

Ultrastructural study of the vascular response in small early gastric cancer

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Summary. The microvasculature of the stroma of four cases of small early gastric cancer (EGC) was investigated by conventional electron microscopy. Severe damage to small and large fenestrated capillaries was observed around endothelium-adherent, partially degranulated neutrophils. The findings suggest the existence of neutrophil-mediated injury of endothelial cells during the development of inflammatory responses in small EGC. The severely injured microvessels exhibited increase in vasopermeability, microhaemorrhage, and platelet aggregates. Other microvascular changes included endothelial cell and pericyte activation as well as basal lamina replications, indicative of repeated episodes of endothelial injury, necrosis and regeneration. This new capillary growth within the old basal laminae shared morphological features with a peculiar angiogenic process described in man and animals.

Key words: Small early gastric cancer, Electron microscopy, Tumour microvasculature changes

Introduction

Solid tumours are composed of two distinct but interdependent compartments, the malignant cells themselves and the vascular and connective tissue stroma that they induce and in which they are dispersed. Studies of vascular function in tumours are hampered by the lack of an *in vitro* model of the complex microenvironment present in the tumour stroma. Investigations of the tumour microvasculature therefore must rely on animal models or on histological examination of (human) tumour material (Jain, 1988; Adachi et al., 1993). Few ultrastructural studies on the microvasculature of human gastric carcinomas have been reported (Listrom and Fenoglio-Preiser, 1987; Ohtani and Nagura, 1990; Ohtani, 1992).

Tumour angiogenesis is the growth of new vessels

toward and within a tumour (Folkman, 1990). Such neovascularization may be stimulated by factors released from the tumour cells, tumour-associated inflammatory cells, and/or from the extracellular matrix (Blood and Zetter, 1990; Folkman, 1990).

In the course of an investigation of the inflammatory response in small early gastric cancers (EGCs), we found ultrastructural evidence of neutrophil-mediated tumour cell damage (Caruso et al., 1994). The aim of the second part of this study was to reveal the neutrophil-associated microvascular changes and the type of angiogenic response in small EGCs.

Materials and methods

Surgically-resected specimens were obtained from 4 patients with small EGCs (less than 10 mm in diameter). None of the patients had undergone preoperative irradiation or immunochemotherapy. The pathological diagnosis was made by light microscopy, as described in our previous publication (Caruso et al., 1993). For light microscopy, the tissues were fixed in 10% formalin and prepared for paraffin sectioning. The sections were stained with haematoxylin and eosin.

The four cases of small EGCs 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 postfixed in 1% osmium tetroxide. Semi-thin araldite-embedded sections were made from four to six blocks prepared from each tissue specimen and were stained with Giemsa's reagent. The intramucosal tumour microvasculature observed under the light microscope was subjected to further study under the electron microscope. Selected areas were trimmed, and ultrathin sections were cut and stained with uranyl acetate-lead citrate; they were then examined with a Siemens 101 electron microscope.

Results

The two men and two women with small EGCs ranged in age from 49 to 71 years. The lesions were

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localized in the gastric antrum. Macroscopically, one case was of type I, two cases were of type IIb, and one case was of type IIc.

According to the WHO classification (Watanabe et al., 1989), 3 small EGC cases were of the tubular type and 1 case was of the tubular type with a focal signet-ring cell component.

The vascular alterations included endothelial cell hypertrophy with focal luminal obliteration. Vascular dilatation and sometimes tight intraluminal packing of erythrocytes and granulocytes were observed.

At ultrastructural level, the microvasculature of intramucosal small EGC was represented mainly by

small and large capillaries of fenestrated type. Activation of endothelial cells was extensive. Such endothelial cells showed a large open nucleus with dispersed chromatin, prominent nucleolus, and increased synthetic organelles (rough endoplasmic reticulum, ribosomes and vesicular-like profiles) (Fig. 1). Mitoses and cytoplasmic sproutings were not seen. Pericytes were found adjacent to the capillary wall; they underwent nuclear and cytoplasmic hypertrophic changes identical to those affecting endothelial cells (Fig. 2). Pericyte processes partially surrounded the capillaries and were often separated from endothelial cells by basal membrane-like material (Fig. 3).



