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Ultrastructural observations on the microvasculature in advanced gastric carcinomas

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Summary. The ultrastructural features associated with vascular permeability in 9 cases of advanced gastric carcinomas were studied, and compared with that of control non-neoplastic mucosa. Tumour microvasculature showed features in common with those of control mucosa, including complete basal lamina, well-developed interendothelial junctions, fenestrations and caveolae. Some tumour blood vessels showed endothelial cell swelling accompained by luminal narrowing and perivascular fibrosis. In 2 out of 9 cases, there were endothelial attenuation with numerous fenestrations and vesiculo-vacuolar organelles. The vesiculo-vacuolar organelle is a recently described cytoplasmic structure found in the endothelial cells lining tumour microvessels and normal venules and which provides an important pathway for extravasation of circulating macromolecules. Our ultrastructural data suggest that advanced gastric carcinomas share with experimental tumour models in vivo only some morphologic features associated with hyperpermeability including fenestration, endothelial attenuation and vesiculo-vacuolar organelles. The implications of perivascular fibrosis on the delivery of immune cells to gastric carcinomas are discussed.

Key words: Advanced gastric carcinoma, Electron microscopy, Vesiculo-vacuolar organelles, Perivascular fibrosis

Introduction

Tumour vasculature is characterized by its tortuous architecture (Skinner et al., 1990), irregular blood flow (Jain, 1988) and increased permeability relative to normal vessels (Dvorak et al., 1995). The hyperpermeability is largely attributable to the secretion by tumours of a cytokine: vascular endothelial growth factor (Senger et al., 1993). The therapy of solid tumours with conventional chemotherapeutics, drug delivery preparations and immunomodulatory agents directed against the tumour cells is corrupted by a major barrier presented by the tumour vasculature. Permeability of the tumour blood vessels for transport of small molecules and macromolecular drug-carrier conjugates is only sufficient in the blood vessels at the tumour-host interface (Netti et al., 1999).

In an our previous ultrastructural study, we described the microvessels changes in small early gastric cancer (Caruso et al., 1996). In this paper, we further extend our ultrastructural observations to study the microvasculature of advanced gastric carcinomas and to discuss the morphological findings consistent with microvascular permeability according to the current literature.

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Materials and methods

Nine patient, who were found to be normal at the endoscopic examination of the stomach, were chosen as control. The clinicopathologic findings of these patients, already used as controls in another study, were published elsewhere (Bagnato et al.,1995).

Surgically resected specimens were obtained from the 9 patients with advanced gastric carcinoma. All surgical specimens for light microscopy were fixed in 10% formalin and embedded in paraffin. Haematoxylin and eosin staining was used for general evaluation of tissue morphology.

For electron microscopy, endoscopic specimens or small pieces of the fresh tumour tissue were immediately fixed in 3% phosphate-buffered glutaraldeyde pH 7.2 and post-fixed in 1% osmium tetroxide. From 4 to 6 blocks were prepared for electron microscopy from each of the surgical specimens containing tumour tissue. Endoscopic biopsies yelded 2-3 tissue blocks each containing only antral mucosa. Histology ranged from normal, congestion and oedema to various degrees of activity. *Helicobacter pylori* infection was not detected. Semi-thin sections of these araldite-embedded blocks 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). Whole areas of each section were observed. A total of 180 microvessels were identified within the tumour, whereas 60 microvessels were studied in non-neoplastic control mucosa.

Results

Control gastric mucosa

The eight women and one man, chosen as controls, ranged in age from 27 to 60 years.

The ultrastructure of the microvasculature in our control mucosa was similar to that reported in the literature (Gannon et al., 1984; Lehnert et al., 1985; Listrom and Fenoglio-Preiser, 1987). Endothelial cells were joined together by well-developed junctions, were enveloped by pericytes and by a continuous basal lamina. The thin and elongated endothelial cells contained few organelles, although pinocytotic vesicles were numerous. Weibel-Palade bodies were occasionally found. In the attenuated part of the capillary endothelium, fenestrations appeared frequently in groups. Sometimes, short finger-like endothelial protrusions, bulging into the capillary lumen, were visualized, and the number of endothelial plasmalemmal caveolae was markedly increased (Fig. 1).

Gastric carcinomas

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 classification (Laurèn, 1965). All the cases showed invasive growth beyond the muscularis mucosae.

