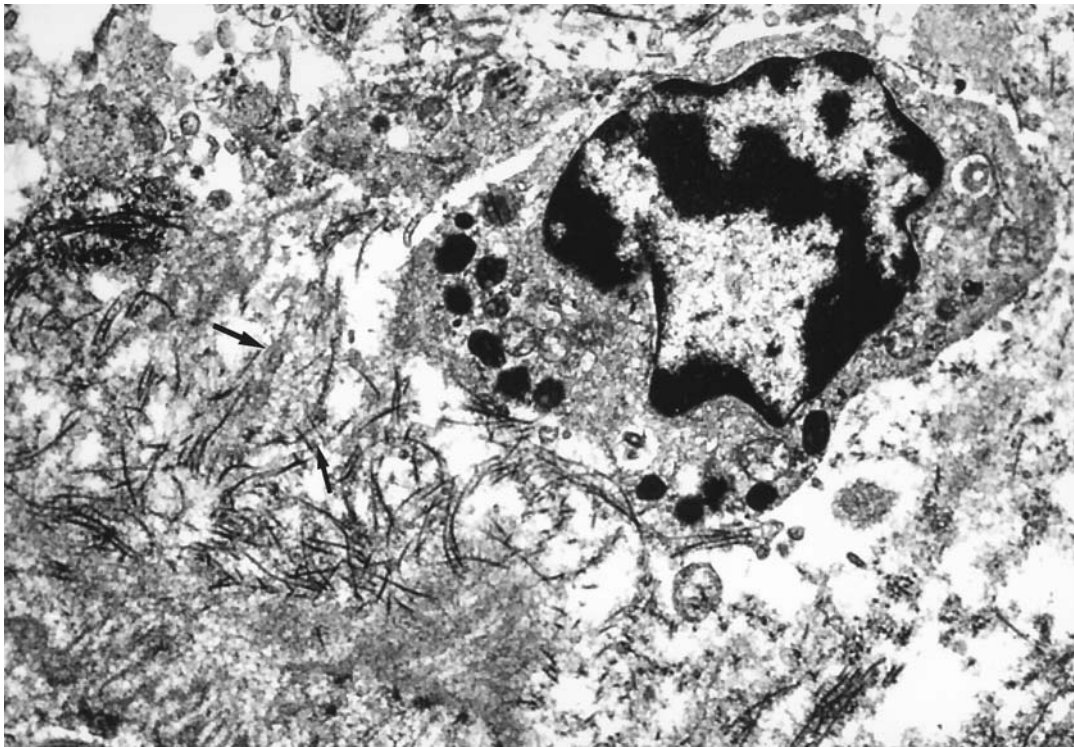


Figure 7. Subendothelial space characterized by the presence of dispersed long-spacing collagen (arrows), basal lamina, collagen fibres and mast cell. Some collagen fibres establish several points of contact with the cell membrane of a mast cell. x 12,000

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