Fig. 1. Fenestrated-type endothelium (E) of the tumour stroma. Activated endothelial cell showing an increase in rough endoplasmic reticulum, ribosomes, and vesicular profiles (arrowheads). x 15,000

Pericytes were also bordered at their outer side by layered basal membrane-like material and by collagen fibres which were never found at their inner side opposite the endothelial cells (Fig. 1). Cellular debris was trapped within the layers of basal lamina (Fig. 4). Inflammatory cell emigration was the most striking

feature. Neutrophils adhering slightly to the endothelium either retained their ovoid shape or became flattened along the contacts with the endothelium. At times, the escaping cell inserted a cytoplasmic process through a gap between the endothelial lining and the basement membrane and passed into the subendothelial space (Fig.

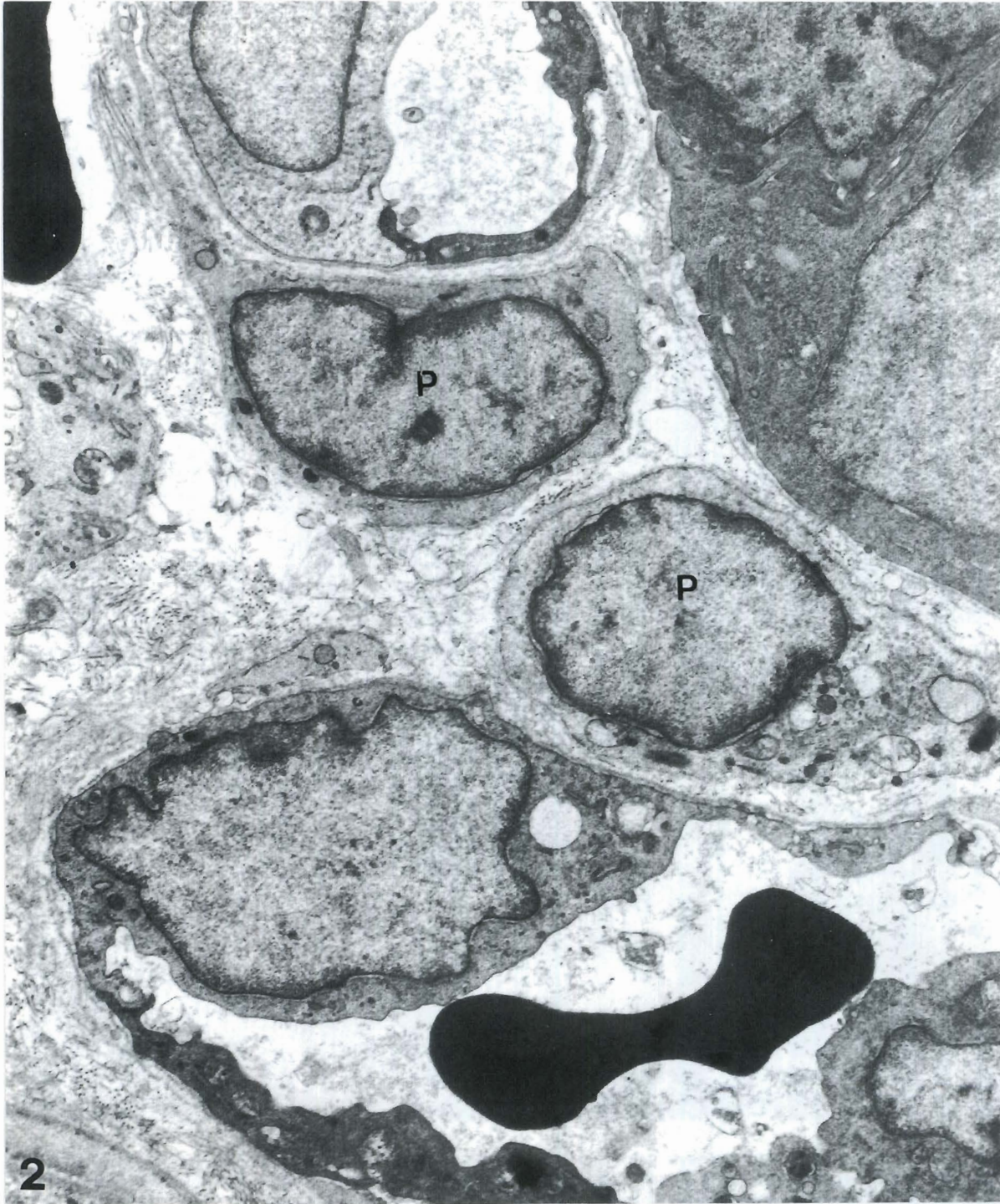


Fig. 2. Two activated pericytes (P) are found adjacent to the capillary wall in the tumour stroma. They show a large open nucleus with dispersed chromatin, and an abundant amount of cytoplasm. Their cell body is surrounded by basal lamina material. x 10,000

