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The microvasculature of the 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumour

I. Vascular patterns as visualized by scanning electron microscopy of corrosion casts

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Summary. We examined the microvasculature of the 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumour by scanning electron microscopy of corrosion casts. An elaborate vascular envelope predominantly consisting of sinusoidal and venular vessels was formed around each tumour nodule. These vessels exhibited various abnormal features, whereas arterioles appeared normal. The abnormal vessels possessed many globular outpouches, possibly representing the site of angiogenesis. An additional capillary layer was seen in the marginal boundary between the tumour and host tissue. The lack of centrifugally extruding vessels in this layer may indicate a poor potency for vascular spread of tumour cells into the adjacent normal tissue. Loop-like or glomerular ingrowths were frequently found on the inner aspect of the vascular capsule, which eventually developed into a dense intranodular plexus. Intranodular vessels often showed focal narrowing, tapering and/or rupturing, possibly due to increased tissue pressure caused by proliferating tumour cells. Those surrounding necrotic portions were extremely dilated with occasional periodic varicosities. The features may be associated with the lessening of the tissue pressure resulting from tumour cell collapse.

Key words: 7,12-Dimethylbenz(a)anthracene – Experimental mammary tumour – Angiogenesis – Corrosion casts – Scanning electron microscopy

Introduction

Solid tumours prompt continuous vascular proliferation by the angiogenic factors (TAFs) they produce. The resulting vessels, in turn, control the growth rate, metabolism and behaviour of the tumours (Wood 1973; Folkman 1985). Tumour vessels differ in their architecture from those in the adjacent normal tissues and vary considerably between different tumours (Reinhold and Van Den Berg-Blok 1983). Tumour-specific vasculature is important in diagnosis and also relevant to the radio-, chemo- and thermal therapy.

We conducted a series of ultrastructural investigations of the tumour vessels of the 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumour. This can readily be developed by a single administration of the chemical within a relatively short period of time (Huggins et al. 1961) which facilitates the physiopharmacological study of tumour blood flow. Perfusion characteristics, vascular resistance and reactivity to vasoactive substances have been intensively studied (Wiig et al. 1981; Hultborn et al. 1983; Tveit et al. 1984; Weiss et al. 1985). However little information is available as to the structural features of the vessels, with the exception of a light microscopical observation using an injection method (Peters et al. 1980).

The present paper reports the vascular pattern of the DMBA-induced mammary tumour as examined with scanning electron microscopy (SEM), using the microcorrosion cast method (Murakami 1971).

Materials and methods

Adult female Sprague-Dawley rats weighing 150–190 g were fed with 20 mg DMBA dissolved in sesame oil through a gastric tube. We used tumours which developed 4–15 weeks after drug administration. The animals were anaesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and were perfused with heparinized phosphate-buffered saline (pH 7.3) via the left ventricle. After perfusion fixation with phosphate-buffered 3% glutaraldehyde (pH 7.3), microcorrosion casts of tumour vessels were prepared according to the method described by Murakami (1971). In short, a low-viscosity resin, Mercox CL-2R (Dainippon Ink, Tokyo, Japan), was substituted for the fixative by cardiac perfusion. After polymerizing the resin, the tumours were dissected out and were macerated in 20% sodium hydroxide. Vascular casts were dried by the *t*-buthyl alcohol method (Inoue and Osatake 1988), were platinum sputter coated and were examined in a Hitachi S-500A scanning electron microscope. Part of the fixed specimen was embedded in epon-epoxy resin, cut and stained with toluidine blue for light microscopic observation.