Tumour vasculature appeared highly heterogeneous within individual tumours and among tumours. Tumour microvasculature was surrounded by a continuous basal lamina (Fig. 2). Endothelial cells contained caveolae, mitochondria, multivesicular bodies and exhibited fenestrations (Figs. 2, 3). The junctions between contiguous endothelial cells were sometimes short, oriented perpendicularly to the direction of blood flow, and comprised cell membranes that were apposed throughout their entire length. More commonly, however, interendothelial junctions were long, diagonally oriented and tortuous (Fig. 2). Bundles of collagen fibres partially surrounded tumor microvessels (Fig. 2). Well-formed collagen fibres (Fig. 3) or collagen fibrils (Fig. 4) could be found in the subendothelial space. Blood vessels with endothelial cells undergoing apoptosis were occasionally observed (Fig. 4). In some microvessels, red blood cells were across the cell wall in areas where no interendothelial junction was visible (Fig. 5). In 2 out of 9 cases, endothelial cells showed abundant clear cytoplasm containing microfilament, mitochondria, and vesiculo-vacuolar organelles (Figs. 6, 7). Luminal microprocesses formed an intercellular canalization (Fig. 8). Numerous fenestrations and endothelial attenuation were also present (Fig. 8). Basal lamina appeared intact,but in some microvessels it was dense and thickened (Fig. 9).

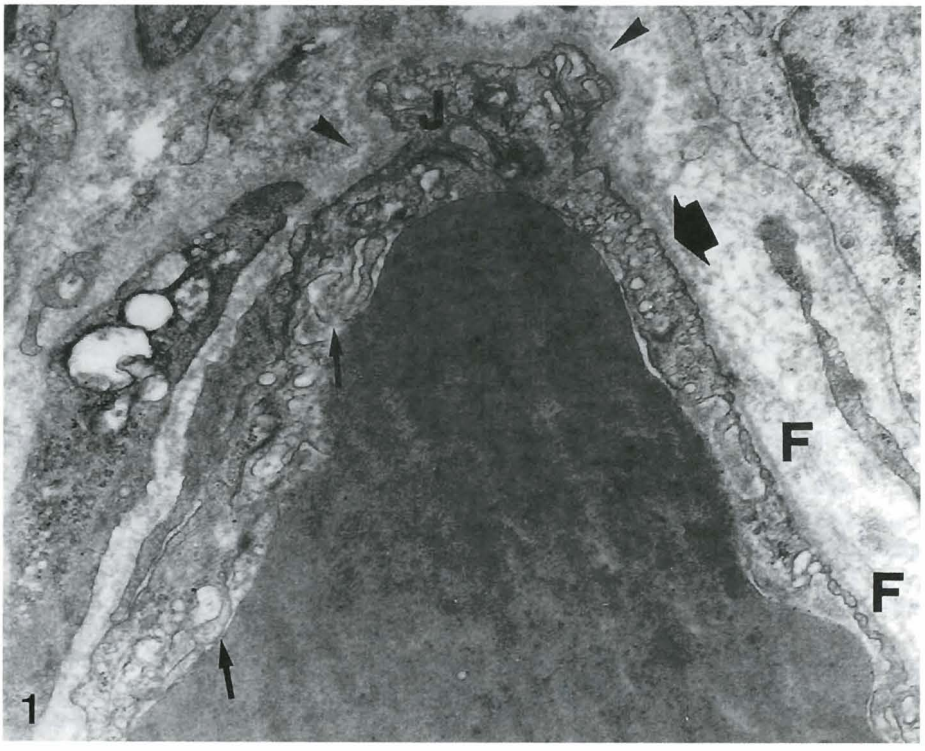


Fig. 1. Gastric mucosa of a control patient. The capillary displays an enlarged lumen and a continuous basal lamina (arrowheads). The endothelial cells are irregular with numerous digitations (thin arrow) to the luminal surface. Fenestrations (F), caveolae (large arrow) and intercellular junctions (J) are also illustrated. x 12,000