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4). Between the emigrating neutrophil and the endothelial lining lifted up by the penetrating pseudopod, there was a close contact (Fig. 4). Neutrophils in intimate contact with damaged endothelial cell showed focal degranulation (Fig. 4). With respect to adjacent undamaged and/or activated endothelial cells, the individual endothelial cell in contact with the neutrophil showed cytoplasmic lucency and cell swelling (Fig. 3). Exuding neutrophils often showed polarization of

primary and secondary granules (Fig. 3). Pericyte necrosis was not observed, even around vessels that exhibited endothelial cell damage. Direct anatomical contacts were also observed between platelets and damaged endothelium (Fig. 5).

Some severely damaged capillaries contained aggregates of platelets and erythrocytes, and were partially surrounded by invasive adenocarcinoma cell (Fig. 6). Extravasated interstitial red blood cells were

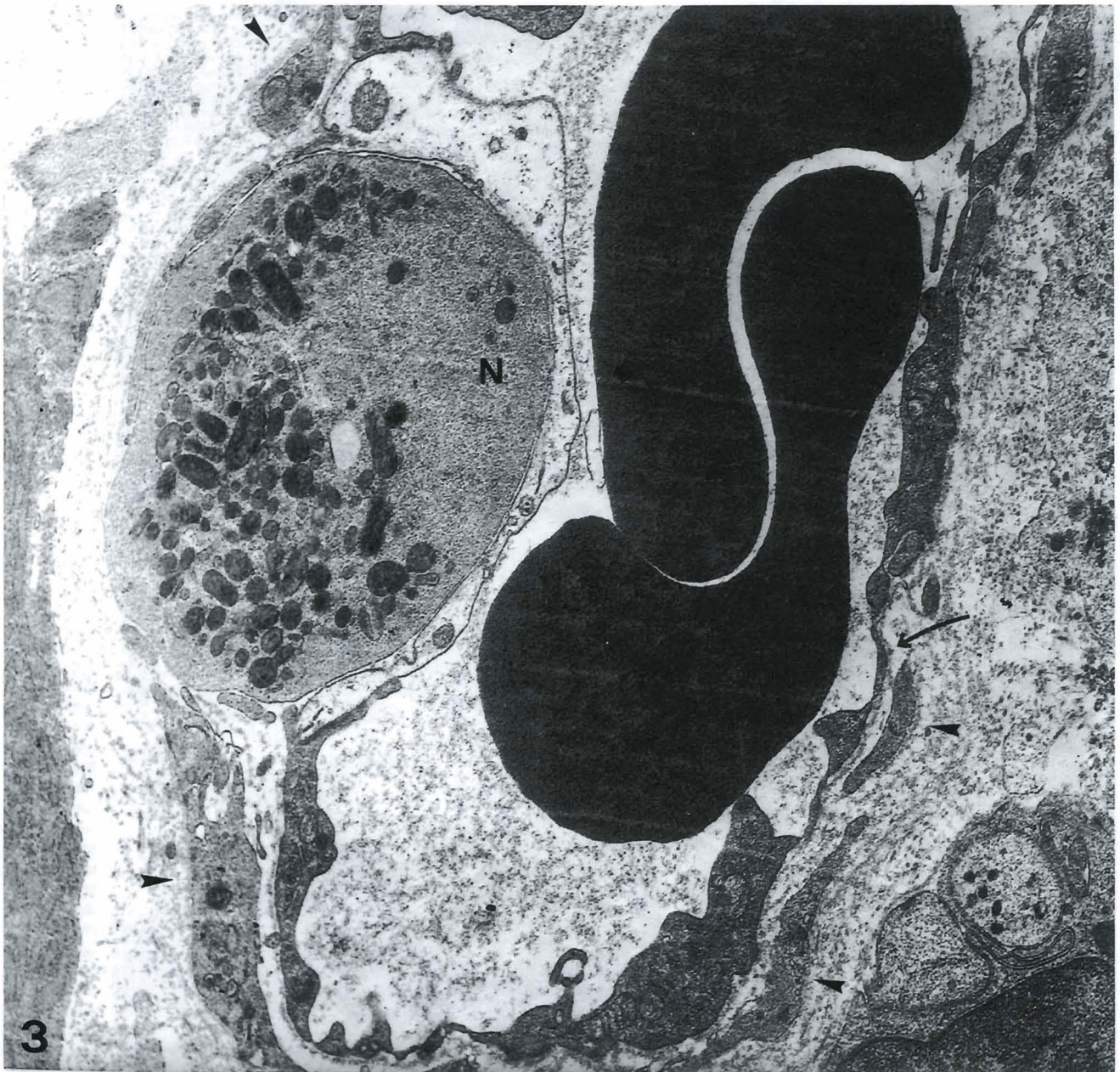


Fig. 3. A large capillary is partially surrounded by pericyte processes (arrowheads). Basal lamina (arrow) separates pericyte processes from endothelium. Direct contact between exuding neutrophil (N) and endothelial cell with cytoplasmic lucency, adjacent to undamaged endothelial cells. Note the polarization of primary and secondary granules of the neutrophil. x 12,000

