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Angiomorphology of the human renal clear cell carcinoma

A light and scanning electron microscopic study

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Summary. The vascular system of human renal clear cell carcinoma was studied using light microscopy of silicon rubber-injected specimens and scanning electron microscopy of conventionally prepared tissue and vascular corrosion casts. The system was found to exhibit the following features: (1) a well developed superficial vascular coat showing different pattern on the anterior and on the posterior side of the tumour, (2) an internal vascular network composed of altered and displaced pre-existing vessels, numerous newly formed ones and those recruited from adjacent structures, (3) quantitative prevalence of dilated veins and distended capillaries, (4) a remarkable proliferative reaction of stellate veins, (5) characteristic features of the intratumour vasculature in the form of avascular nodules surrounded by basket-like capillary plexuses and separated by well vascularized "septa", (6) a relatively less dense vascularization of central tumour areas, frequently exhibiting necrotic foci, and the highest density of vessels in areas close to the superficial vascular coat, and (7) morphological evidence for a continuous remodelling of the tumour vasculature. The observed patterns of the vascular system seem to provide a pathway for further tumour expansion.

Key words: Renal clear cell carcinoma – Blood vessels – Angiogenesis – Corrosion casts – SEM

Introduction

Human renal clear cell carcinoma is a solid tumor arising from the proximal tubular cell histogenetically (Oberling et al. 1960; Terreros et al. 1986; Thoenes et al. 1986). The usually well vascularized tumour grows spontaneously in the form of an irregular mass composed of nodules exhibiting acinar appearance. It also shows high interstitial pressure, being therefore included, according to Falk's classification to the group of "tense" tumours (Falk 1978, 1982). This high pressure influences both the shape and functional state of the tumour vessels and is an important factor responsible for the ischaemic, necrotic and haemorrhagic foci frequently occurring within the central areas (Osteaux and Jeanmart 1979; Grunt et al. 1986b).

The vascular system of renal clear cell carcinoma comprises both pre-existing, mostly pathologically altered renal vessels, as well as new vessels resulting from angiogenesis (Osteaux and Jeanmart 1979; Shubik 1982; Reinhold and Van der Berg-Blok 1983). This form of vascularization which affects not only elements of the proper renal circulation including the capsular plexus, but also those from adjacent structures, is utilized by the tumour for its further expansion and spread (Lang 1973; Warren et al. 1978). Irrerspective of its mode of growth, the tumour spreads by direct expansion or by dissemination into blood and lymphatic vessels (Holland 1973). The hematogenous route, however, seems to play a much more important role since solid tumours are usually devoid of their own lymphatic system (Warren 1979b; Gullino 1982; Vaupel and Muller-Klieser 1983).

One of the principal sources of our knowledge concerned with the vascularization of the spontaneously growing renal celar cell carcinoma is angiography and inferior venocavography, performed in vivo. More detailed information on the vascular organization of normal and pathologically altered kidneys have been gained from experimental microangiographic studies performed on

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animals and, however, man combined with comparative histological investigations at the light and electron microscopic levels (Ericsson et al. 1966; Fischer and Horvat 1972; Warren and Chauvin 1977; Osteaux and Jeanmart 1979; Pitz et al. 1987). Nevertheless, as far as the understanding of the vascular network topography is concerned, the essential limitations of these methods are obvious: they do not allow the demonstration of the 3-dimensional structure of the vascular system. The recently developed method of vascular corrosion casting (Murakami 1971) is especially well suited for such a detailed morphological analysis of vascular systems (Miodoński et al. 1981; Lametschwandtner et al. 1984). Moreover, scanning electron miscroscopy (SEM) of vascular corrosion casts gives a quasi-3-dimensional image in which arteries, veins and capillaries can be discerned with high accuracy, at least in mammals (Miodoński et al. 1976).

The aim of this study was to visualize the angiomorphology of the human renal clear cell carcinoma using scanning electron microscopy of vascular corrosion casts, accompanied by conventional scanning electron microscopy of critical point-dried tissue and by light microscopy of silicon rubber-injected specimens.

Materials and methods

15 surgically removed human kidneys with advanced renal adenocarcinoma (histopathologically confirmed as renal clear cell carcinoma, Fig. 1 b) were used for this study. The kidneys were immediately placed in prewarmed (37° C) heparinized saline and perfused via the renal artery with approx. 1000 ml of the same saline containing 3% Dextran, m.w. 70000 (Gannon 1978). Each kidney was next prefixed by perfusion with 500 ml of 0.66% paraformaldehyde/0.08% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 containing 0.2% lignocaine (Paine and Low 1975). The kidneys were then divided into 3 groups and subjected to procedures described below.

Three kidneys were injected with Microfil-MV 122 yellow silicon rubber (Canton Biomedical Products, Boulder, USA). They were transferred to 70% ethanol and cut sagittally into 1.0–1.5 mm thick slices. The slices were dehydrated in graded series of ethanol, cleared in 3 changes of absolute ethanol/methylsalicylate (3:1, 1:1, 1:3), placed in pure methylsalicylate and examined under a stereomicroscope.

6 kidneys were fixed by simultaneous perfusion/immersion with a modified Karnovsky's solution composed of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 (900 ml per kidney). Afterwards, they were cut sagitally and large blocks ($2 \times 2 \times 1$ cm) from different regions of the tumors were excised and kept in the same fixative for 20 h at 4° C. After a thorough rinse in the cacodylate buffer, tissue blocks were postfixed with 2% osmium tetroxide for 9 h at 4° C, dehydrated in ethanol and aceton and critical point-dried in liquid carbon dioxide. The dried blocks were coated with carbon and gold and examined in a Jeol JSM-35-CF scanning electron microscope at 15–25 kV.

6 kidneys were injected with 250–300 ml of Mercox CL-2R resin (Japan Vilene Comp. Ltd., Tokyo) and left in a warm water bath (60° C) for several h to allow for the polymerization of the resin. They were next repeatedly macerated in 15–20% sodium hydroxide at 37° C and washed with warm (60° C) tap water for a few days. The obtained casts were washed for another few days in multiple changes of distilled water, cleaned with 5% trichloroacetic acid, again washed with distilled water and frozen-dried. The casts were examined under a stereomicroscope. Next, they were again frozen in distilled water, fractured along the desired planes in order to expose the intratumour vasculature and frozen-dried. The obtained small samples were coated with gold and examined in the scanning electron microscope specified above. The entire procedure has been described in detail elsewhere (Miodoński et al. 1981).