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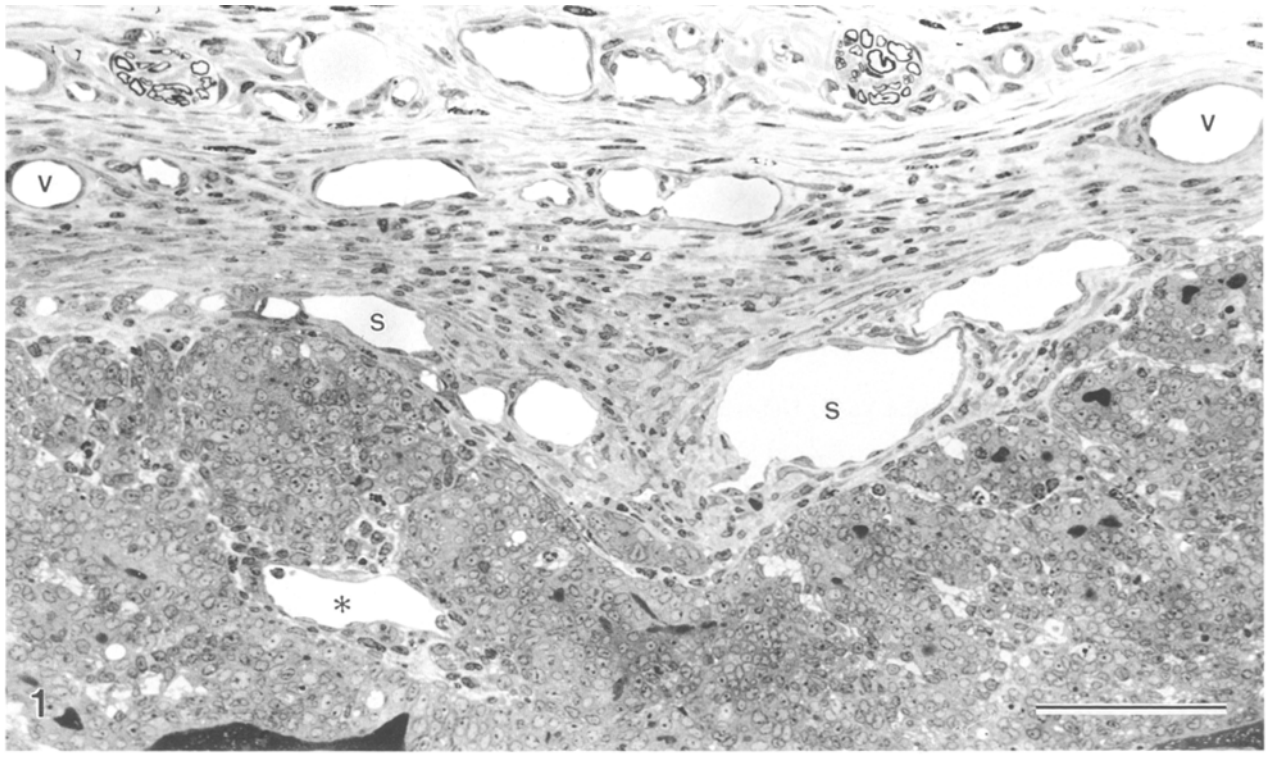