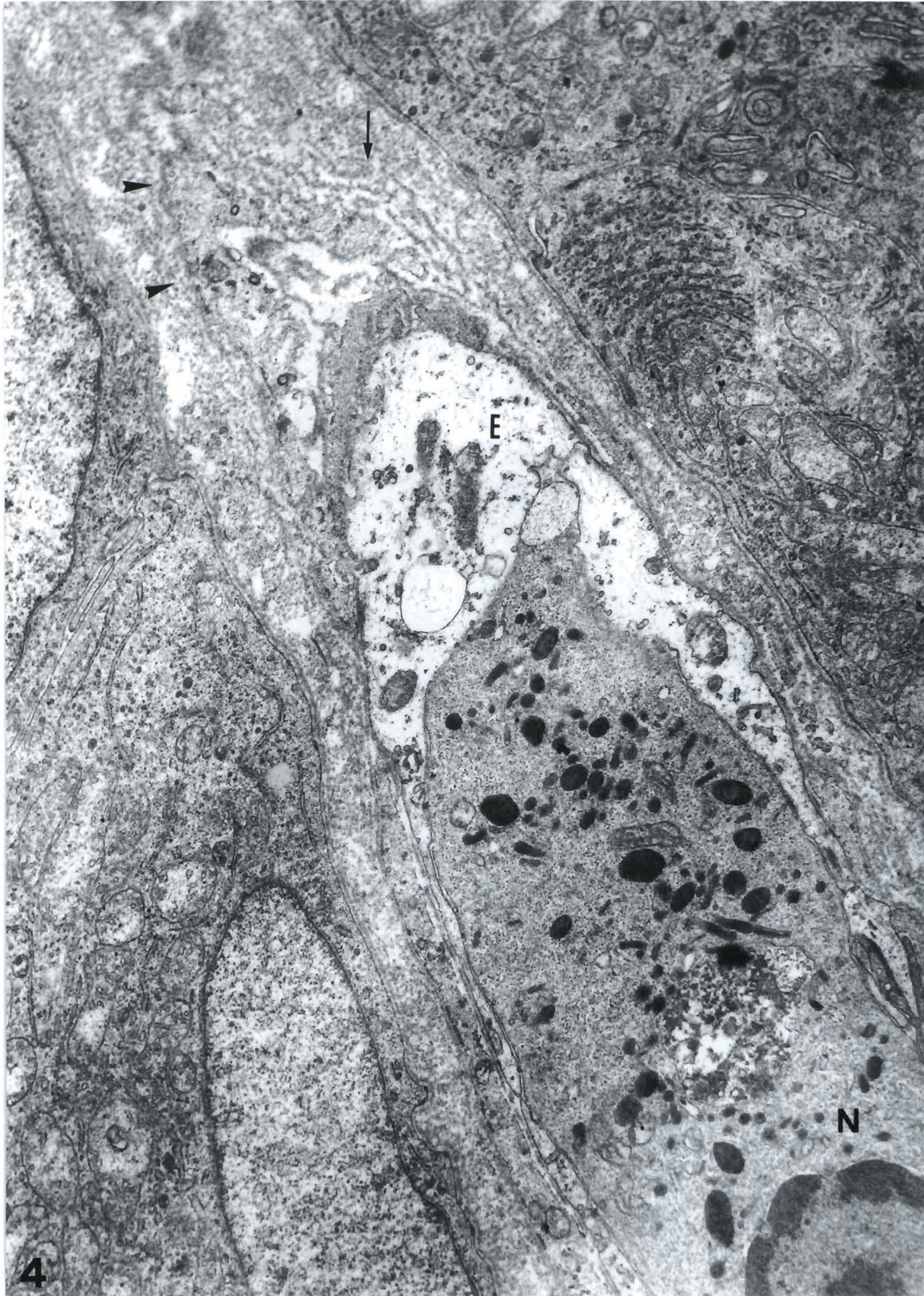


Fig. 4. A partially-degranulated neutrophil (N) establishes areas of intimate contact with a severely damaged endothelial cell (E). Note the marked reduplication of basal lamina (arrow). Some of the cell debris containing spaces between basal lamina layers has the configuration of endothelial cell (arrowheads). x 12,000

present (Fig. 6).

Discussion

In the four cases of intramucosal small EGCs the microvasculature consisted of small and large capillares that were ultrastructurally analogous to fenestrated capillaries of normal gastric mucosa (Gannon et al., 1984; Lehnert et al., 1985; Raschke et al., 1987). According to tumour blood vessel classification proposed by Jain (1988), these intratumoural capillaries were of fenestrated type.

Our data showed focal endothelial necrosis in

association with neutrophil exudation. Although endothelial injury may occur through a variety of mechanisms, the neutrophil has been suggested to play an important role in the genesis of endothelial injury in inflammatory states (Harlan et al., 1985). The wide area of contact between adherent neutrophils and endothelial cells appears to be essential for the induction of vascular damage, because the adjacent endothelial cells not contacted by neutrophils are morphologically well preserved. Furthermore, neutrophils in the lumen showed ultrastructural features of focal degranulation. The findings suggest the existence of neutrophil-mediated injury of fenestrated capillaries during the