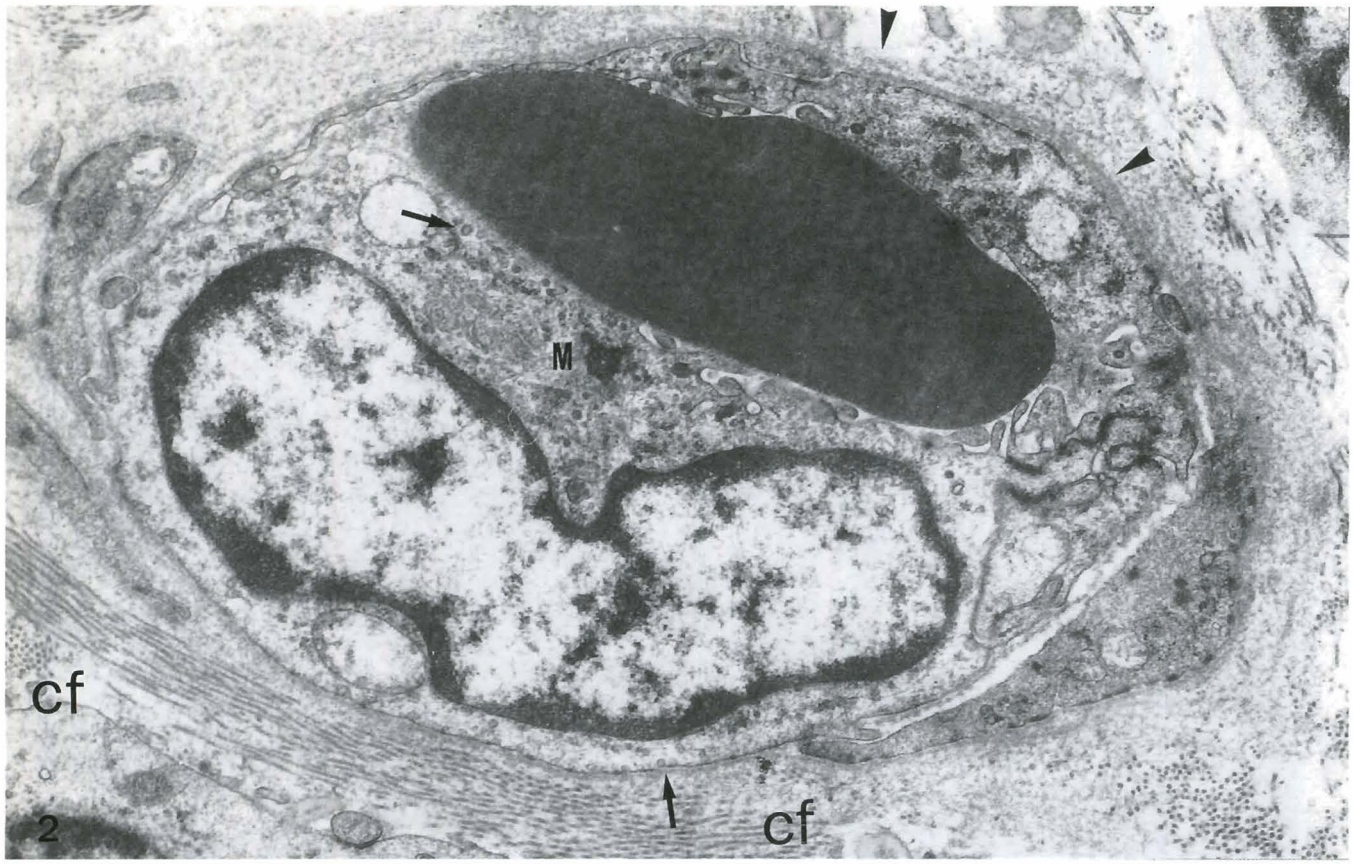


Fig. 2. Fenestrated-type endothelium of the tumour stroma with intact basal lamina (arrowheads). Prominent cytoplasmic organelles include mitochondria, caveolae (arrows) and dark multivesicular bodies (M). Bundles of collagen fibers (cf) partially surround tumour microvessel. x 8,000