Results

When viewed under low magnifications in light microscopy, kidney slices injected with silicon rubber reveal clear-cut angioachitectonic differences between the normal renal parenchyma and the vascular network of the tumour (Fig. 1 a). Tumour tissue is separated from the normal, albeit compressed renal tissue by a well developed vascular coat. The peripheral areas of the tumour show the highest density of blood vessels arranged in a form of avascular nodules surrounded by thin vascular sheaths and separated by narrow, condensed vascular strands or septa. The deeper parts are relatively less densely vascularized and show the presence of irregularly distributed vessels of variable shape, size and course. These areas of the tumour most frequently contain necrotic foci combined with haemorrhagic plaques, although the latter can also be occasionally encountered in the vicinity of the superficial vascular coat.

Scanning electron microscopy of conventional preparations reveals groups of tumour cells often arranged in a form of trabeculae, sheets and tubules, separated by usually inconspicuous stromal tissue containing numerous thin-walled capillary and/or venous vessels (Fig. 2). The vessels are surrounded and compressed by tightly packed tumour cells, showing very irregular shapes in consequence and sometimes degenerative features in the form of round intraendothelial indentations, approx. 5 μm in diameter (Fig. 3). Deformations caused by compression are especially well pronounced in venous vessels which acquire a sinusoidal character (Fig. 3). They are tightly surrounded by cancer cells which are in a direct contact either with the vessel wall, or with a delicate layer of collagen fibers covering their adluminal surface (Fig. 4). The endothelial lining of medium-size and smaller vessels exhibits signs of proliferation: irregular distribution, variable size, overlapping and a rather

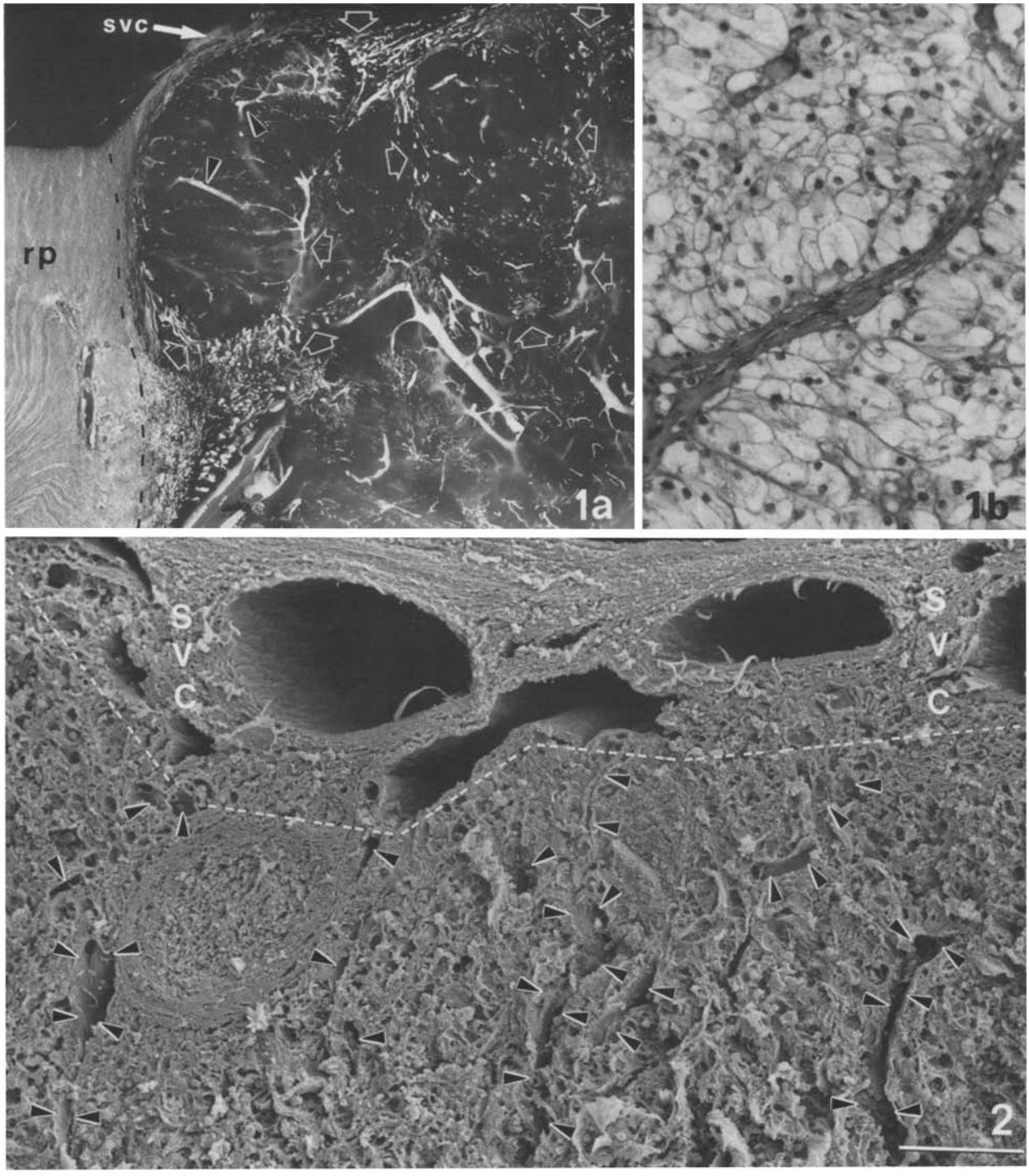


Fig. 1. a Panoramic view showing the border between tumour tissue and normal renal parenchyma (*interrupted black line*). Note the superficial vascular coat (*svc*) and the nodular pattern of tumour, with nodules separated by vascular septa (*arrows*), directed towards the center of the tumour. A few larger, strongly altered vessels can be observed within the peripheral nodule (*arrowheads*, compare with Fig. 7). Microfil injection. ×3. **b** Light micrograph of tumour parenchyma, showing typical appearance of the renal clear cell carcinoma. Paraffin section, H & E, ×540

Fig. 2. A peripheral region of the tumour: the superficial vascular coat (*svc*) composed of large and medium-size vessels covers tumour parenchyma arranged in a form of solid trabeculae separated by numerous vascular profiles of variable diameter (*arrowheads*). Bar = 100 μm, SEM, ×200