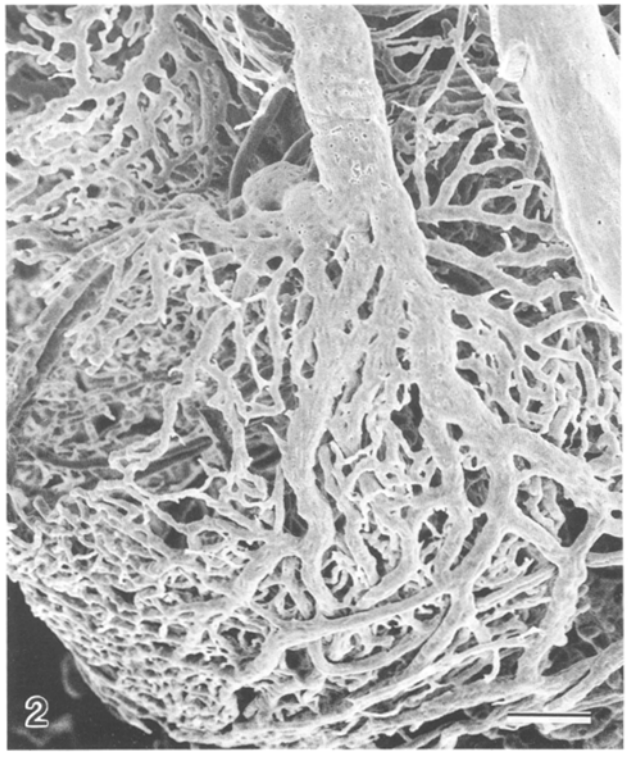

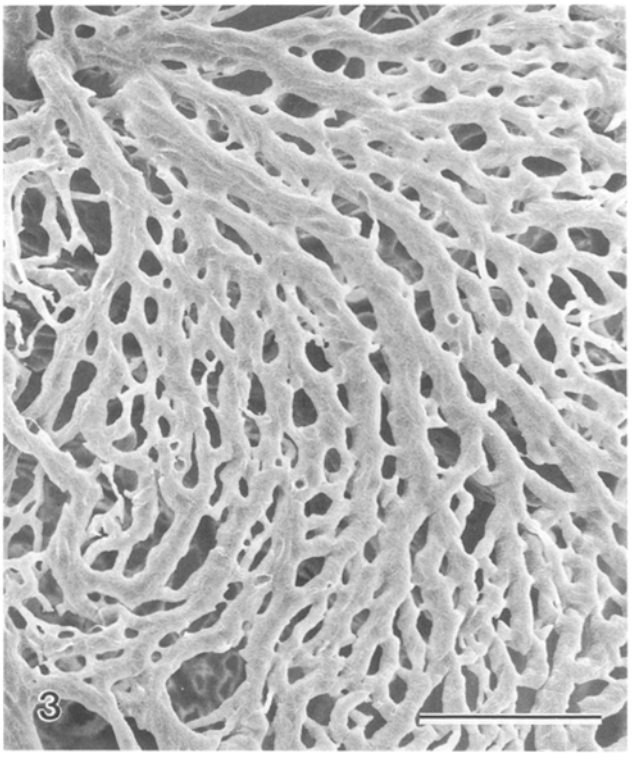

Fig. 1. Histological section of the 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumour. Proliferating microvessels occur in the connective tissue sheath, forming the vascular envelope around a tumour nodule. They consist mainly of sinusoidal (*s*) and venular (*v*) vessels. The nodule is penetrated by a sinusoidal vessel (*). *Bar* = 100 μm, × 250

Fig. 2. Corrosion cast of the vascular envelope. This encloses a hollow cavity in the tumour tissue which has been totally corroded away. *Bar* = 100 μm, × 110

Fig. 3. Vascular envelope developed between the adjacent nodules, assuming the features of a fenestrated vascular plate. *Bar* = 100 μm, × 240