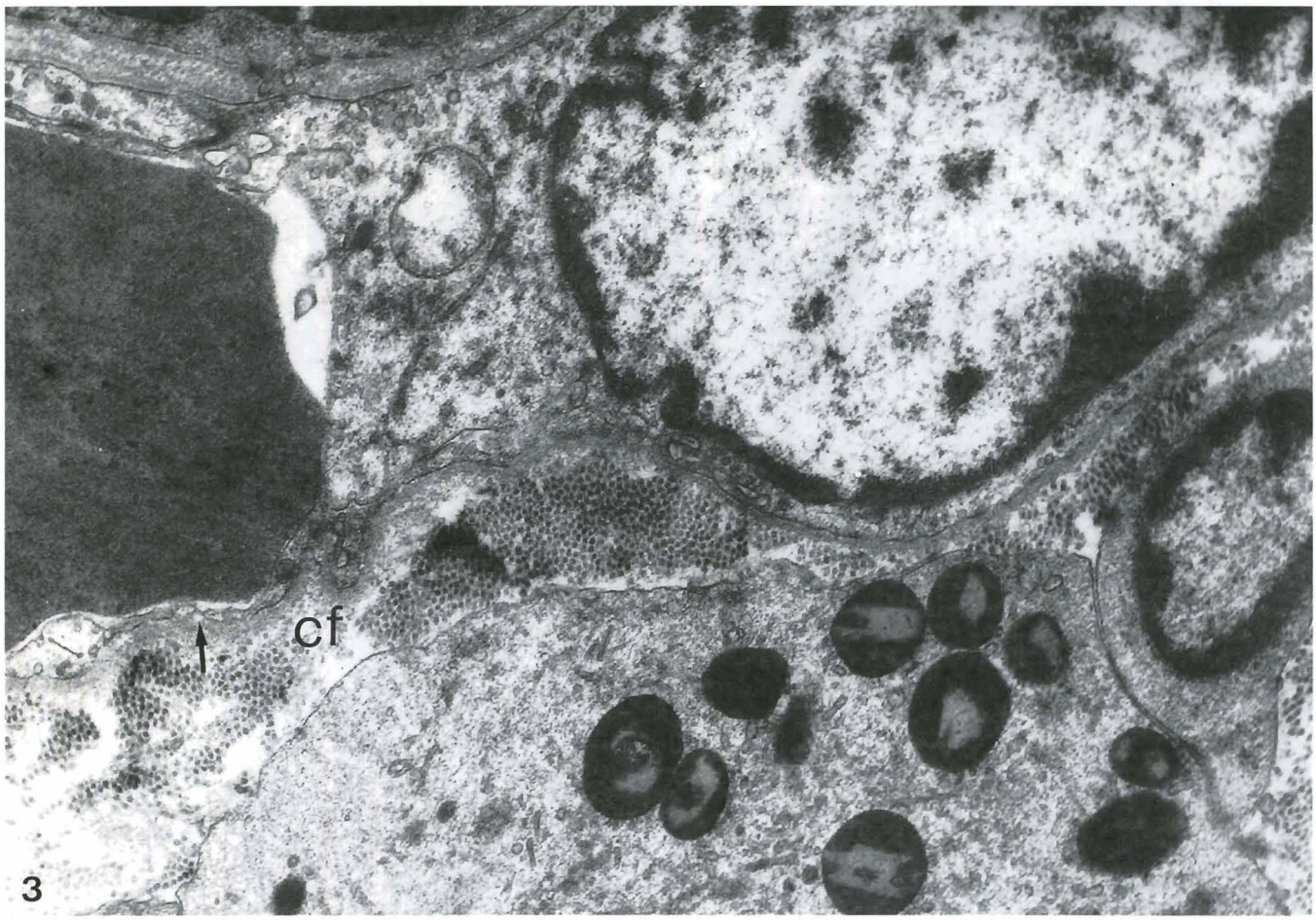


Fig. 3. Well-formed collagen fibers (cf) adjacent to basal lamina of a fenestrated-type endothelial cell (arrow). x 14,000

