Scanning Microscopy

Volume 9 | Number 4 Article 25

9-23-1995

Microvascular Corrosion Casting in the Study of Tumor Vascularity: A Review

M. A. Konerding
Johannes Gutenberg-Universität Mainz

A. J. Miodonski
N. Copernicus Academy of Medicine

A. Lametschwandtner
University of Salzburg

Follow this and additional works at: https://digitalcommons.usu.edu/microscopy

 Part of the Biology Commons

Recommended Citation

Konerding, M. A.; Miodonski, A. J.; and Lametschwandtner, A. (1995) "Microvascular Corrosion Casting in the Study of Tumor Vascularity: A Review," *Scanning Microscopy*: Vol. 9 : No. 4 , Article 25.
Available at: https://digitalcommons.usu.edu/microscopy/vol9/iss4/25

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

Scanning Microscopy, Vol. 9, No. 4, 1995 (Pages 1233-1244) 0891-7035/95$5.00+.25
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

MICROVASCULAR CORROSION CASTING IN THE STUDY OF TUMOR VASCULARITY: A REVIEW

M.A. Konerding[1]*, A.J. Miodonski[2], and A. Lametschwandtner[3]

[1]Inst. of Anatomy, Dept. of Macroscopic Anatomy, Johannes Gutenberg-Universität Mainz, 55099 Mainz, Germany
[2]Department of Otolaryngology, N. Copernicus Academy of Medicine, Kopernika 23a, PL 31-501 Krakow, Poland
[3]Inst. of Zoology, Dept. of Exptl. Zoology, Univ. of Salzburg, Hellbrunnerstr. 34, A-5020 Salzburg, Austria

(Received for publication June 27, 1995 and in revised form September 23, 1995)

Abstract

Tumor blood flow is dependent on the structure and three-dimensional (3-D) architecture of the vascular network. The latter can be best studied by scanning electron microscopy of microvascular corrosion casts. However, literature reviews show that nearly all studies using this technique render comparisons of different tumors more difficult since they are mainly based on descriptive terms that might lead to misunderstandings. Qualitative comparisons of 13 experimental and 3 human primary tumors of different origin show a high degree of similarity in the vasculature. Quantitative analysis of these casts reveals similar ranges of parameters such as diameters, intervascular and interbranching distances. Diameters of vessels with capillary wall structure range from 6 μm to 55 μm in the human primary tumors (renal clear cell carcinoma, basalioma), and from 5 μm to 80 μm in xenografted tumors (sarcomas, colon carcinoma). Intervascular distances in the human primary tumors range from 2 μm to 52 μm, and from 11 μm to 105 μm in the xenografts. Interbranching distances range from 34 μm to 258 μm in the former, and from 11 μm to 160 μm in the latter. Both qualitative and quantitative analyses of tumor microvascular corrosion casts enable pathophysiological conclusions to be drawn and contribute to a better understanding of tumor vascularity.

Key Words: Microvascular corrosion casting, tumor vascularization, tumor angiogenesis, neovascularization, vascular architecture, sprout formation, network formation, scanning electron microscopy, experimental tumor, xenotransplantation.

*Address for correspondence:
Moritz A. Konerding,
Anatomisches Institut, Abteilung für Makroskopie,
Johannes Gutenberg-Universität Mainz,
Becherweg 13,
55099 Mainz, Germany
Telephone number: (49) 6131-392549
FAX number: (49) 6131-394710

Introduction

The importance of the blood vessel system in solid tumors for their growth beyond 1-2 mm^3, i.e., a clinically relevant size, is generally accepted (Folkman, 1985). This has given rise to an increasing interest in this system as a direct target for tumor therapy, i.e., vascular targeting (Denekamp, 1984; Folkman, 1972). Furthermore, its importance as a route for delivery of anticancer drugs (chemo- and immunotherapies) or photosensitizers (photodynamic laser therapy), as well as its modulatory influences on radiotherapy and hyperthermia, the former greatly depending on the amount of oxygen available, the latter on heat transfer, are evident.

The tumor blood vessels derive from two sources, i.e., pre-existing host vessels, and newly formed ones from tumor angiogenesis (neoangiogenesis). Tumor angiogenesis is defined as the growth of new vessels from pre-existing (mature) ones, exclusively from capillaries and/or venules, by means of sprouting as well as dilatation and elongation of established mature and newly evolved immature ones.

For selected reviews on the role of endothelial cells, extracellular matrix (ECM) components and tumor cells in tumor angiogenesis, see Carey (1991); Fajardo (1989); Folkman (1985); and Folkman and Shing (1992); Klagsbrun and D'Amore (1991).

The vasculature in solid tumors is most frequently studied using the transparent rabbit ear chamber, the hamster cheek-pouch, rabbit cornea, and the chick chorioallantoic membrane (CAM) assays (for reviews see Auerbach *et al.*, 1991; Folkman, 1985; Peterson, 1979). These allow for intravital light microscopy and enable detailed analyses of sprouting events to be made like in immature tissue (Rhodin and Fujita, 1989). Despite the tumor biological and therapeutic implications, studies on primary tumors and comparisons of different tumor models, which confirm their reliability and validity, are still scarce.

Numerous studies on the energy metabolism of solid tumors (Vaupel *et al.*, 1987, 1989) have pointed out the functional importance of the blood vessel system and

Tables 1a and 1b. Vascular corrosion cast/SEM studies on solid tumors. **Table 1a.** Carcinomas.

Tumor type	Grown in	Location	Exp. (e) / Spont. (s)	Reference(s)
Squamous cell carcinoma (pubic)	mice	rump	e; tumor cell inoculation	Egawa and Ogata, 1979
human squamous cell carcinoma (head,neck)	nude mice	anterior axillary line	e, xenotransplanted excised human tissue slices	Konerding, 1990; Steinberg *et al.*, 1990
Ehrlich ascites carcinoma	mice	rump	e, tumor cell inoculation	Egawa and Ogata, 1979
Carcinoma	rats	urinary bladder	e, chemically induced	Tatematsu *et al.*, 1978, 1984; Cohen *et al.*, 1980
Carcinoma	Syrian hamster	cheek-pouch	e, chemically induced	Lurie *et al.*, 1983
Carcinoma	rat	colon	e, chemically induced	Skinner *et al.*, 1990
Carcinoma	man	larynx	s	Miodonski *et al.*, 1980, 1992; Kus *et al.*, 1981
Basalioma	man	skin	s	Staindl and Lametschwandtner, 1981
Renal clear cell carcinoma	man	kidney	s	Bugajski *et al.*, 1989; Miodonski *et al.*, 1992
B-16 murine melanoma	mice	right flank, skin	e, tumor cell inoculation	Walmsley *et al.*, 1987
Human amelanotic melanoma	nude mice	anterior axillary line	e, excised human tumor tissue slices	Konerding *et al.* 1989b, 1989c, 1989d; Konerding, 1990
Human melanoma	nude mice	anterior axillary line	e, excised human tumor tissue slices	Konerding and Steinberg, 1989; Konerding, 1990
Colonic adenocarcinoma	man	colon	s	Gaudio *et al.*, 1982
Human colonic adenocarcinoma (LS174T)	nude rat	knee, hind leg, skin	e, tumor cell inoculation	Ahlström *et al.*, 1988; Christofferson and Nilsson, 1992
Human colonic adenocarcinoma (LS174T)	nude rat	endometriu m	e, tumor cell inoculation	Christofferson and Nilsson, 1992
Adenocarcinoma	rat	e, kidney	excised human tumor tissue slices xenotranspl.	Shah-Yukich and Nelson, 1988
Lewis lung carcinoma	mice	axillary region	e, tumor cell inoculation	Grunt *et al.*, 1986a, 1986b; Grunt, 1992