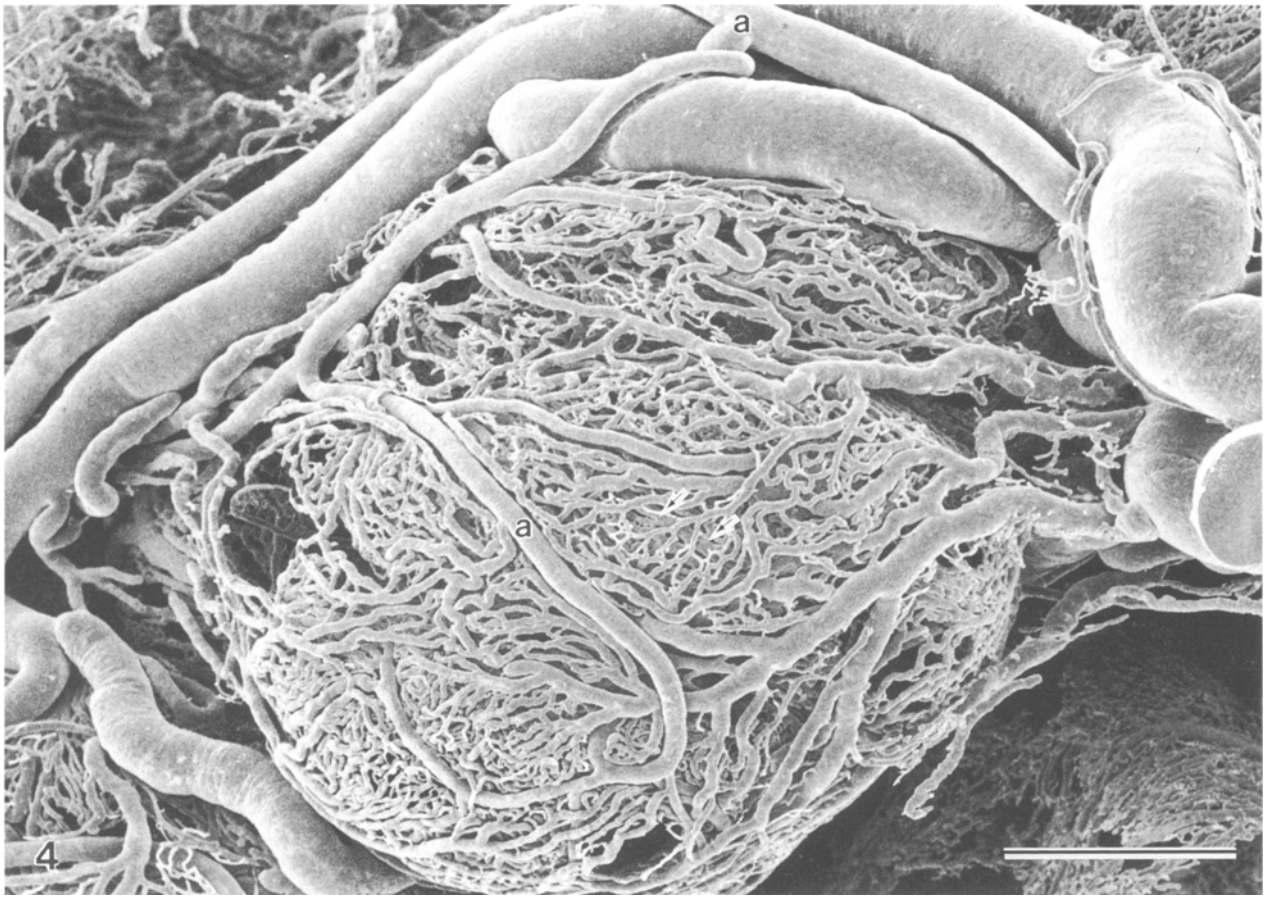

Fig. 4. Corrosion cast of the vascular envelope. An additional vascular layer has appeared in the boundary between the tumour and the host tissue, where capillaries less than 15 μm in diameter were predominant. No extruding capillaries were present. Sinusoidal vessels (*arrows*) underlying a capillary bed can be seen. *a*, Arteriolar vessels. *Bar* = 500 μm, × 60

Results

On light microscopy the DMBA-induced mammary tumours were multinodular, consisting of spherical nodules invested individually by a distinct connective tissue sheath. Proliferating vessels occurred in the sheath, which tended to be arranged in a layer, forming the "vascular envelope" around the nodule (Fig. 1). They consisted mostly of sinusoidal and venular vessels with fewer capillaries and arterioles. Large nodules were penetrated by highly dilated sinusoidal vessels, but their central necrotic area appeared to be avascular.

SEM of vascular corrosion casts demonstrated that the vascular envelope enclosed a hollow internal cavity from which the tumour tissue had been totally corroded (Fig. 2). The envelope consisted mostly of sinusoidal vessels (15–40 μm in diameter) and larger venules which anastomosed freely in an elaborate two-dimensional network. As shown in Fig. 3, the envelope often assumed a fenestrated vascular plate rather than a network; this feature resembled the casts of monkey choriocapillaries (Shimizu and Ujiie 1978). Here the vessels were arranged radially with numerous lateral connections less than 5 μm thick.

An additional vascular layer occurred in the boundary between the tumour and the host tissue, which consisted predominantly of capillaries less than 15 μm in diameter, as shown in Fig. 4. A few arteriolar vessels were found in the network. Host arterioles, 40–50 μm in diameter, and venules were presented adjacent to the nodule. The underlying sinusoidal vessels could be seen through the capillary meshes. There appeared to be no vessels present which extruded centrifugally into the host tissue.

The venules of the vascular envelope showed such abnormalities as pit- or slit-like perforations of the casts and short collaterals (Fig. 5). Direct offshoots occurred with blind ends and multiple branchings. In contrast, arterial vessels appeared almost normal.

Intranodular vessels were also sinusoidal, varying in shape and density between nodules. In the small nodule, vessels ranging from 20 to 80 μm formed a loose trabecula, which exhibited loop formation, focal dilation and attenuation (Fig. 6). In larger nodules, vessels were more densely arranged (Fig. 7). They took an angular course with focal narrowing and tapering and/or rupturing with horn-like or club-like ends (Figs. 8, 9).