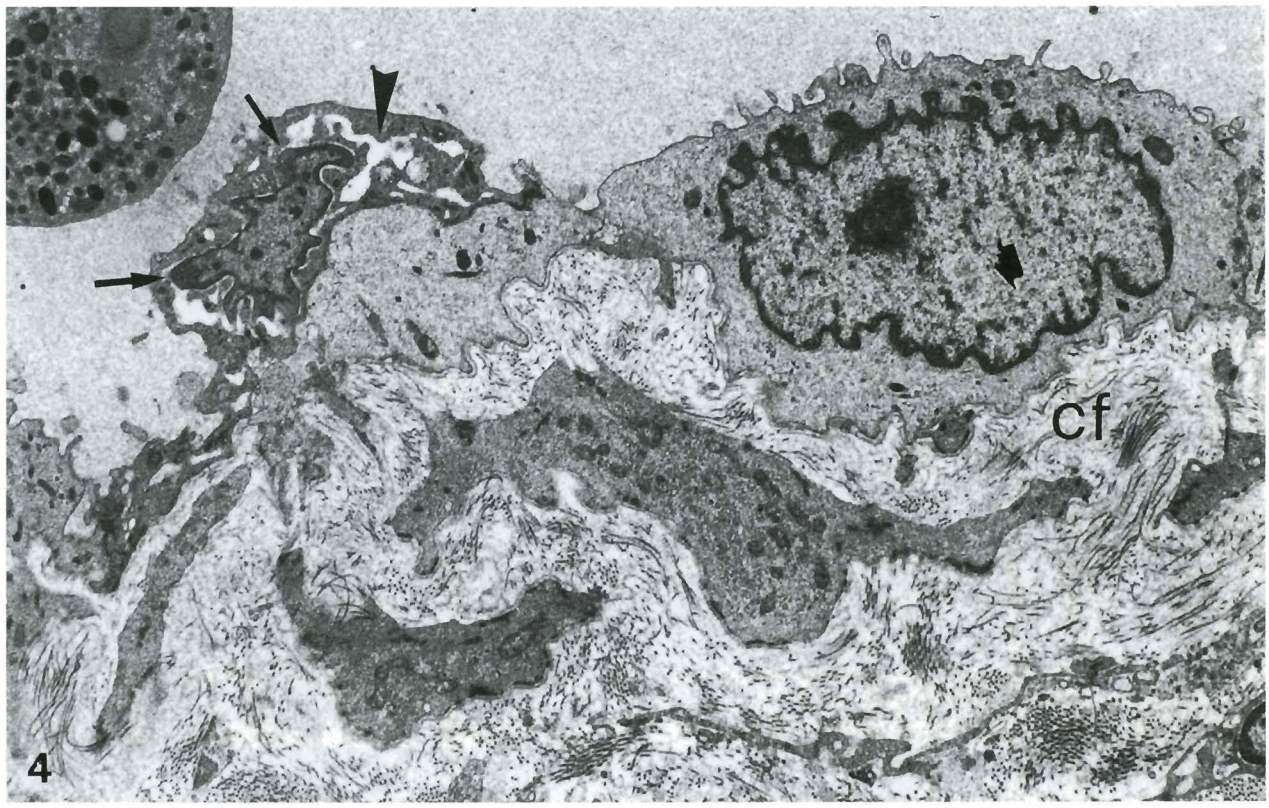


Fig. 4. Isolated endothelial cell at the luminal surface of a blood vessel shows signs of initial apoptotic changes such as margination and condensation of chromatin (thin arrows) as well as extensive vacuolization (arrowhead). A nuclear body (large arrow) is identified in the adjacent endothelial cell. Perivascular collagen fibers (cf) reach subendothelium. x 8,000

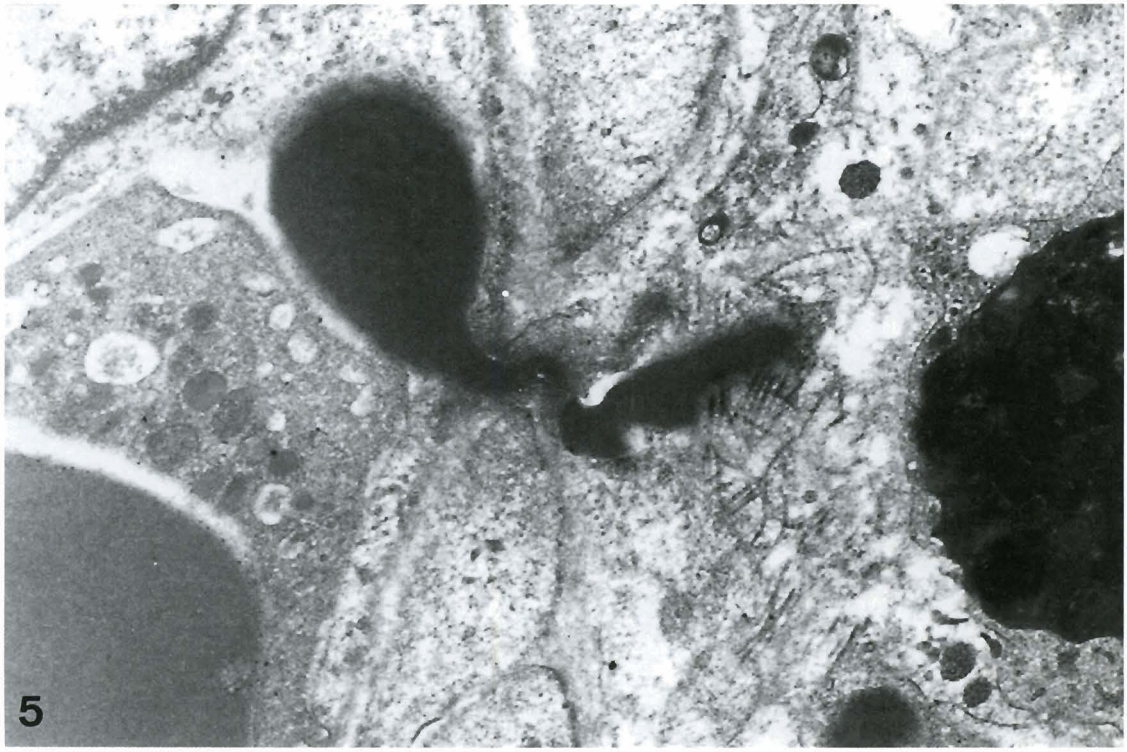
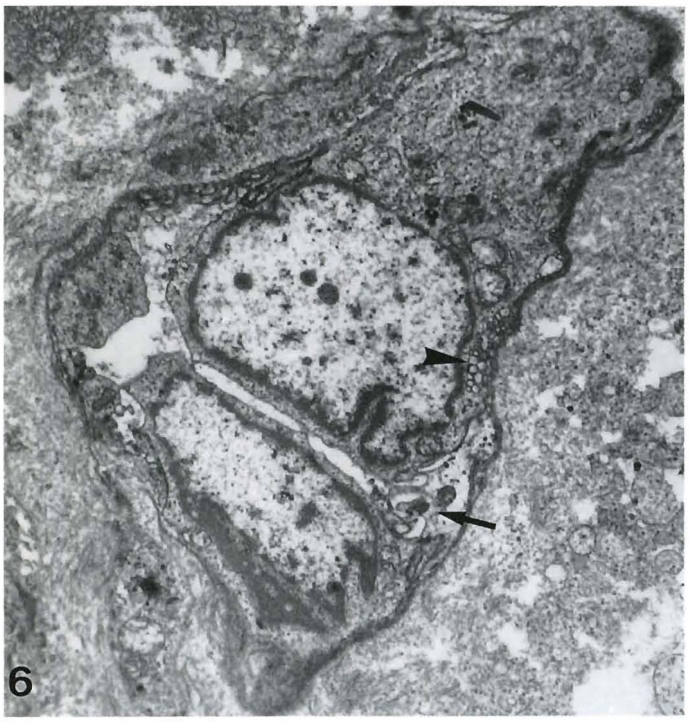