stress the need for further thorough investigations of its functions in terms of transport capacities for nutrients, oxygen, catabolite removal, delivery of therapeutic substances, and heat transfer.

Blood flow, in physiological terms, as a limiting parameter for tumor energy metabolism and thus tumor growth is basically defined by physical constraints, i.e., the construction of the vascular network. The way the blood vessels are formed (i.e., resulting geometry), their dimensions, together with their wall composition and structure, as well as intravascular and extravascular conditions finally determine the efficiency of the system.

However, at least partly due to the limitations of the methods used, our knowledge of these architectonic terms is still limited. This is, intravital microscopy is effective in the observation of two-dimensional vascular networks as given in most angiogenesis assay systems. However, it cannot visualize the tremendous hetero-

Table 1b. Sarcomas.

Tumor type	Grown in	Location	Exp. (e) / Spont. (s)	Reference(s)
Ascites hepatoma (7924)	rat	trunk	e, tumor cell inoculation	Egawa and Ogata, 1979
Ascites hepatoma (109A)	rat	trunk	e, tumor cell inoculation	Egawa and Ogata, 1979
VX-2 tumor	rabbit	ear	e, tumor cell inoculation	Maruhashi, 1984
Angiosarcoma	syrian hamster	liver	e, chemically induced	Malick and Toth, 1977
VX-2 tumor	rabbit	muscle, hind leg	e, tumor cell inoculation	Shah-Yukich and Nelson, 1988;
Human leiomyosarcoma (8 different types)	nude mice	anterior axillary line	e, xenografted human tumor tissue slices	Konerding *et al.*, 1989a, 1989b, 1989c; Konerding, 1990, 1991
Human neurofibrosarcoma	nude mice	anterior axillary line	see above	Konerding, 1990
Human liposarcoma	nude mice	anterior axillary line	see above	Konerding, 1990

geneity of the tumor vascular system found even in different areas of the same tumor. Classical injection methods (Spalteholtz, 1914) with light microscopic evaluation are time-consuming, require laborious reconstruction work and are still of limited value, unless sophisticated computerized techniques are used.

In view of these considerations, we consider scanning electron microscopy (SEM) of microvascular corrosion casts (Murakami, 1971) to be a powerful method providing information on the geometry of the tumor vascular system in terms of a network defined primarily by the number of vessels, their branching behaviour (modes, angles, frequencies, and interbranching distances), and their characteristic course as a whole.

During its twenty years of application, the microvascular corrosion cast/SEM method has proven to be an extensive, powerful technique to convincingly document the three-dimensional (3-D) arrangement of the vascular system with (a) a high depth of focus, (b) a reasonably high resolution allowing visualization of the microvascular bed as well as the reliable differentiation between arteries and veins (Miodonski *et al.*, 1976), and (c) minimal risks in producing artifacts (Christofferson and Nilsson, 1988, 1992; Gannon, 1978; Hodde and Nowell, 1980; Hodde *et al.*, 1990; Konerding, 1991; Lametschwandtner *et al.*, 1989; Miodonski *et al.*, 1981; Murakami *et al.*, 1983).

Several studies using scanning electron microscopy of microvascular corrosion casts have shown that this method is suitable for studies of blood vessel development both in physiological vascular systems, e.g., the uterus or the placenta (Kaufmann *et al.*, 1985) and in tumors (Christoffersson and Nilsson, 1992).

However, until now, published work on the tumor vascular system as revealed by the vascular corrosion cast/SEM method is mainly descriptive. The terms used are subjective, qualitative, and sometimes prone to misunderstandings and misinterpretations.

This situation underlines the need for a more objective, quantitative evaluation of the solid tumor vasculature. Quantitative vascular casting studies are done both at the light microscopic level (Anderhuber *et al.*, 1989; Less *et al.*, 1991) and at the SEM level (Hossler and West, 1988; Kratky *et al.*, 1989; Lametschwandtner *et al.*, 1989; Zeindler *et al.*, 1989).

Therefore, the aim of the present study is (a) to document the studies performed using microvascular corrosion casting/SEM of different tumors, (b) to review descriptive terms used in these studies to characterize the structure of the tumor vascular bed, and (c) to highlight vascular structures common to or different in distinct tumor types.

Materials and Methods

This study is based on previous work carried out by us (Bugajski *et al.*, 1989; Grunt *et al.*, 1985, 1986a, 1986b; Konerding, 1990; Konerding and Steinberg, 1987, 1989; Konerding *et al.*, 1989a, 1989b, 1989c, 1989d; Kus *et al.*, 1981; Miodonski *et al.*, 1980, 1981; Staindl and Lametschwandtner, 1981; Steinberg *et al.*, 1990) and others. In total, 15 different types of carcinomas and 14 different types of sarcomas were considered (see Table 1).

Table 2. Listing of parameters able to be quantified.

Parameters to be quantified	Descriptive terms / attribute
Diameter	thin, thick, small, great, widened, small-sized, medium-sized, delicate, dilated, constricted, flattened, varying, differing
Length	short, long, irregular, stretched, extended
Surface	smooth, rough, ruffled, wrinkled, irregular, varicose
Endothelial cell (nuclei) imprints	oval, ovoid, fusiform, shallow, deep, round, irregular, numerous, scattered
Volume	voluminous
Cross section (area, perimeter, shape factor)	no reports
Branching	often, seldom, rare, multiple, frequent
- mode	bifurcated, trifurcated
- angle	acute, oblique
- interbranching distance	short, long, variable, constant
- order	low, high, few, several
Parameters partially to be quantified:	
Course	sinus wave-like, undulating, wave-like, wavy, winded, tortuous, helix-like, spirally, spirally-twisted, spiralled, corkscrew-like
Mesh shape	regular, irregular, polygonal, ovoid, rectangular, square
- size	small, large, medium
Number of vessels composing the mesh	
Parameters not to be quantified:	
Course	riverine, meandering, irregular, chaotic, abnormal
Looping	short, long, twisted, convoluted

Additionally, the available literature on microvascular corrosion casting of tumor vessels was reviewed.