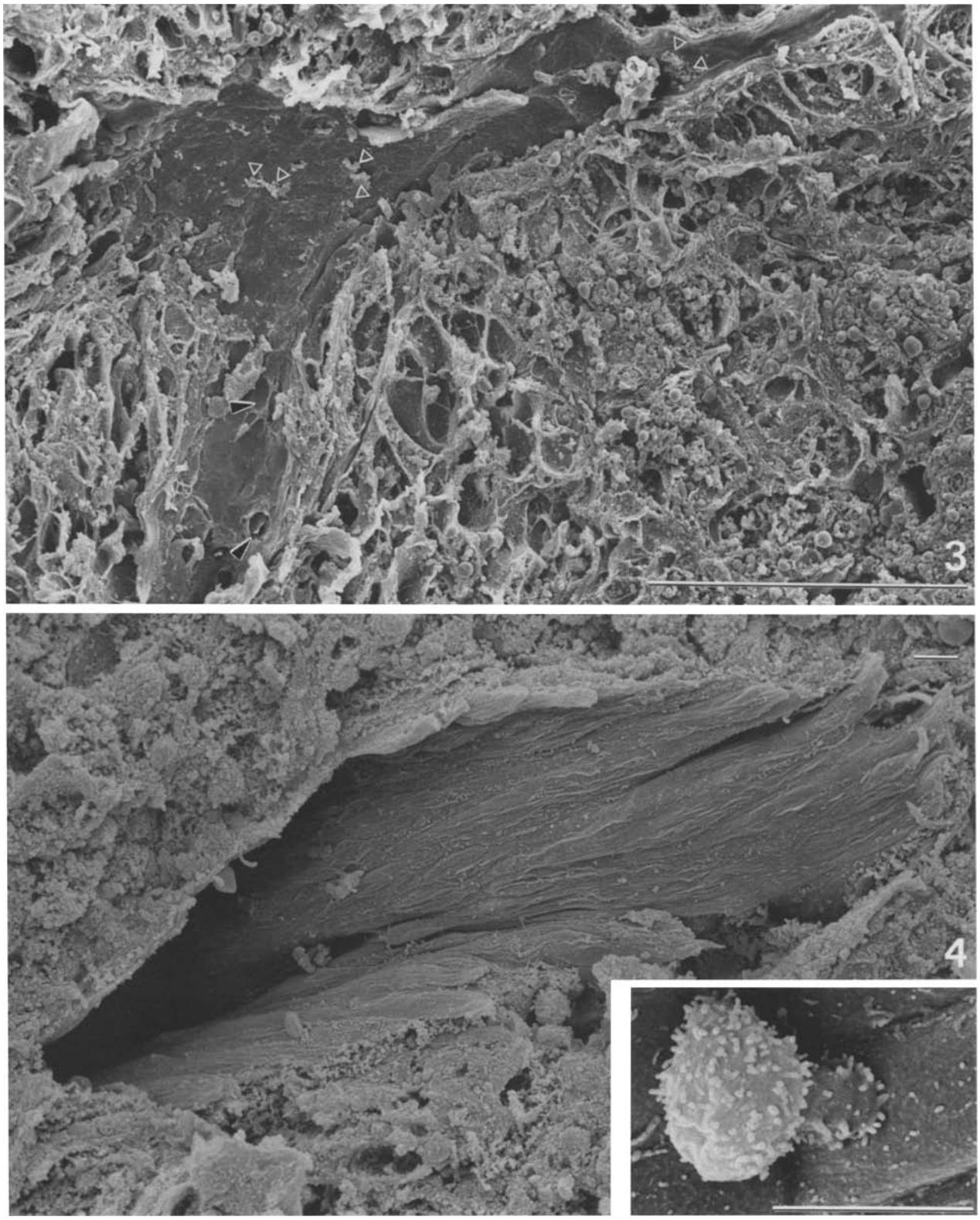
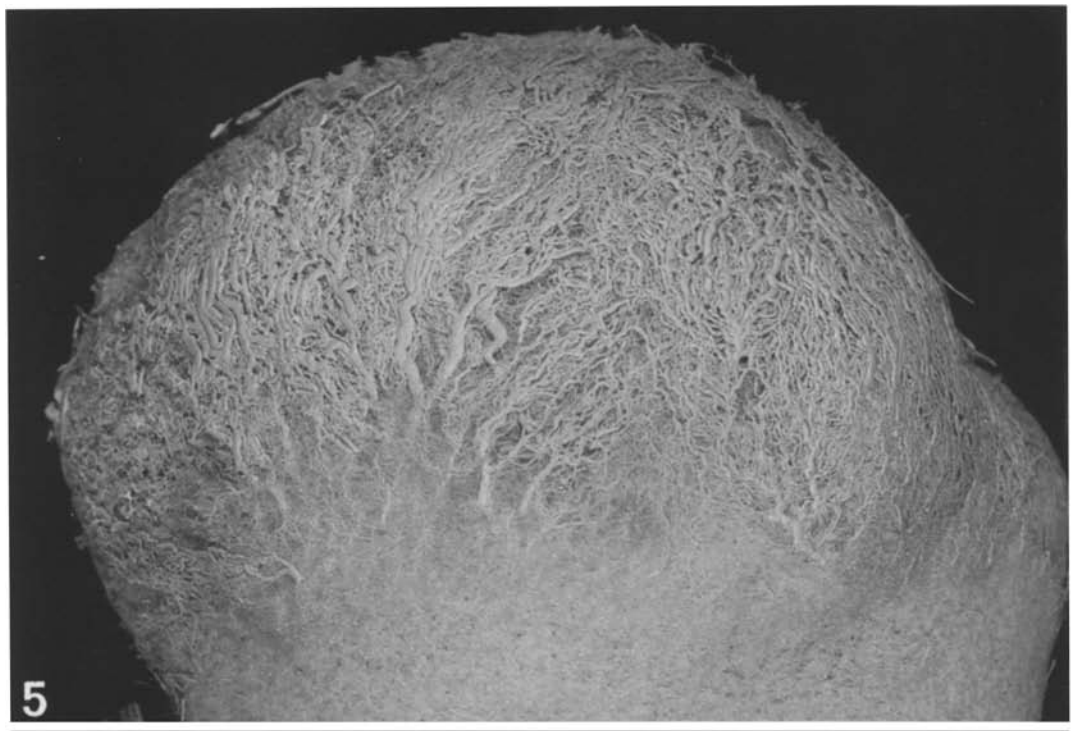