Fig. 5. A red blood cell is across the endothelial cell wall in an area where no interendothelial cell junction is visible. x 20,000

Solid capillary buds, characterized by marked enlargment of endothelial cells without the formation of a lumen (Fig. 9), were noted in both intestinal and diffuse types of advanced gastric carcinomas.



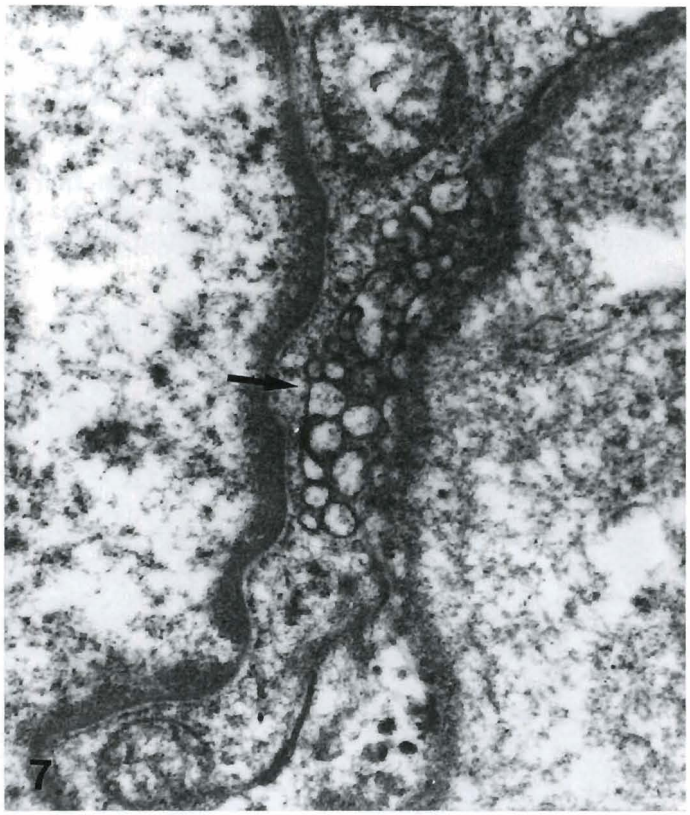


Fig. 6. Plump endothelial cells form slit-like lumina representing intercellular canalization. Luminal endothelial membranes show microprocesses forming a complex network of filiform, finger-like projections (arrow). These endothelial cells possess abundant clear cytoplasm containing microfilaments, mitochondria, dense bodies and vesiculo-vacuolar organelles (arrowhead). x 8,000

Fig. 7. Detail of the figure 6 showing vesiculo-vacuolar organelles (arrow). x 20,000