The methods used for microvascular corrosion casting/SEM studies have been described in detail previously (Grunt *et al.*, 1986a, 1986b; Konerding *et al.*, 1989b; Lametschwandtner *et al.* 1989; Miodonski *et al.*, 1989).

Results

Microvascular corrosion casting/SEM studies of tumor vascular patterns. Which tumors are studied?

Table 1 shows that only five spontaneously occurring human tumors (larynx carcinoma, renal clear cell carcinoma, basalioma of skin, rectal carcinoma, and glioma; see references cited in Table 1) were studied. Most work, however, has involved experimental tumors, i.e., either chemically induced, inoculated or xenografted ones of various origin. This clearly underlines the necessity of further studies on spontaneous human tumors.

Descriptive terms

Descriptive terms used in SEM studies of cast tumor blood vessels characterize vessels at three levels: (a) at the level of the individual vessel, (b) vascular "unit", and (c) three-dimensional network (vascular pattern, feature) resulting from the combination of (a) and (b). At all levels, terms may either emphasize a static (anatomical) or dynamic (physiological) aspect.

In this context, an individual vessel is defined as belonging to one of the following categories: artery, arteriole, capillary, venule, vein, sinusoid (for classification, see, Rhodin, 1974; for luminal cast classification, see, Miodonski *et al.*, 1976, 1981) running freely over a given length including its first branching. Several

individual vessels form "vascular units", whereby each consists at least of a feeding arteriole, capillaries and a draining venule. Vascular units finally form the 3-D network.

SEM analysis of cast tumor vasculature predominantly uses descriptive terms for these three levels of vessel network (see Table 2, left column), although this method allows numerous parameters to be quantified (see Table 2, right column) leading to a more objective assessment of tumor vascular systems. The examples listed in Table 2 (left column) show that most qualitative terms used and most studies do not contribute to a better understanding and definition of the tumor vascular system but, rather, enhance confusion. Above all, these subjective terms render comparisons of different tumor vascular systems more difficult and do not significantly contribute to vascular network analysis.

Comparison of vascular features of carcinomas (CAs) and sarcomas (SAs)

Vascular corrosion casts of spontaneous human renal clear cell carcinoma and basalioma (skin) as well as human squamous cell carcinoma and sarcoma xenografts (soft tissue, mostly leiomyosarcomas) share the following common features:

(a) **Plexuses of flatly orientated vessels with abrupt changes in diameter and blind endings (Figs. 1a-1c)**: These plexuses predominantly border avascular tumor areas. They consist of frequently wide venules and sinusoidal capillaries with diameters varying between 6 and 55 μm in human primary tumors (renal clear cell carcinoma, basalioma) and from 5 to 80 μm in xenografts (colon carcinomas and sarcomas).

Venules and sinusoidal capillaries are intensively interconnected. Intervascular distances range from 2 and 52 μm (primary tumors) and from 11 μm to 105 μm in the xenografted tumors. The interbranching (joining) distances ranged from 11 to 258 μm. It should be noted, however, that diameters, and intervascular and interbranching distances mainly lie within the lower dimensional ranges.

In summary, vascular plexuses are found in all tumors studied, i.e., carcinomas and sarcomas. They show only negligible qualitative and quantitative differences, making a differentiation between carcinomas and sarcomas based on this feature impossible.

(b) **Vessel outpouchings (Figs. 2a-2c)**: These lead to an increased (luminal) surface area in any case. Depending on the location of the particular vessel in which these outpouchings are found, they may contribute to at least two phenomena: (i) elongation of parent vessels and/or sprouting, or (ii) enlargement of vessel diameter (perimeter, surface, volume). Both lead to adaptive changes in pre-existing and newly formed vessels and permanently remodulate the tumor vascular bed.

Discussion

There is a strong need for a detailed knowledge of tumor vascularization since the vascularity determines, amongst others, both the blood flow and tumor biology as well as the efficacy of therapeutic measures, e.g., radiation therapy or hyperthermia. However, little is known about the 3-D architecture of tumor vascularity. In this context, microvascular corrosion casting is a superior method compared to other morphological techniques (Konerding, 1991; Lametschwandtner *et al.*, 1989).

Literature searches show that there are already numerous papers dealing with SEM of tumor vascular corrosion casts. However, most of these studies were carried out on a subjective, descriptive level (cf Table 1). Quantitative data are almost entirely missing although measurements of vessel (luminal) diameters, lengths and diameters of endothelial cell nuclei imprints are feasible. Even data such as branching angles, the most important parameter for 3-D networks, are missing. Thus, comparisons of different tumor vascular systems, e.g., that of an experimental tumor with that of a spontaneous human tumor are nearly impossible or, at best, restricted to a subjective basis. Such comparisons are of importance in order to determine to what extent results of experimental studies on tumor vascularity can be extrapolated to other models or even to the human primary tumor.

On the other hand, literature reviews reveal a lack of studies on the 3-D appearance of tumor vascularity in human primary tumors. The only exceptions are the reports by Bugajski *et al.* (1989), Grunt *et al.* (1985) and Miodonski *et al.* (1980).

Another scope of this study was to compare both descriptive findings as well as quantitative data of different tumor vascular systems. From our results, it becomes evident that there is no tumor type specific vascular bed (Lewis, 1927); at least not in the carcinomas and sarcomas considered here. From our findings and those of others (Cohen *et al.*, 1980; Lurie *et al.*, 1983; Tatematsu *et al.*, 1978, 1984) we conclude that (a) the location of the tumor, (b) the biology of the particular tumor cell types (carcinoma, basalioma, melanoma, leiomyosarcoma as diagnosed by histopathology), and (c) the organo- and tissue-typical organization of the preexisting vascular bed together define the individual vascular bed. Thus, the tumor vascular bed arises as a result of multiple interactions between tumor properties