Fig. 3. Opened profile of a compressed, thin-walled vein. Note moulding of the luminal surface which exhibits signs of endothelial degeneration (*arrowheads*) and the presence of platelet aggregates (*triangles*). Bar = 100 μm, SEM, × 540

Fig. 4. A proliferative reaction of the endothelial lining in a small, thin-walled vein. The endothelial cells are differentiated in size and show irregular distribution, mutual overlapping and the presence of scarce microvilli. Cancer cells remain in a direct contact with the vessel wall. Inset: a lymphocyte attached to the surface of the endothelial cell. Bar = 10 μm, SEM, × 3400



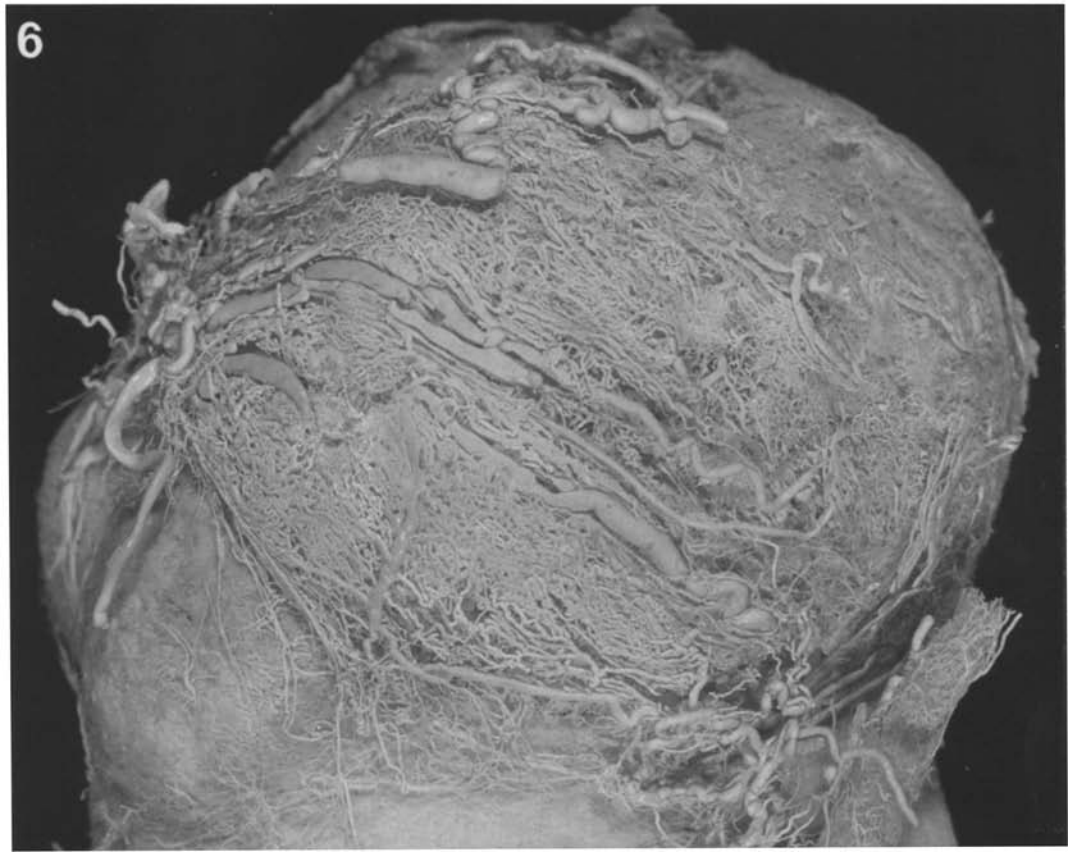


Fig. 5. The superficial vascular coat covering the anterior surface of tumour growing on the lower pole of the right kidney. Note a dense vascular network assuming a nearly meridional orientation and mainly composed of pathologically altered stellate veins. Corrosion cast, ×3.5

Fig. 6. The superficial vascular coat covering the posterior surface of the tumour shown in Fig. 5. Note distinct angioarchitectonical differences in the vascular pattern. Corrosion cast, ×3.5