Focal loop-like or glomerular ingrowths were often

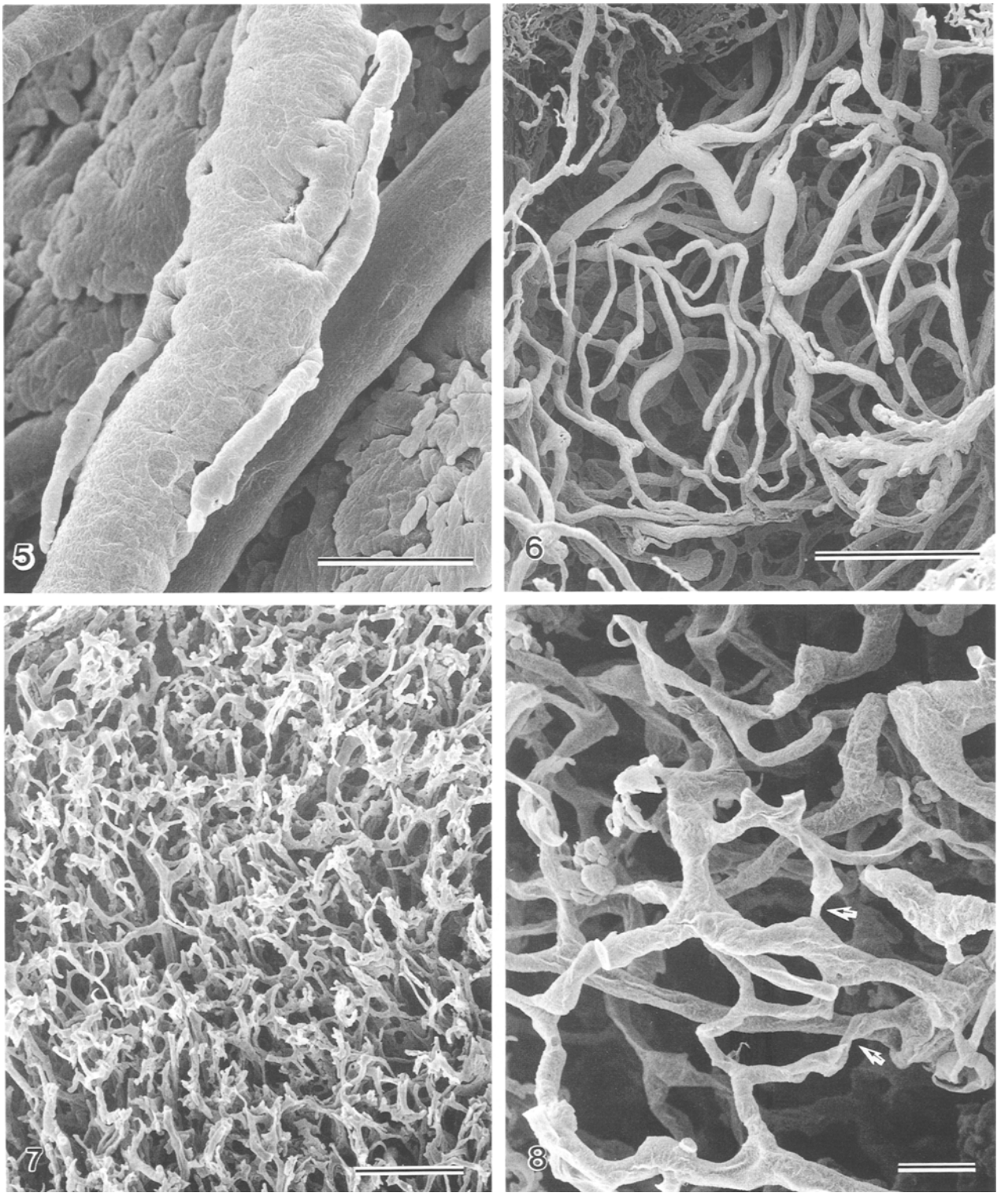

Fig. 5. Venules of the vascular envelope showing various abnormalities. Pit or slit-like perforations of the cast, short collaterals and direct offshoots with blind ends. *Bar* = 50 μm, × 520