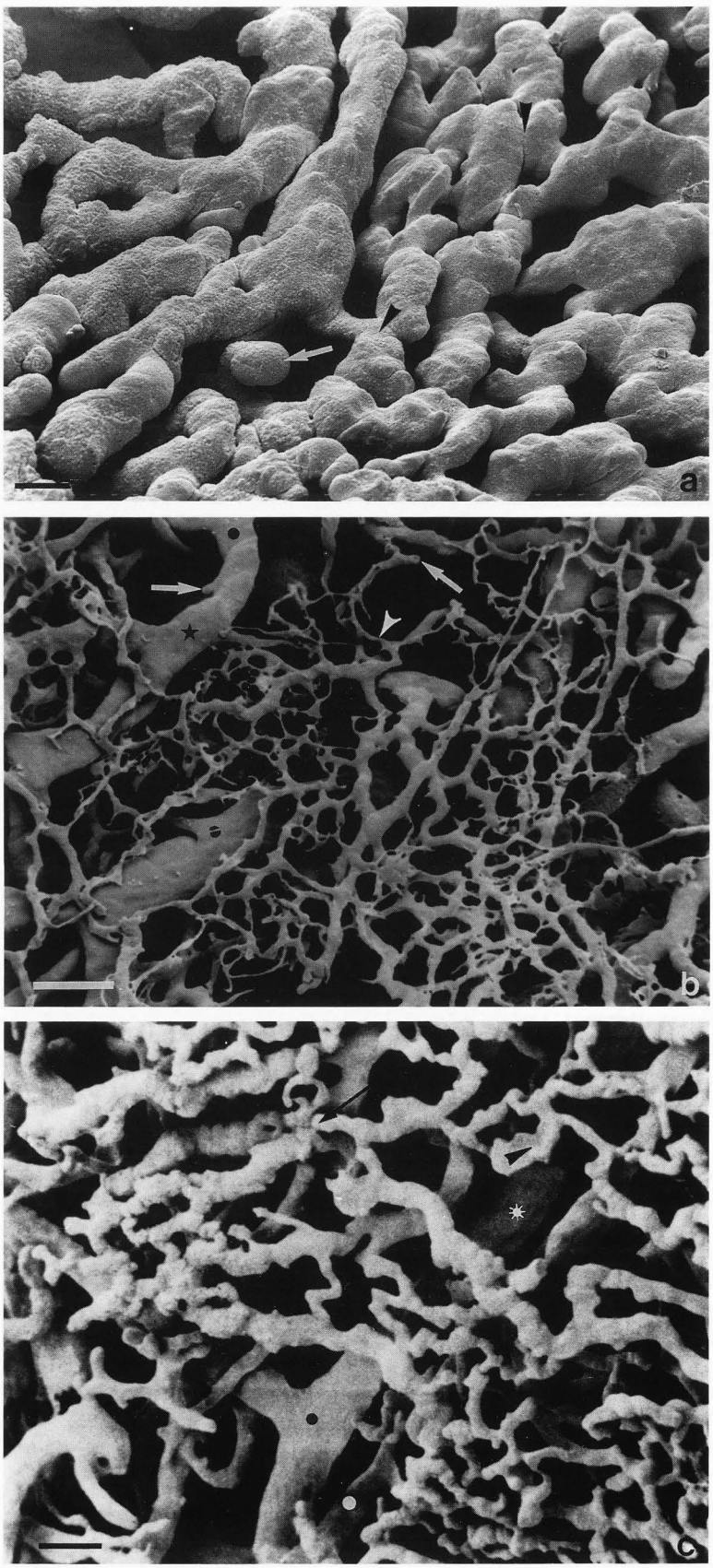

Figure 1. Architecture of tumor sinusoids: comparison of vascular networks in human primary (**a**), human xenografted (**b**), and experimental tumors (**c**). Microvascular corrosion casts. Note the multiform, partially multilayered plexus-like arrangement of the sinusoidal vessels with varying diameters (arrowheads) and numerous blind ends (arrows). In the background feeding and draining, respectively, venous vessels (asterisks) with typical flattenings (circles). Also note the architectural similarity of these plexuses in terms of lacking hierarchy and varying diameters in tumors of different origin. (**a**) Human larynx carcinoma; bar = 50 μm. (**b**) Xenograft of human undifferentiated head-neck-cancer on nude mouse, 15 days p.t.; bar = 150 μm. (**c**) Lewis lung carcinoma on rat, 20 days after inoculation; bar = 150 μm.