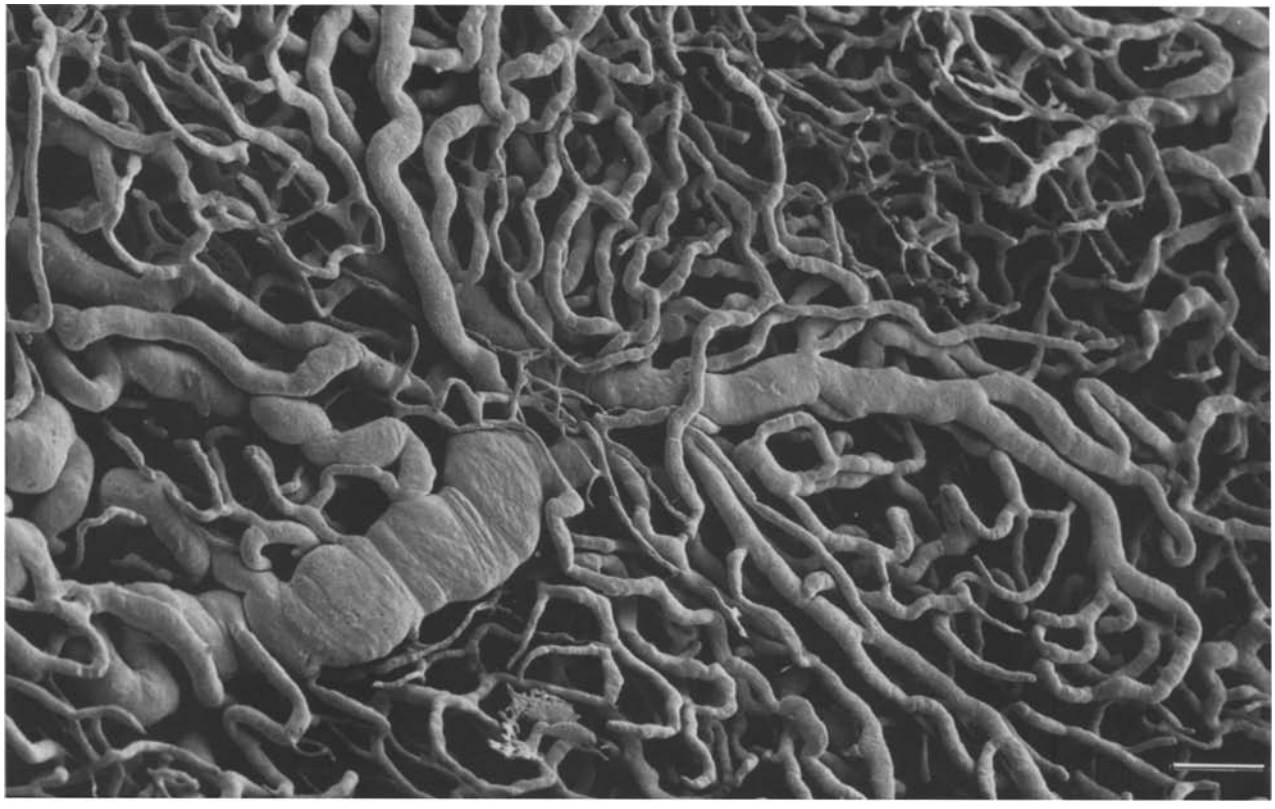


Fig. 7. Stellate vein altered by a marked proliferative reaction. Note flattening, irregular dilatation and tortuosity of larger and smaller branches. The latter ones form a dense plexus with irregular meshes. Corrosion cast. Bar = 100 μm, SEM, × 13

smooth abluminal surface scantily decorated with microvilli and occasional platelet aggregates (Fig. 4). In contrast, larger arteries and veins exhibit normal endothelial patterns and frequently contain leukocytes glued to their abluminal surface either single (Fig. 4, inset) or in small clusters.

Vascular corrosion casts reveal very clearly the structure of the superficial vascular coat, which is particularly well developed in large tumours protruding out of the kidney. A striking feature of the coat is the different vascular pattern on the anterior and posterior side of the tumour, observed in all the specimens examined. The anterior surface of the tumour, facing the peritoneum, is covered by a dense and quite regular network of medium-size vessels, mostly veins, which together with their smaller branches exhibit "riverine" course of a generally vertical orientation (Fig. 5). In contrast, the posterior surface of the tumour, facing the iliopsoas muscle, shows the presence of a much more irregular vascular coat with a characteristic horizontal arrangement of large vessels (mostly veins, but also arteries) derived from both the tumour vasculature and collateral circulation (Fig. 6).

The veins of the vascular coat include main branches of the stellate and – to a lesser extent – capsular veins, greatly altered by angiogenetic processes on the one hand and by mechanical deformations (stretching, compression and displacement) on the other (Figs. 5–7). The proliferative reaction of stellate veins, especially evident on the anterior tumour surface, gradually decreases towards the unaffected part of the kidney (Fig. 5). The arteries of the vascular coat exhibiting wavy courses (Fig. 6) represent displaced and deformed capsular arteries as well as arteries derived from the recruited capsular collateral circulation and from adjacent structures.

These large components of the vascular coat cover a dense capillary plexus with irregular meshes, expanded in the background. The plexus is formed by strongly dilated and rippled capillary-like twigs of stellate and capsular veins, and, much less frequently, of local arteries (Figs. 5–7). The spatial coexistence of dilated arterial and venous capillaries which are connected at random with large vessels of the coat creates favorable conditions for the development of arterio-venous shunts.