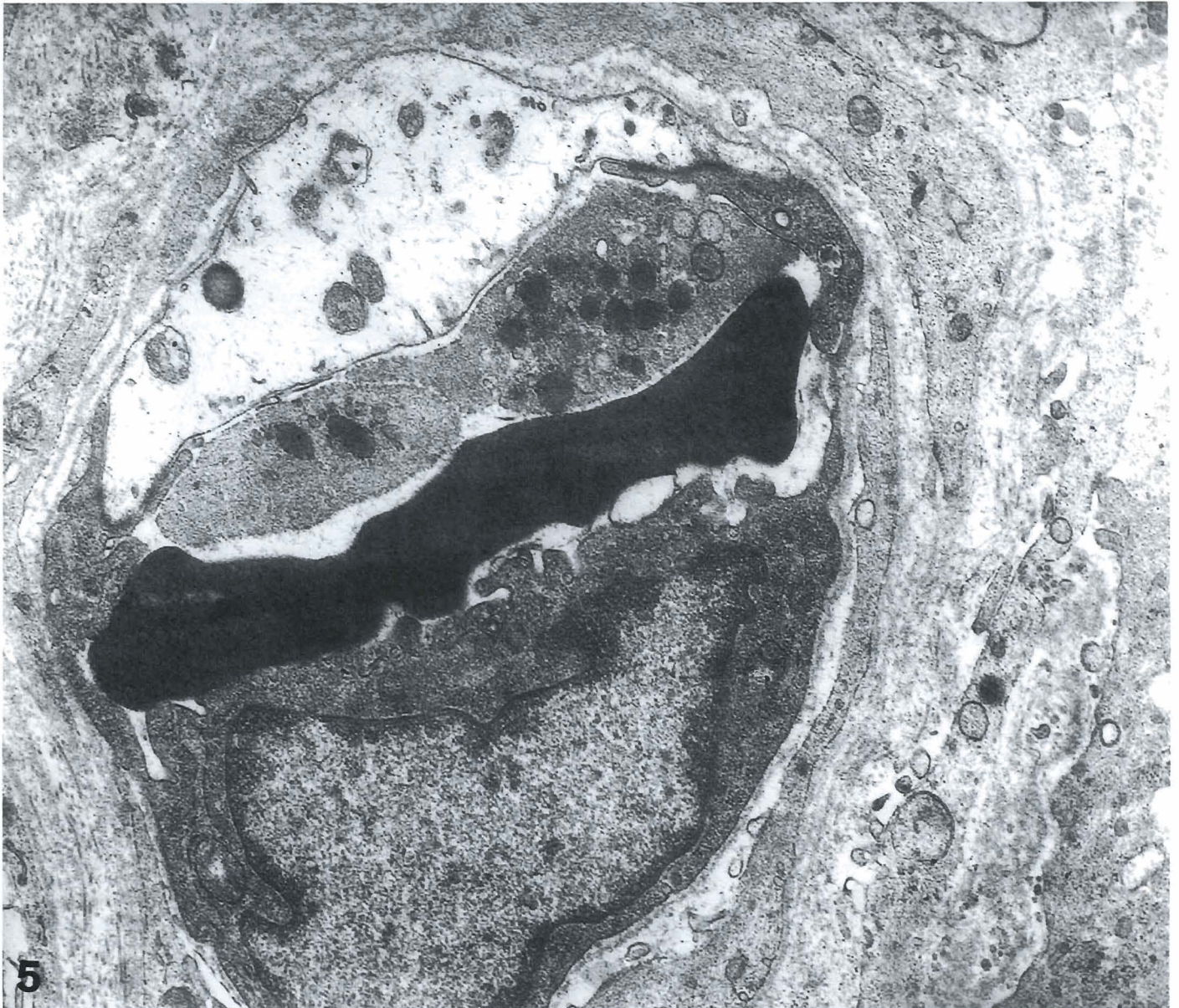


Fig. 5. Platelets are adherent to damaged endothelial cell. x 15,000



Fig. 6. Erythrocyte and platelet aggregates are present in the lumen of a small capillary, which is partially surrounded by an adenocarcinoma cell showing intracytoplasmic lumen. Red blood cells is also visible in the tumour stroma. x 12,000

development of inflammatory responses in small intramucosal EGCs.

Focal endothelial necrosis results in an altered internal barrier. Extravasated red blood cells adjacent to microvessels with compromised barrier function serve as markers for loss of vascular contents to the tissue spaces.

Our findings of replicating basal lamina are diagnostic of repeated focal injury and repair of endothelial cells (Vracko, 1974), because remnants of endothelial cells were found between different layers of the basal lamina of the microvasculature. They also suggest the presence of a peculiar form of angiogenesis characterized by a new capillary growth within the old basal lamina of necrotic vessel (reviewed by Diaz-Flores et al., 1994). The type of angiogenetic reaction is not specific for tumours (Dvorak et al., 1980a,b), because it has also been described in neovascularization of tissue graft (Dvorak et al., 1979), in allergic contact dermatitis (Dvorak et al., 1976) and in Crohn's disease (Dvorak et al., 1980b). It is assumed that features suggestive of active angiogenesis such as vascular sprouting, endothelial cell mitosis and migration occur frequently throughout the tumour vasculature (Folkman, 1985). However, we were unable to