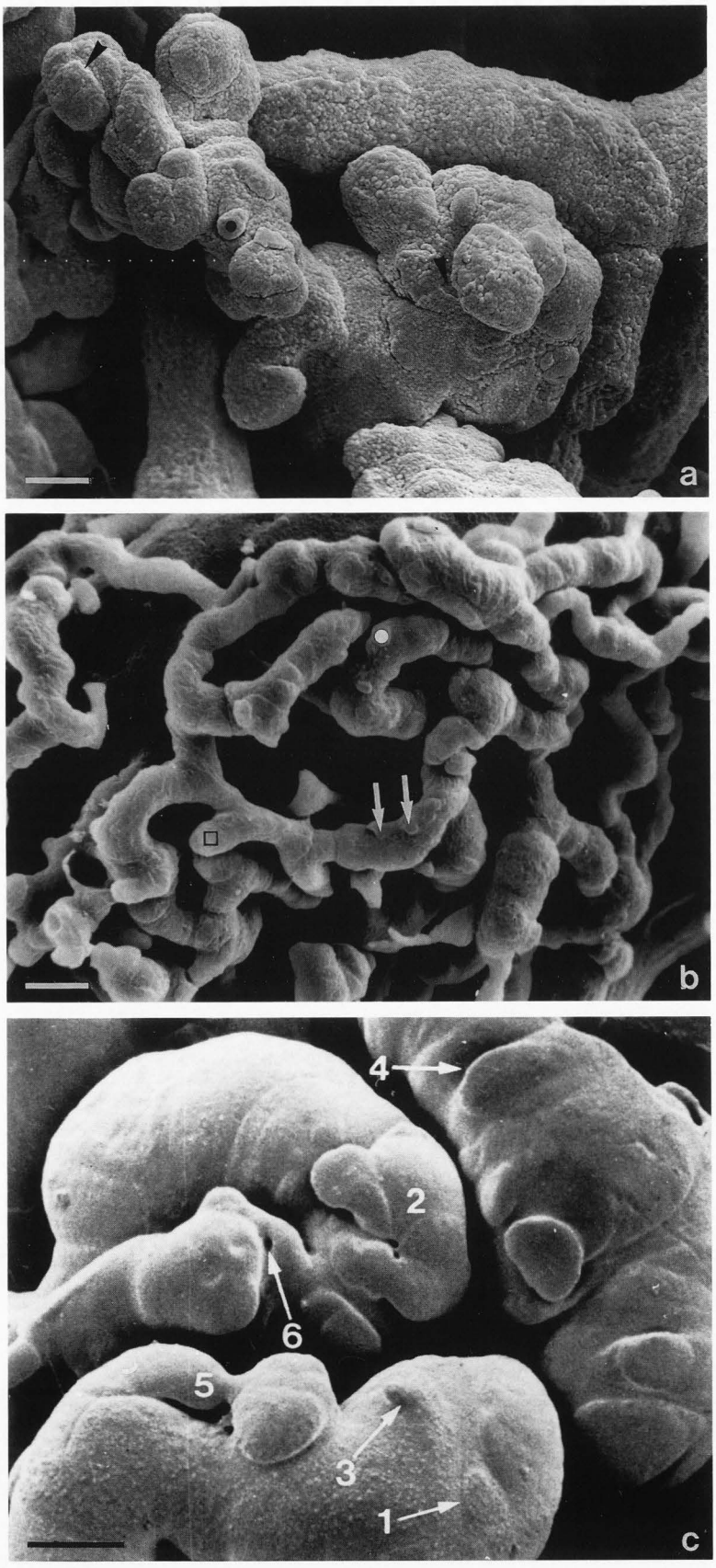

Figure 2. Angiogenic features and remodelling of the tumor vascularity as seen in microvascular corrosion casts of a human primary (**a**), human xenografted (**b**), and experimental tumor (**c**). Note the common appearance of furrows (arrowheads, 1 and 4 in **c**), flaps (arrows, 2 in **c**), bulges (asterisks, 3 in **c**) and fungiform protrusions (circles) indicating early steps of new vessel stretch formation and collateral formation (△ in **b**, 5 in **c**). Apparently, these features can also be involved in vascular diameter enlargement. Note the hole (6) between parent vessel and collateral. (**a**) Nude mouse, 15 days p.t.; bar = 50 μm. (**b**) Xenograft of human undifferentiated head-neck-cancer on nude mouse, 15 days p.t.; bar = 50 μm. (**c**) Lewis lung carcinoma on rat, 18 days after inoculation; bar = 20 μm.

(production of tumor angiogenesis factors, TAFs; metabolism, immune surveillance etc.), nature of the pre-existing host blood vessels (quantity and quality, arrangement, differences in endothelial cell biology etc.), and the behaviour of the extracellular matrix (biochemical and biophysical properties).

The finding that carcinomas and sarcomas, irrespective of whether they occur spontaneously (as cancer of larynx, renal clear cell carcinoma, and basalioma of skin) or are experimentally induced by xenografting (in the case of colon carcinoma, melanoma, and human leiomyosarcomas), or if they are xenotransplanted to the same location (right axillary line behind forelegs), or exposed to (spontaneous human carcinomas) or protected from host immune responses (xenotransplants), share very similar vascular features, e.g., vascular plexuses made up of vessels with capillary wall structure and wide diameters, are in line with this and agree with the statements of Vaupel and Gabbert (1986). The described lack of architectonic characteristics parallels the finding of a lack of structural, tumor-type specific characteristics of different tumor vascular systems (Konerding *et al.*, 1992a, 1992b).

In our opinion the quantitative measurements of vessels of the flat plexuses bordering avascular tumor areas (Figs. 1a-1c) reflect a real situation. A distension of these vessels by the casting procedure is unlikely to occur (Grunt *et al.*, 1986a).

The risk of artifactual, incomplete filling of the blood vascular system is the most often mentioned contention against the use of this method. This problem can possibly be augmented by arteriovenous anastomoses and, above all, by partially or completely compressed vessels that may no longer be pervious to the casting medium. Such compressions can, for instance, be caused by high interstitial pressures (Vaupel and Gabbert, 1986). Thus, morphometry of tumor corrosion casts might indeed lead to a too low a vascular density; however, those compressed or occluded vessels, that were not cast, cannot be of any nutritive importance. Vice versa, cast vessels do not imply a guarantee of functionality but, instead, only the pre-requisite for a nutritive blood flow.

The lack of nutritive blood flow in distinct tumor areas with high vascular densities can be explained in terms of capillary elongations, insufficient numbers of supplying arteries and the frequent occurrence of venovenous sinusoidal systems (Konerding *et al.*, 1989a; 1992b). This morphologically determined insufficient nutritive blood flow, despite high vascular densities and surprisingly low intercapillary distances, explains the missing correlation of vascular density to necroses previously described (Steinberg *et al.*, 1991).

Summing up, our studies have shown that SEM of tumor microvascular corrosion casts may yield more qualitative and quantitative information on architectural and pathophysiological properties of the tumor vascular system than has been published up to now.

References

Ahlström H, Christofferson R, Loerelius LE (1988) Vascularization of the continuous human colonic cancer cell line LS 174 T deposited subcutaneously in nude rats. APMIS (Acta Pathol Microbiol Immunol Scand) **96**, 701-710.

Anderhuber F, Weiglein A, Pucher RK (1989) Ein Beitrag zur Hirndurchblutung des Menschen anhand von Gefäßausgüssen des Karotissystems (A contribution to the human cerebral blood flow based on vascular casts of the carotis system). Acta Anat. **136**, 42-48.

Auerbach R, Auerbach W, Polakowski I (1991) Assays for angiogenesis: a review. Pharmacol. Ther. **51**, 1-11.

Bugajski A, Nowogrodzka-Zagorska M, Lenko, J, Miodonski AJ (1989) Angiomorphology of the human renal clear cell carcinoma. A light and scanning electron microscopic study. Virchows Archiv A Pathol. Anat. **415**, 103-113.

Carey DJ (1991) Control of growth and differentiation of vascular cells by extracellular matrix proteins. Annu. Rev. Physiol. **53**, 161-177.

Christofferson RH, Nilsson BO (1988) Angiogenesis in a human cancer growing in the nude rat endometrium. In: Angiogenesis as Induced by Trophoblast and Cancer Cells. Ph.D. Thesis. University of Uppsala, Sweden. pp. 73-81.

Christofferson RH, Nilsson BO (1992) Microvascular corrosion casting in angiogenesis research. In: Scanning electron Microscopy of Vascular Casts: Methods and Applications. Motta PM, Murakami T, Fujita H (Eds.). Kluwer Academic Publ., Boston. pp. 27-37.

Cohen SM, Tatematsu M, Shinohara Y, Nakanishi K, Ito N (1980) Neovascularization in rats during urinary bladder carcinogenesis induced by N-(4-5-nitro-2-furyl)-2-thiazoyllformamide. J. Natl. Cancer Inst. **65**, 145-148.

Denekamp J (1984) Vascular endothelium as the vulnerable element in tumors. Acta Radiol. Oncol. **23**, 217-225.

Egawa J, Ogata T (1979) Vascular structure of experimental tumors. Appearances in scanning electron microscope. Acta Radiol. Oncol. **18**, 367-379.

Fajardo LF (1989) The complexity of endothelial cells. A review. Am. J. Clin. Pathol. **92**, 241-250.

Folkman J (1972) Anti-Angiogenesis: New concept for therapy of solid tumors. Ann. Surg. **175**, 409-416.

Folkman J (1985) Tumor angiogenesis. Adv. Cancer Res. **43**, 175-203.

Folkman J, Shing Y (1992) Angiogenesis. J. Biol. Chem. **267**, 10931-10934.