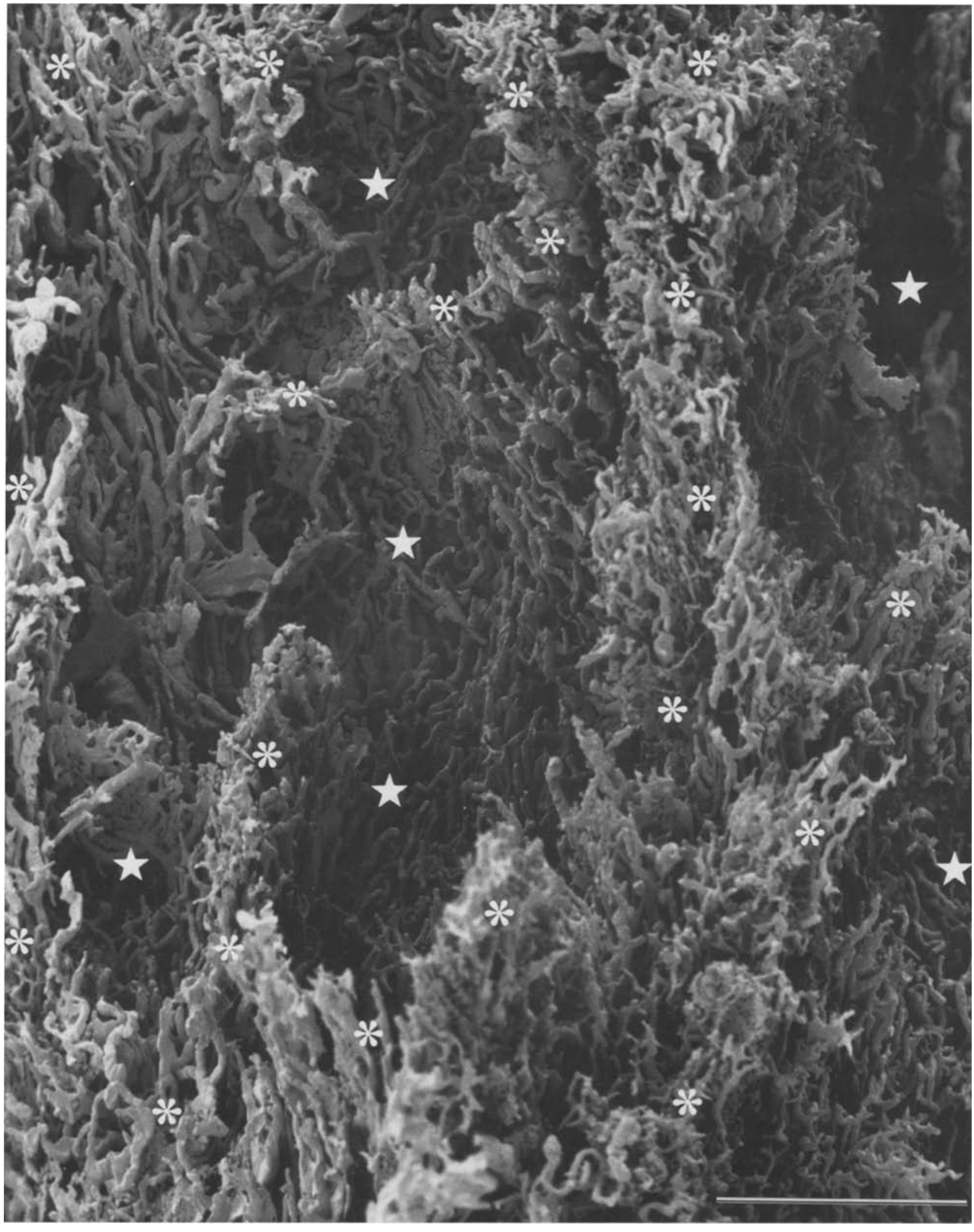
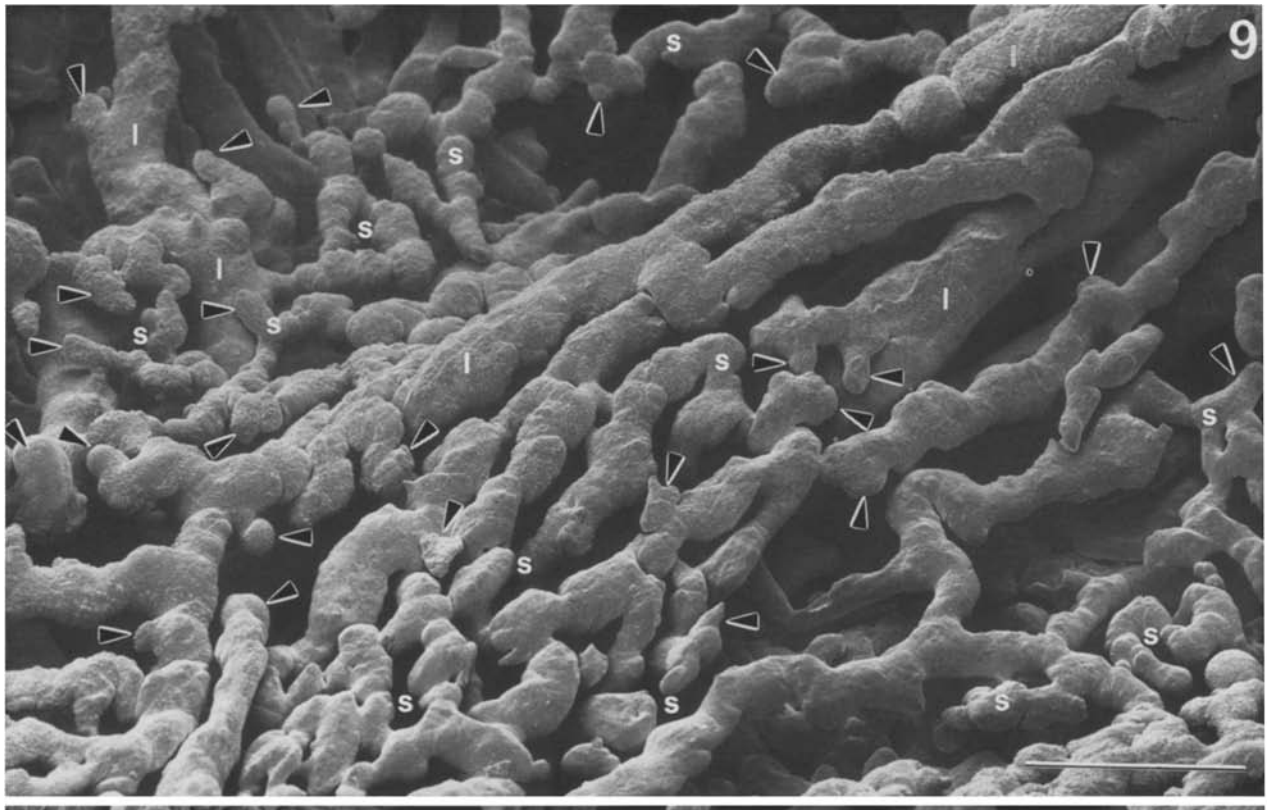


Fig. 8. A panoramic view of the intratumour vasculature: the highraising vascular territories (*rosettes*) are interspersed with adjacent avascular spaces (*asterisks*). Corrosion cast. Bar = 1 mm, SEM, × 143