demonstrate these phenomena in the tumour stroma of small EGCs. Previous studies have shown angiostatic capacities of pericytes (Orlidge and D'Amore, 1987). In our cases, activated pericytes surrounded endothelial cells and probably contributed to the inhibition of proliferation and migration of the endothelial cells.

In tumours, ischemic necrosis often occurs, because of insufficient angiogenesis (Folkman, 1985) or deficient blood supply (Jain, 1988). Neutrophil exudation and platelet aggregates are factors that may compromise the blood flow (Ryan and Majno, 1977; Schmid-Schonbein et al., 1981; Schmid-Schonbein, 1987; Jain, 1988), contributing to tumour tissue ischemia and necrosis in our four cases of small EGC.

The demonstration of neutrophil-endothelium interaction in the tumour stroma may have important implications to cancer therapy (Wu et al., 1992). For instance, gene therapy (Rosenberg, 1991; Colombo and Forni, 1994) would best succeed in tumours in which the leukocytes used for the therapy would adhere to and migrate through endothelial cells.

In conclusion, our ultrastructural data suggest the existence of neutrophil-mediated injury of endothelial cells during the development of inflammatory responses in small EGCs. The microvascular inflammatory reaction is associated with a peculiar form of angiogenesis, characterized by a new capillary growth within the old basal laminae. The possibility that this moderately angiogenic phenotype is related to the early stage of examined tumours deserves further studies.

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