Gannon BJ (1978) Vascular casting. In: Principles and techniques of scanning electron microscopy. Biological Applications. Vol. 6. Hayat MA (eds.). Van Nostrand Reinhold, New York. pp. 170-193.

Gaudio E, Pappalardo G, Pannarale L, Pintucci S, Resta S, Ripani M (1982) Tridimensional observations (by S.E.M.) on the microcirculation of adenocarcinoma of the recto-sigmoid junction in man. Ital. J. Surg. Sci. **12**, 221-225.

Grunt TW (1992) The fundamental vascular structures in epithelial cancer demonstrated in an experimental lung carcinoma. In: Scanning Electron Microscopy of Vascular Casts: Methods and Applications. Motta PM, Murakami T, Fujita H (Eds.). Kluwer Academic Publ., Boston. pp. 331-344.

Grunt TW, Lametschwandtner A, Staindl O (1985) The vascular pattern of basal cell tumors: Light microscopy and scanning electron microscopic study on vascular corrosion casts. Microvascular Res. **29**, 371-386.

Grunt TW, Lametschwandtner A, Karrer K, Staindl O (1986a) The angioarchitecture of the lewis lung carcinoma in laboratory mice. Scanning Electron Microsc. **1986**; II, 557-573.

Grunt TW, Lametschwandtner A, Karrer K (1986b) The characteristic structural features of the blood vessels of the lewis lung carcinoma. Scanning Electron Microsc. **1986**; II, 575-589.

Hodde KC, Nowell JA (1980) Übersicht über Mikromazerations-Innenabdrücke für die Rasterelektronenmikroskopie (Review of imprints of intervascular casts for SEM). Beitr. elektronenmikrokop. Direktabb. Oberfl. **13**, 295-316.

Hodde KC, Steeber DA, Albrecht RM (1990) Advances in corrosion casting methods. Scanning Microsc. **4**, 693-704.

Hossler FE, West RF (1988) Venous valve anatomy and morphometry: Studies on the ducklet using vascular corrosion casting. Amer. J. Anat. **181**, 425-432.

Kaufmann P, Bruns U, Leiser R, Luckhardt M, Winterhager E (1985) The fetal vascularisation of term human placental villi. II Intermediate and terminal villi. Anat. Embryol. Berl. **173**, 203-214.

Klagsbrun M, D'Amore PA (1991) Regulators of angiogenesis. Annu. Rev. Physiol. **53**, 217-239.

Konerding MA (1990) Das Gefäßsystem xenotransplantierter humaner Tumoren. Deskriptiv-morphologische und experimentelle Untersuchungen (The vascular system of xenotransplanted human tumors. Descriptive morphological and experimental studies). Habilitationsschrift (Ph.D. thesis). Univ. of Essen, Germany. pp. 1-217.

Konerding MA (1991) Scanning electron microscopy of corrosion casting in medicine. Scanning Microsc. **5**, 851-865.

Konerding MA, Steinberg F (1987) Die Morphologie von Xenotransplantaten humaner Melanome und Sarkome - REM-Untersuchungen unter besonderer Berücksichtigung der Gefäßverteilung (The morphology of xenotransplanted melanomas and sarcomas - SEM studies with special references to the vascular distribution). Beitr. Elektronenmikr. Direktabb. Oberfl. **20**, 281-288.

Konerding MA, Steinberg F (1989) Scanning and transmission electron microscopic studies on the vascular system of xenotransplanted human tumours on nude mice. In: Cells and Tissues: A Three-Dimensional Approach by Modern Techniques in Microscopy. Progress in Clinical and Biological Research, Vol. 295. Motta PM (ed.). Alan R. Liss, New York. pp. 475-480.

Konerding MA, Steinberg F, Streffer C (1989a) Ultrastructural studies on the vascular system of untreated and hyperthermia treated xenotransplanted tumors. In: Hyperthermic Oncology. Sugahara T, Saito M (eds.). Taylor & Francis, New York. pp. 344-346.

Konerding MA, Steinberg F, Budach V (1989b) The vascular system of xenotransplanted tumors: Scanning and light microscopic studies. Scanning Microsc. **3**, 327-336.

Konerding MA, Steinberg F, Streffer C (1989c) The vasculature of xenotransplanted human melanomas and sarcomas on nude mice. I. Vascular corrosion casting studies. Acta Anat. **136**, 21-26.

Konerding MA, Steinberg F, Budach V, Steffens C (1989d) Scanning electron microscopic studies on the vascular system of xenotransplanted tumours. Strahlenther. Onkol. **165**, 503-504.

Konerding MA, van Ackern C, Hinz S, Steinberg F, Streffer C (1991) Ultrastructural studies of tumour angiogenesis in human xenotransplanted tumours. Int. J. Radiat. Biol. **60**, 49-53.

Konerding MA, Steinberg F, van Ackern C, Budach V, Streffer C (1992a) Comparative ultrastructural studies of the vascularity in different human xenografted tumours. In: Contributions to Oncology, Vol. **42**: Immunodeficient Mice in Oncology. Fiebig HH, Berger DP (eds.). Karger, Basel, Switzerland. pp. 169-179.

Konerding MA, van Ackern C, Steinberg F, Streffer C (1992b) Combined morphological approaches in the study of network formation in tumor angiogenesis. In: Angiogenesis. Key Principles - Science - Technology - Medicine. Steiner R, Weisz PB, Langer R (eds.). Birkhäuser, Basel. pp. 40-51.

Kratky RG, Zeindler CM, Lo DKC, Roach MR (1989) Quantitative measurement from vascular casts. Scanning Microsc. **3**, 937-943.

Kus J, Miodonski A, Olszewski E, Tyrankiewicz R (1981) Morphology of arteries, veins, and capillaries in cancer of the larynx, SEM study on microcorrosion casts. J. Cancer Res. Clin. Oncol. **100**, 271-283.

Lametschwandtner A, Weiger T, Bernroider G (1989) Morphometry of corrosion cast. In: Cells and Tissues: A Three-dimensional Approach by Modern Techniques in Microscopy. Progress in Clinical and Biological Research, Vol. 295. Motta PM (ed.). Alan R. Liss, New York. pp. 427-433.

Less JR, Skalak TC, Sevick EM, Jain RK (1991) Microvascular architecture in an mammary carcinoma: Branching patterns and vessel dimensions. Cancer Res. **51**, 265-273.

Lewis WH (1927) The vascular patterns of tumours. Johns Hopkins Bull. **41**, 156-175.

Lurie AG, Tatematsu M, Nakatsuka T, Rippey RM, Nobuyuki I (1983) Anatomical and functional vascular changes in hamster cheek pouch during carcinogenesis induced by 7, 12-dimethylbenz(a)anthracene. Cancer Res **43**, 5986-5994.