Fig. 6. Within the small nodule, vessels ranging from 20 to 80 μm form a loose trabecula with loop formation, focal dilatation and attenuation. *Bar* = 500 μm, × 55

Fig. 7. In the larger nodule, sinusoidal vessels form a highly dense intranodular plexus. *Bar* = 200 μm, × 90

Fig. 8. Vessels within the larger nodule take an angular course with focal abrupt narrowings (*arrows*). *Bar* = 50 μm, × 250

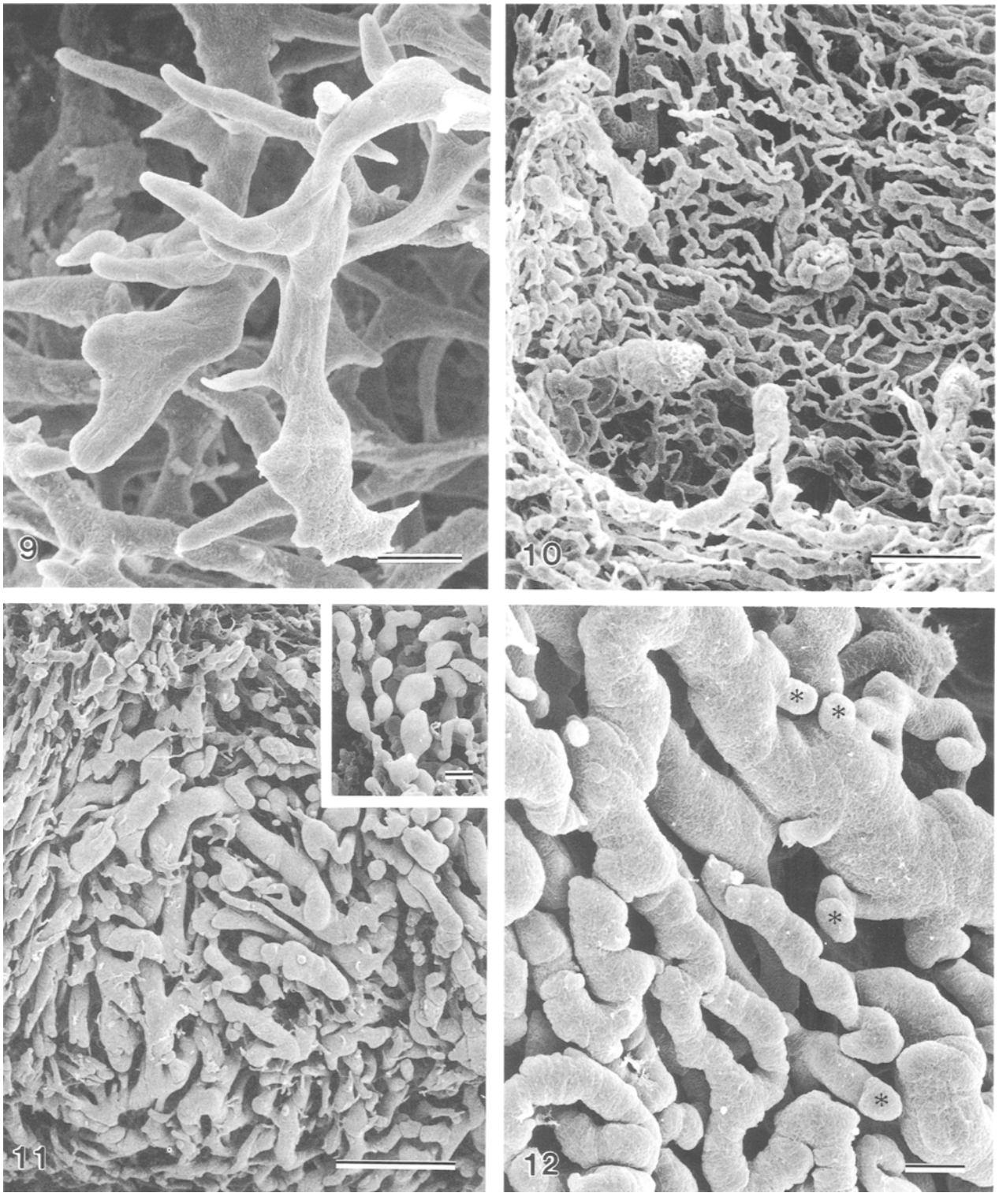

Fig. 9. Vessels within the larger nodule exhibit tapering and/or rupturing with horn-like or club-like ends. *Bar* = 50 μm, × 280