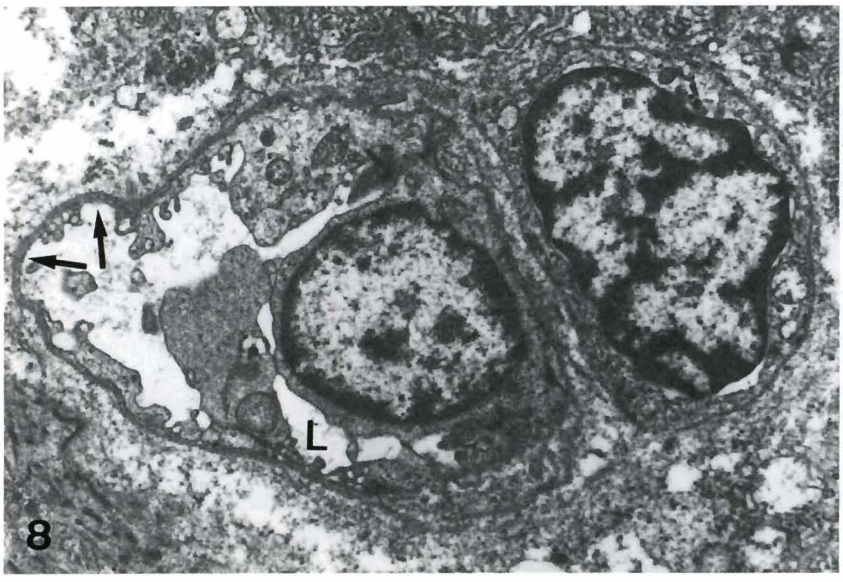


Fig. 8. Tumour vessel surrounded by complete basal lamina and showing extremely attenuated endothelium (arrow). A narrow luminal space (L) is seen between two endothelial cells. x 8,000

Discussion

In contrast to normal microvessels, those that supply tumours are strikingly hyperpermeable to circulating macromolecules such as plasma proteins (Dvorak et al., 1995). Only in an animal experimental model one can study permeability in the tumour microvasculature. Because our morphologic data were collected on surgically resected human specimens, no direct evidence of microvascular permeability could be provided .

The present observations demonstrate that small blood vessels of advanced gastric carcinomas have features in common with those of control mucosa, including a complete basal lamina, well-developed interendothelial junctions, fenestrations and caveolae. Our data suggest that the microvascular bed within advanced gastric carcinomas displayed ultrastructural features related rather to a decreased permeability of tumour microvasculature than to an increased one. Tumour microvasculature was surrounded by complete basal lamina and endothelial cells were joined by well-developed and complex tight junctions. Endothelial cells showed a variable number of fenestration and caveolae,but bundles of collagen fibers adjacent to subendothelial basal lamina seemed to compromise vascular permeability. Furthermore, the distribution of