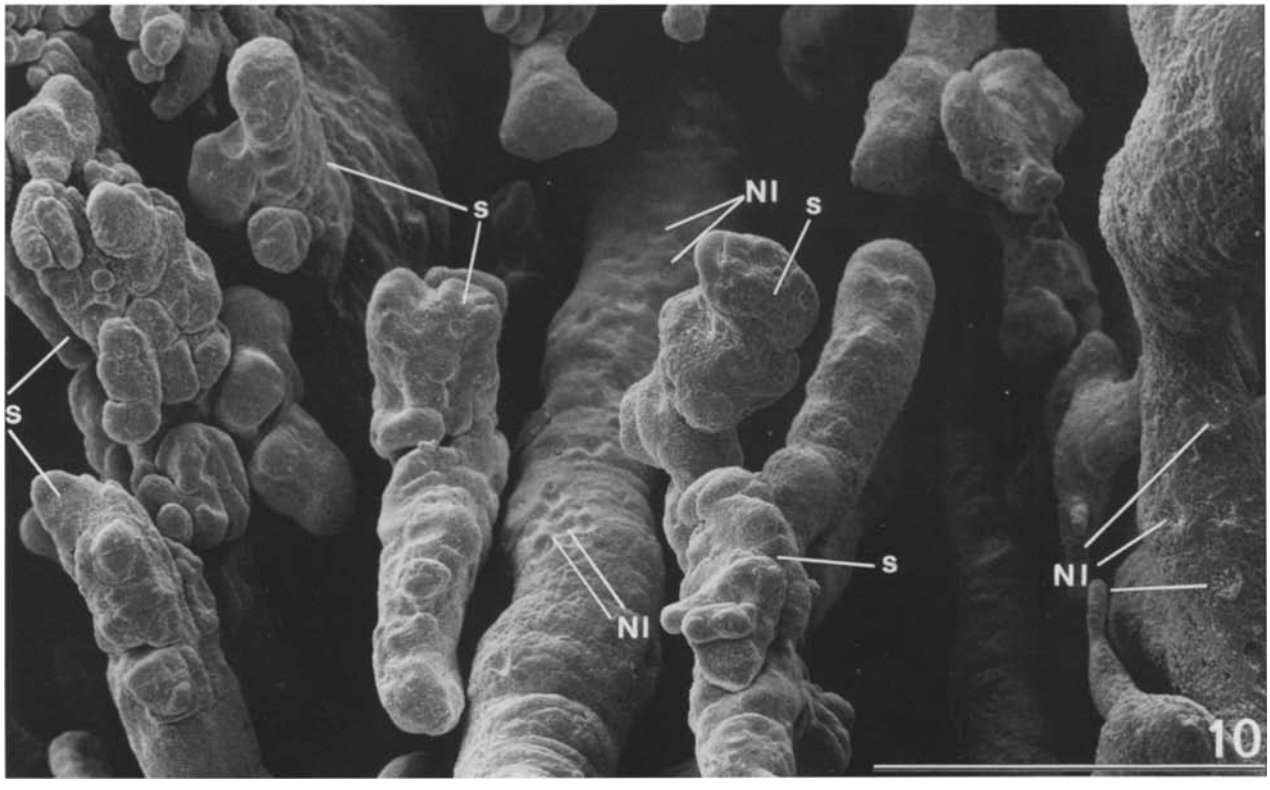


Fig. 9. A fairly regular and flat vascular plexus surrounding a ballshaped avascular space. The plexus is built of smaller (*s*) and larger (*l*) vessels which show the presence of globular or finger-like outgrowths (*arrowheads*). Corrosion cast. Bar = 100 μm, SEM, × 300

Fig. 10. A fragment of vascular territory, mainly composed of dilated and irregularly arranged veins. Note the presence of sinusoidal dilatations (*S*) with outer surfaces decorated by globular swellings or protrusions. Nuclear impressions (*NI*) are also visible. Corrosion cast. Bar = 100 μm, SEM, × 510

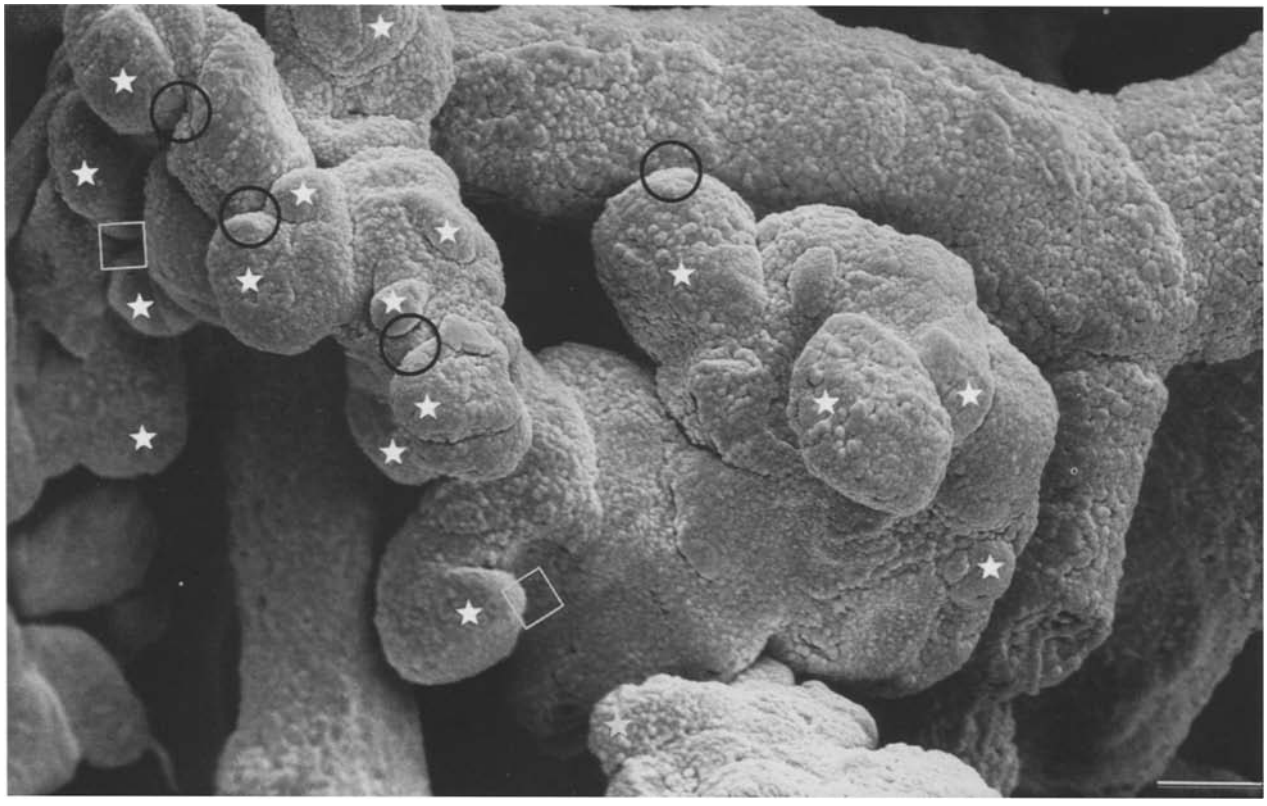


Fig. 11. A higher magnification of veins with outgrowths of a variable shape and diameter on their outer surface (*asterisks*). Black circles and white squares indicate outgrowths prior to fusion with each other or with their parent vessel. Corrosion cast. Bar = 10 μm, SEM, × 1400

The internal vasculature of the tumour shows a remarkably heterogenous pattern, caused not only by steadily growing, densely packed neoplastic cells but also by tumour-induced angiogenesis. As a rule, the vasculature shows the presence of high-raising vascular territories interspersed with adjacent avascular spaces (Fig. 8). Such image observed in the casts of small fragments of the vasculature reflects the nodular structure of the tumour observed in the silicone rubber-injected specimens. The avascular nodules, approx. 0.9–1.2 mm in diameter, are surrounded by their own vascular plexuses mainly built of small vessels with globular endings and flattened, distended lacunae. Their surface shows the presence of numerous globular or finger-like outgrowths indicating the angiogenic response (Fig. 9). The vessels, representing altered elements of the peritubular plexus, are interconnected and also communicate with the interposed larger vessels by anastomoses of various calibre.