Malick L, Toth B (1977) Injection replication of the vasculature of angiosarcomas in liver of Syrian golden hamster. Scanning Electron Microsc. **1977**; II, 533-540.

Maruhashi K (1984) Changes of the tumor vessels following intra-arterial infusion therapy using a combination of anti-cancer agents and dexamethasone. Nippon Geka Hokan **53**, 193-212.

Miodonski A, Hodde KC, Bakker C (1976) Rasterelektronenmikroskopie von Plastik-Korrosions-Präparaten, morphologische Unterschiede zwischen Arterien und Venen (Scanning electron microscopy of plastic corrosion casts: Morphological differences between arteries and veins). Beitr. Elektronenmikroskop. Direktabb. Oberfl. **9**, 435-442.

Miodonski A, Kus J, Olszewski E, Tyrankiewicz R (1980) SEM studies on blood vessels in cancer of the larynx. Arch. Otolaryngol. **106**, 321-332.

Miodonski A, Kus J, Tyrankiewicz R (1981) SEM blood vessels casts analysis. In: Three-Dimensional Microanatomy of Cells and Tissue Surfaces. Didio JA, Motta PM, Allen DJ (eds.). Elsevier, Amsterdam, Netherlands. pp. 71-87.

Miodonski A, Kus J, Bär T (1989) Application of the corrosion casts method to the anuran eye and mammalian cochlea. In: Cells and Tissues: A Three-Dimensional Approach by Modern Techniques in Microscopy. Progress in Clinical and Biological Research. Vol. 295, Motta PM (eds.). Alan R. Liss, New York. pp. 527-535.

Miodonski AJ, Kus J, Nowogrodzka-Zagorska M, Olzewski J, Bugajski A (1992) Angiomorphology of the human larynx and renal carcinoma. In: Scanning Electron Microscopy of Vascular Casts: Methods and Applications. Motta PM, Murakami T, Fujita H (eds.). Kluwer Academic Publ., Boston. pp. 345-362.

Murakami T (1971) Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. histol. Jap. **32**, 445-454.

Murakami T, Ohtani O, Ohtsuka A, Kikuta A (1983) Injection replication and scanning electron microscopy of blood vessels. In: Biomedical Research: Applications of Scanning Electron Microscopy, Vol. 3. Hodges GM, Carr KE (eds.). Academic Press, London, UK. pp. 1-30.

Orita T, Nishizaki T, Kamiryo T, Aoki H, Harada K, Okamura T (1988) The microvascular architecture of human malignant glioma. A scanning electron microscopic study of vascular cast. Acta Neuropathol. **76**, 270-274.

Peterson HI (1979) Tumor blood flow compared with normal tissue blood flow. In: Tumor Blood Circulation: Angiogenesis, Vascular Morphology and Blood Flow of Experimental and Human Tumors. Peterson HI (ed.). CRC-Press, Boca Raton, FL. pp. 103-114.

Rhodin JAG (1974) Histology. A Text Atlas. Oxford University Press, Oxford, U.K. p. 331-369.

Rhodin JAG, Fujita H (1989) Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analysis. J. Submicros. Cytol. Pathol. **21**, 1-34.

Shah-Yukich AA, Nelson AC (1988) Methods in laboratory investigation. Characterization of solid tumor microvasculature: A three-dimensional analysis using the polymer casting technique. Lab. Invest. **58**, 236-244.

Skinner SA, Tutton PJM, O'Brian PE (1990) Microvascular architecture of experimental colon tumors in the rat. Cancer Res. **50**, 2411-2417.

Spalteholtz W (1914) Über das Durchsichtigmachen von menschlichen und tierischen Präparaten nebst Anhang über Knochenfärbung (How to prepare transparent human and animal specimens, with supplement on bone staining). Second revised edition. Hirzel, Leipzig. pp. 2-37.

Staindl O, Lametschwandtner A (1981) Die Angioarchitektur solid-zystischer Basaliome. Eine rasterelektronenmikroskopische Untersuchung an Korrosionspräparaten (The angioarchitecture of solid cystre basaliomes. A scanning electron microscopy study on corrosion casts). Hals-Nasen-Ohrenheilk. **29**, 112-117.

Steinberg F, Konerding MA, Streffer C (1990) The vascular architecture of tumors: histological, morphometrical and ultrastructural studies. J. Cancer Res. Clin. Oncol. **116**, 517-524.

Steinberg F, Konerding MA, Sander A, Streffer C (1991) Vascularisation, proliferation and necrosis in untreated human primary tumours and untreated human xenografts. Int. J. Rad. Biol. **60**, 161-168.

Tatematsu M, Cohen SM, Fukushima S, Shirai T, Shinihara Y, Ito N (1978) Neovascularization in benign and malignant urinary bladder epithelial proliferative lesions of the rat observed *in situ* by scanning electron microscopy and autoradiography. Cancer Res. **38**, 1792-1800.

Tatematsu M, Lurie AG, Rippey RM, Conran PB, Arai M, Fukushima S, Ito N (1984) Possible mechanistic roles of anatomical and functional vascular changes in rat urinary bladder carcinogenesis induced by N-butyl-N-(4-hydroxybutyl) nitrosamine. Cancer Res. **44**, 278-284.

Vaupel P, Gabbert H (1986) Evidence for and against a tumor type specific vascularity. Strahlenther. Onkol. **162**, 633-638.

Vaupel P, Fortmeyer HP, Runkel (1987) Blood flow, oxygen consumption, and tissue oxygenation of human breast cancer xenografts in nude rats. Cancer Res. **47**, 3496-3503.

Vaupel P, Kallinowski F, Schlenger K, Fortmeyer HP (1989) Blood flow and oxygen consumption rates of human gynecological tumors xenografted into rnu/rnu-rats. Strahlenther. Onkol. **165**, 502-503.

Walmsley JG, Granter SR, Hacker MP, Moore AL, Ershler WB (1987) Tumor vasculature in young and old hosts: scanning electron microscopy of microcorrosion casts with microangiography, light microscopy and transmission electron microscopy. Scanning Microsc. **1**, 823-830.

Zeindler CM, Kratky RG, Roach MR (1989) Quantitative measurement of early atherosclerotic lesions on rabbit aortae from vascular casts. Atherosclerosis **76**, 245-455.

Discussion with Reviewers

A. Castenholz: Concerning "descriptive terms" used in tumor angiology, you mentioned vascular units consisting of feeding arterials, capillaries and venules. Such units, thus, correspond to the consisting of feeding arterioles, capillaries and venules, and thus, to the "classic" type of capillary branching. In tumor tissue, do other patterns also exist, e.g., as those reported by Chambers and Zweifach (1944) in rat mesentery, which is based on the occurrence of anterio-venous bridges from which the capillaries laterally branch off? Since the aspect of nutritive blood flow and other physiological parameters are addressed in your approach, the knowledge of the organization of sinusoids prevailing in a certain tumor seems to be of special interest.