Fig. 10. Inner aspect of the sinusoidal layer. Focal loop-like or glomerular ingrowths are found. *Bar* = 100 μm, × 180

Fig. 11. Aggregation of extremely dilated sinusoidal vessels facing the central necrotic cavity. *Bar* = 500 μm, × 40. *Inset*: A series of varicosities of intranodular vessels adjacent to the necrotic portion. *Bar* = 200 μm, × 25

Fig. 12. Bulbous vascular outpouches (*) in the vascular envelope. *Bar* = 20μm, × 500

found on the inner aspect of the sinusoidal layer (Fig. 10), which appeared to develop further into a dense intranodular plexus. Extremely dilated sinusoidal vessels more than 100 μm in diameter were found to be aggregated around a central necrotic cavity (Fig. 11). They often formed a series of varicosities more than 150 μm in diameter (inset of Fig. 11).

Bulbous outpouches 10–20 μm in diameter were encountered along the vessels' length in the vascular envelope (Fig. 12). They occurred more frequently along sinusoidal vessels and venules than capillaries, and none were found in arterioles.

Discussion

The formation of an elaborate vascular envelope appears to be one of the common features of tumour vessels, as can be deduced from previous studies of a wide variety of experimental and spontaneous tumours (Tatematsu et al. 1978; Egawa et al. 1979; Grunt et al. 1986a, b; Bugajski et al. 1989). The vascular pattern, however, varies considerably between tumours; the variability may be a result of the combination of several factors including the growth pattern and the effectiveness of the angiogenic stimulus. It also has relevance to invasiveness and metastatic potency of individual tumours (Wood 1973; Warren 1979a, b). In various lesions, the vascular envelope has been shown to consist of multiple layers, each differing in architecture and vascular composition. The vascular envelope of the DMBA-induced tumour was much simpler in structural organization than those of other tumour types, consisting of a single layer of sinusoidal vessels, although an additional capillary layer was encountered in the marginal boundary. Unlike in most malignant lesions (Egawa et al. 1979; Grunt et al. 1986a) there were no centrifugally extruding vessels. This suggest that our tumour is benign, in the sense that tumour cells may be largely arrested to spread vascularly into the adjacent normal tissue.

Sinusoidal and venular vessels of the vascular envelope exhibited various morphological abnormalities, whereas arteriolar vessels were apparently normal. Arteriolar vessels are essentially inert, being comparatively little affected by TAFs (Intaglietta et al. 1977), whilst venules are apparently responsive (Reinhold and Van Den Berg-Blok 1983). The predominance of dilated sinusoidal vessels and venules over arteriolar vessels may account for sluggish tumour blood flow which crisscrosses the mass (Endrich 1979).

Focal attenuation, tapering and/or rupturing with blind ends of the intranodular vessels may be due to high tissue pressure within the tumours compressing the vessels (Folkman and Cortan 1976). The increased tissue pressure may also result in increased hypoxia and subsequent central necrosis (Wiig et al. 1980; Reinhold and Van Den Berg-Blok 1983). Extreme dilatation with occasional periodic varicosities of the vessels surrounding a necrotic cavity may be the result of lessened tissue pressure caused by the collapse of tumour cells (Reinhold and Van Den Berg-Blok 1983).

The developmental sequence of intranodular vessels has not been fully elucidated. Thompson et al. (1989) have suggested that newly formed vessels are incorporated into tumours by the infiltration and expansion of the surrounding tumour tissue. We found loop-like or glomerular vascular ingrowths on the inner aspect of the vascular envelope, indicating that the intranodular vessels can be formed by vascular penetration.

Bulbous or club-like outpouches have been found and have been interpreted as representing vascular sprouts in previous SEM studies of proliferating vessels (see Yasugi et al. 1989). Since developing vessels are highly vulnerable, particularly at their growing points, the luminal shape of vascular sprouts can easily be deformed even at the controlled injection pressure of the plastic. We are currently performing transmission electron microscopic analysis of serial ultrathin sections to elucidate the actual shape and structure of vascular sprouts.

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