The inclined surfaces of the avascular spaces that pass into highrising vascular territories are composed of (1) markedly altered capillaries of the peritubular plexus, (2) displaced and deformed interlobular veins, and (3) interlobular arteries, almost unchanged morphologically. The outer surfaces of veins and capillaries are decorated by numerous short vascular sprouts, varying in form and diameter, typical for angiogenesis. They often join each other, forming loops directed towards the avascular space.

The vascular territories extend from the periphery of the tumour, where they fuse with the superficial vascular coat, to its central areas, towards which they are generally directed. They are composed of interlobular, interlobar, as well as distal segments of arcuate veins and arteries. The vessels are displaced and strongly deformed by compression and stretching, densely packed and irregularly arranged. In this region, the veins also show the presence of numerous vascular outgrowths of different shape and diameter. They mostly assume a form of lacunar or sinusoidal dilatations covered by multiple globular swellings or protrusions (Figs. 10, 11). As revealed by systematic analysis of their shape in various tumour areas, these protrusions represent the consecutive stages of a process leading to their fusion. Such fusion can occur not only between two or several closely located protrusions, but also between the protrusion and its own parent vessel (Fig. 11). The subsequent incorporation of these outgrowths into the walls of

the vessels leads to their dilatation and changes in both shape and diameter. Moreover, the outgrowths located on separated but closely running vessels frequently fuse to form anastomoses.

Discussion

The configuration of the vascular network of solid tumours such as renal clear cell carcinoma is influenced by forces generated during their progressive growth. Growing tense tumours push and displace the vascular network by their advancing perimeter, getting their blood supply mainly from the periphery (Falk 1978, 1982) where a superficial vascular coat is formed. The different vascular patterns observed in this study on the anterior and posterior side of the lesion can be explained by the different characteristics of the adjacent tissues. A regular arrangement of blood vessels on the anterior side, facing the peritoneum, results from remodeling of the local renal vasculature, whereas the large vessels observed on the posterior side probably include vasculature derived from renal capsule and the adjacent iliopsoas muscle.

Blood vessels forming the dense superficial vascular coat, as well as their intermediate segments directed towards the center of the tumour, are stretched both tangentially and radially (Falk 1982; Grunt et al. 1986b) and become undulated or contorted. At the same time, the central segments of tumour vessels are compressed perpendicularly to their long axis and stretched radially. This process is induced by growing interstitial pressure and leads to pathological alterations of the endothelial lining observed in this and in other studies (Vaupel and Muller-Klieser 1983; Hammersen et al. 1985; Grunt et al. 1986b). The damage cannot, however, be compensated by the proliferation of endothelial cells (Reinhold and Van der Berg-Blok 1983; Grunt et al. 1986b). In consequence, the blood flow within these vessels decreases, thus causing an irregularly distributed but focal anoxia and ischaemia (Gullino 1982; Vaupel and Muller-Klieser 1983). Within these areas of the tumour, mostly located centrally, foci of necrosis combined with haemorrhagic plaques often occur. Similar necrotic foci, however, can also be encountered in the most richly vascularized regions of the tumour, in the vicinity of the superficial vascular coat, where the formation of new vessels is very strong. It has already been found that angiogenic factor(s) can diffuse in the tissue for a distance of a few millimeters (Folkman 1975) and endothelial cell divisions have been observed only at the tumour edge (Denekamp 1984). We found it remarkable that the tumour itself did not proliferate and regrow into the areas of necrosis (Thompson et al. 1987). Therefore we can presume that angiogenic factor(s) can operate most actively at the periphery of the tumour most probably in cooperation with other biologically active substances such as plasminogen activator, PGE_1 and PGE_2 discharged by activated monocytes/macrophages. The action of such factor(s) within deeper areas of tumour seems to be ineffective most probably due to vascular compression by a high interstitial pressure. It is therefore likely that a diffusible angiogenic factor(s) released at the periphery of renal clear cell carcinoma will exert its effect most effectively on vascular elements located in the vicinity of the tumour. Such territories, not yet invaded by malignant cells will be "prepared" for infiltration by the formation of new vessels.

The vascular bed of renal clear cell carcinoma exhibits a preponderance of venous vessels over arteries, which show no significant morphological alterations (Warren 1979a; Hammersen et al. 1985). Further exponential growth depends to a certain degree however, on the formation of new vessels which appear as incompletely developed, delicate and highly permeable endothelial tubes (Ausprunk 1979; Warren 1979a; Vaupel and Muller-Klieser 1983). This study provids morphological evidence for intense angiogenesis in different areas of the tumour, demonstrating typical vascular outgrowths to be present on cast surfaces of veins and capillaries. These represent a progressive proliferation and fusion of the endothelial elements in the course of formation of new vessels (Grunt et al. 1986a). The angiogenesis-factor-induced proliferation is especially pronounced in stellate veins which drain the superficial half of the renal cortex. They are formed by the distal segments of numerous interlobular and superficial cortical veins collecting fine radicles of the peritubular plexus. These thin-walled vessels, most sensitive to angiogenic stimulation, are transformed and annexed into the 3-dimensional framework of the tumour vascular bed.

The development of markedly dilated vessels, very common in the renal clear cell carcinoma, combined with a subsequent slowing down of blood flow may create favourable conditions for a long-lasting action of angiogenic factor(s) upon endothelial cells (Osteaux and Jeanmart 1979; Warren 1979; Reinhold and Van der Berg-Blok 1983). Hence, the tumour-induced angiogenesis is also responsible for the adaptative changes of vessels and remodelling of the vascular network, as confirmed in our material by the presence of numerous outgrowths observed on cast surfaces of capillaries and veins. These findings imply that further development of solid tumours following formation of new vessels may depend not only on oxygen and nutrient supply, but also on the pres-

ence of an outer endothelial surface covered by basal lamina and organized in a form of a 3-dimensional network permitting malignant cells to adhere and promoting their migration (Vlodavsky and Gospodarowicz 1981; Lacovara et al. 1984). Hence, the perivascular migration of tumour cells at the periphery of the tumour may also be a route of expansion (Vlodavsky and Gospodarowicz 1981; Nicosia et al. 1986; Thompson et al. 1987).

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