Authors: Numerous physiological studies on the tumor blood flow postulate that arteriovenous anastomoses play a significant role in the distribution of tumor blood volume. However, we have not seen such features in our casts.

A. Castenholz: Among other features, malignant tumors are characterized by invasive growth. Are you able to assess this phenomenon qualitatively and quantitatively by proof of special structural features such as an accumulation of extravasates in your specimens?

Authors: Primarily, invasive growth is not correlated with an increase of extravasates. Of course, those are more frequently seen in tissues with increased new vessel formation. However, extravasates are not a characteristic feature in tumor angiogenesis, but can also be seen in other forms of angiogenesis, such as wound healing and inflammation.

D.E. Schraufnagel: On Figure 2b the cast vessels may suggest that vessels develop forming small thin-lumened channels that later expand. How frequent is this compared to more broad based budding? Or, are the two processes the same? How do sinusoidal structures fit into the angiogenic scheme?

Authors: In general, new vessel formation in tumors (Konerding *et al.*, 1991; 1992b) as well as in normal tissues (Rhodin and Fujita, 1989) starts after degradation of the basement membrane with a migration of endothelial cells that engulf a small lumen which is connected to the parent vessel and which progressively enlarges. So the mentioned two processes reflect only different stages of new vessel formation. This is also true for the formation of the quoted sinusoidal structures: their diameter increases after sprouting. However, because of their structural properties and lacking hierarchy they do not contribute significantly to a nutritive blood flow.

D.E. Schraufnagel: How would you define a sinusoid?

Authors: "Sinusoidal vessels" or "sinusoids" are defined by their capillary wall structure with increased diameter and consecutively slow blood flow. This is, however, also true for sinuses, e.g., in the spleen or liver. Obviously, the designator "sinusoid" was chosen for those vessels in tumors in order to discern them from the functionally more competent sinuses in normal tissues.

D.E. Schraufnagel: Could you speculate on how angiogenesis takes place structurally? Do you think that most new tumor blood vessels start by broad budding, by forming long thin tubes, or by lateral outfouling of small veins in the size of capillaries? I have seen all of these forms but I am wondering which is more common or whether one may be specific for a model.

Authors: Primarily angiogenesis takes place on the venous side. At least, we (and others!) have not yet

seen sprouting on the arterial side of the vascular system. We share your opinion that all forms occur. Comparisons of primary human and experimental tumors show that they are not specific for a particular model.

R. Christofferson: Do you think it is relevant to measure the luminal diameter of microvascular corrosion casts obtained by injection of a resin ten times as viscous as blood, injected manually at intravascular pressures ranging from 0 to 300 mm Hg? Which vessels are most likely to exhibit artifactually altered diameters after casting?

Authors: Of course, it is difficult to judge to what extent microvascular corrosion casts reflect the real, intravital diameter since physiological alterations of the vascular diameter, which can be as high as 70%, cannot be taken into account. However, this does not apply to vessels void of contractile elements, such as, the majority of tumor vessels which do not show a medial layer nor a significant number of intracellular contractile elements. Comparisons between different tumor casts should thus be possible as long as their vessels share similar structural and functional properties. We consider the quoted range of intravascular pressures much lower within the depicted tumor vessels. The low number of supplying arteries, the abundance of sinusoidal plexuses without hierarchy and the high branching frequency of large caliber vessels together with the high viscosity contradicts any high pressure. This could, of course, only be proven by intravital microscopy or intratumoral vascular pressure measurements. Another fact contradicting the assumption of artifactually increased vascular diameter is that in tumor casts filling artifacts prevail compared to ballooned vessels. So, we assume that in tumor casts the diameter of sinusoids and venules is rather underestimated.

R. Christofferson: The biological purpose for angiogenesis must be to increase the surface for metabolic exchange within a given volume of tumor tissue. Can it be deduced with certainty from casts which vessels are "exchange vessels" (i.e., capillaries and post-capillary venules in transmission electron microscopic terms, borrowed from study of non-malignant tissue), and which vessels that are the mere plumbing to feed "exchange vessels"? And, if you think, such a distinction can be made, how to quantify the surface for metabolic exchange per unit volume tumor tissue?

Authors: The existence of tumor vessels is a mere prerequisite, but not a guarantee for a sufficient nutritive blood flow. Numerous studies have shown that the intravascular pO2 in tumor vessels may be as low as 0-5 mm Hg. Thus, also keeping in mind the lack of hierarchy of the tumor vascular system and the existence of non-endothelialized blood conduits, it does not make sense to differentiate between exchange vessels and others. A distinction based on the morphology of cast vessels is not possible. The missing correlation between vascular densities, tumor growth and necroses (Steinberg *et al.*, 1990, 1991) also contradicts the use of a quantification of the metabolic exchange surface.

R. Christofferson: Is there any marker (morphological, biochemical, and pathognomonic) for angiogenesis that can be used for its quantification?

Authors: To our knowledge, there is no marker which is specific, quantifiable and pathognomonic for angiogenesis.

R. Christofferson: Which parameter in a vascular cast would be proportional to, or in any way dependent on, the tumor blood flow?

Authors: Such parameters are not (yet) known in cast tumor vessels.

R. Christofferson: There are investigations (e.g., Paweletz and Knierim, 1989) reporting that tumor cells themselves may serve as an endothelium, permitting circulation of plasma and erythrocytes. Have you observed such non-endothelialized blood conduits?

Authors: Yes, we have found such non-endothelialized blood conduits to varying extent in all tumors. However, the physiological importance of these conduits remains unclear. In this context, we would like to point out that tumor cells can be obviously integrated into the endothelium (Hammersen *et al.*, 1985).

R. Christofferson: What is the morphological foundation for the hemorrhages always observed within malignant tumors regardless of size? Can hemorrhages be advantageous for the survival of tumor cells?

Authors: We consider vessel wall destruction as the morphological basis for hemorrhages in malignant tumors. There is no indication that these hemorrhages might be of any advantage for the survival of tumor cells unless that the tumor cells use the "opened" vessel wall as a pathway for metastasation.

Additional References

Chambers R, Zweifach BW (1944) The topography and function of the mesenteric capillary microcirculation. Am. J. Anat. **75**, 173-205.

Hammersen F, Endrich B, Messmer K (1985) The fine structure of tumor blood vessels. I. Participation of non-endothelial cells in tumor angiogenesis. Int. J. Microcirc. Clin. Exp. **4**, 31-43.

Paweletz N, Knierim M (1989) Tumor-related angiogenesis. Crit. Rev. Oncol. Hematol. **9**, 197-242.