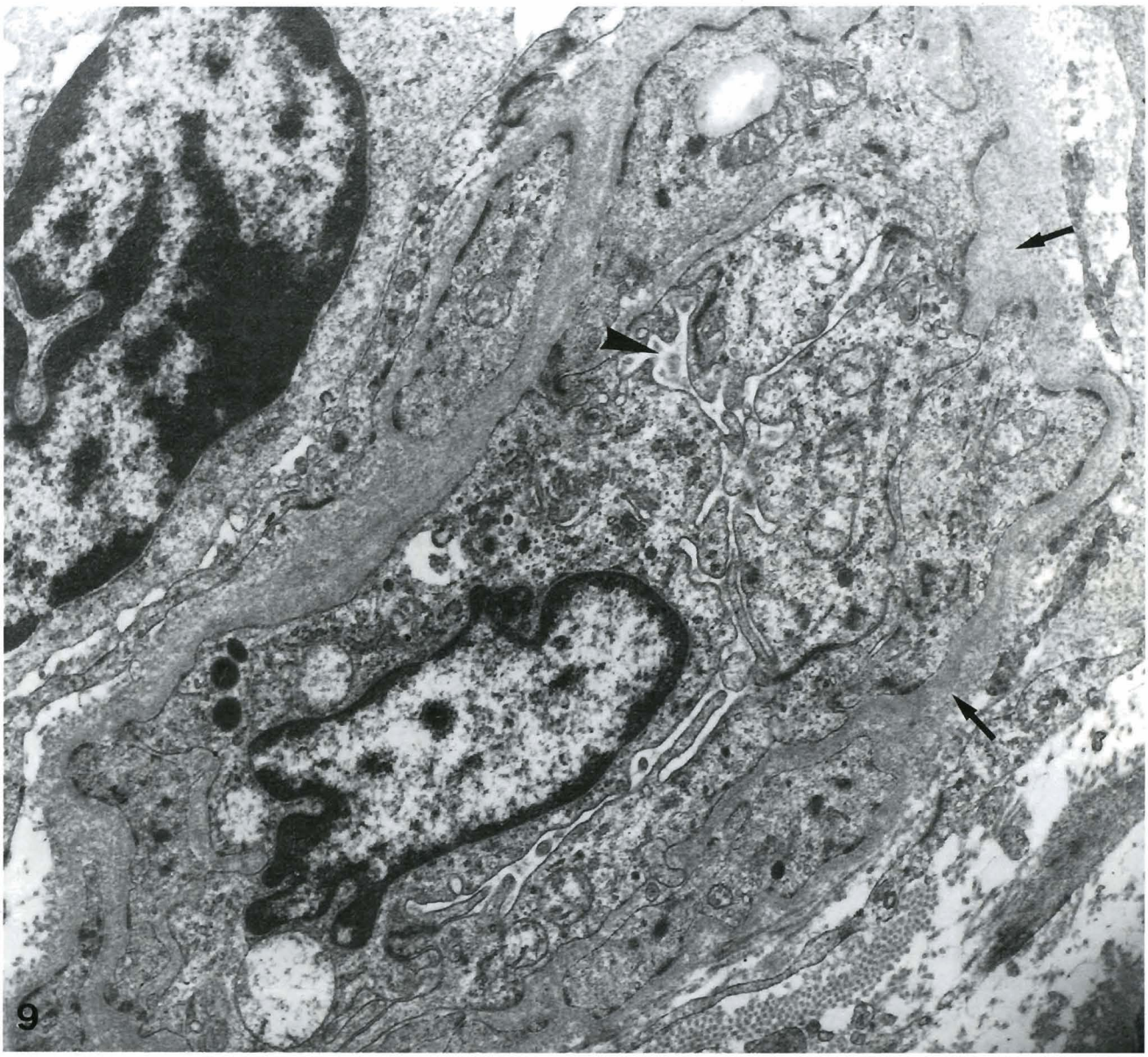


Fig. 9. Blood vessel showing marked enlargement of endothelial cells and slit-like lumen (arrowhead). Basal lamina is dense and thickened (arrows). x 8,000

collagen fibres around blood vessels suggested a process of perivascular fibrosis. These aspects of the tumour microenvironment might compound the impenetrability of activated antitumour lymphocytes, due to the presence of abundant collagen-rich extracellular matrix (Jain, 1988; Ganns and Hanahan, 1998). Moreover, the stiffening properties of fibrillar collagens might be important for vascular growth and stability (Kohn et al., 1992).

In 2 out of 9 cases, tumour microvasculature exhibited vesiculo-vacuolar organelles, that were not observed in control mucosa. The vesiculo-vacuolar organelle is a recently described cytoplasmic structure found in the endothelial cells that line tumour microvessels and normal venules (Kohn et al 1992; Dvorak et al., 1996; Feng et al., 1997, 1999, 2000; Vasile et al., 1999). They provide an important pathway for extravasation of circulating macromolecules, and have been mainly observed in experimental tumours (Kohn et al., 1992; Dvorak et al., 1996; Feng et al., 1997, 1999, 2000; Vasile et al., 1999) and in a few cases of human tumours (Horbelt et al., 1999).

According to Ohtani and Nagura (1990), solid capillary buds, considered to be immature capillaries, were noted in both intestinal and diffuse types of advanced gastric carcinomas.

Occasionally, red blood cells were across the vessel wall in areas not associated with interendothelial junctions. These ultrastructural findings suggest the possibility of transendothelial openings at these sites (Neal and Michel, 1997; Michel and Neal, 1999). Transcellular pathways are the principal type of opening induced in microvascular endothelium by the ionophore A23187, by vascular endothelial growth factor, and by high transmural pressures (Michel and Neal, 1999). In our cases, three distinct morphological features which might be pathways for extravasation were mainly observed in the tumour microvasculature: 1) fenestrae; 2) transendothelial openings; and 3) vesiculo-vacuolar organelles. Our ultrastructural findings consistent with increased permeability in gastric carcinomas have features in common with experimental and human tumours (Roberts and Palade, 1997; Roberts et al., 1998). These include fenestrations, endothelial attenuation and vesiculo-vacuolar organelles. However, our data differ from that described in the above-mentioned tumours (Roberts and Palade, 1997; Roberts et al., 1998), in that incomplete basal lamina and opened intercellular junctions were absent. Our ultrastructural features associated with vascular permeability are also unlike that reported in some tumor vessels, where the opening between defective endothelial cells, composed of disorganized, loosely connected, branched, overlapping or sprouting endothelial cells, explains tumour vessel leakiness (Hashizume et al., 2000).

In summary, these observations provide ultrastructural evidence that advanced gastric carcinomas shares with experimental tumour models in vivo some morphological features that are associated with hyperpermeability. Further research is needed to elucidate these apparent discordant results between the microvascular permeability models and those of ultrastructural studies of human gastric carcinomas.

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