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VASCULAR STRUCTURE OF EXPERIMENTAL TUMOURS

Appearances in scanning electron microscope

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The vascular structure of experimental tumours was investigated using scanning electron microscopy after fixation with the resin cast technique. Four tumour types—squamous cell carcinoma of mouse, Ehrlich ascites carcinoma, AH7974 and AH109A—were transplanted into the subcutaneous tissue of animals. The main features of the capillaries of the resulting tumours were irregularity, tortuosity, sinusoid-like structures, disorderly running capillaries and tapering. These abnormalities occurred in all four types of tumours, even though slight differences were observed. The appearances and locations of the capillaries were almost identical in the tumours. The weight per unit volume decreased when the tumour sizes increased.

Observations on the capillary system of tumours related to tumour growth, effects of radiation and chemotherapeutic agents have been reported (DAVIDSON et coll. 1957, GOLDACRE & SYLVÉN 1962, RUBIN 1967, TANNOK 1968, TANNOK & STEEL 1969, FOWLER et coll. 1970, JOHNSON et coll. 1971. TANNOK & HAYASHI 1972, THOMLINSON 1973). It is generally considered that the vascular system of tumours differs from normal tissues and that it is much more complicated (MARGULIS et coll. 1961, OMAR et coll. 1971, SAEKI et coll. 1971, KAWAMURA & FUJIWARA 1973). Previously, the vascularity of normal tissues and experimental tumours was usually demonstrated

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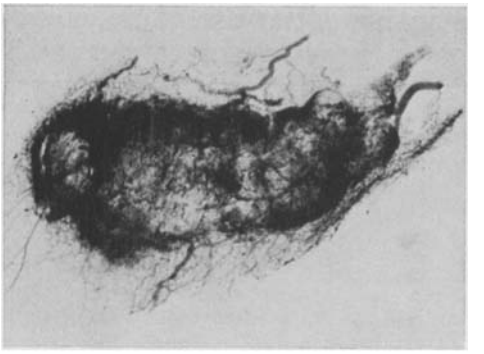
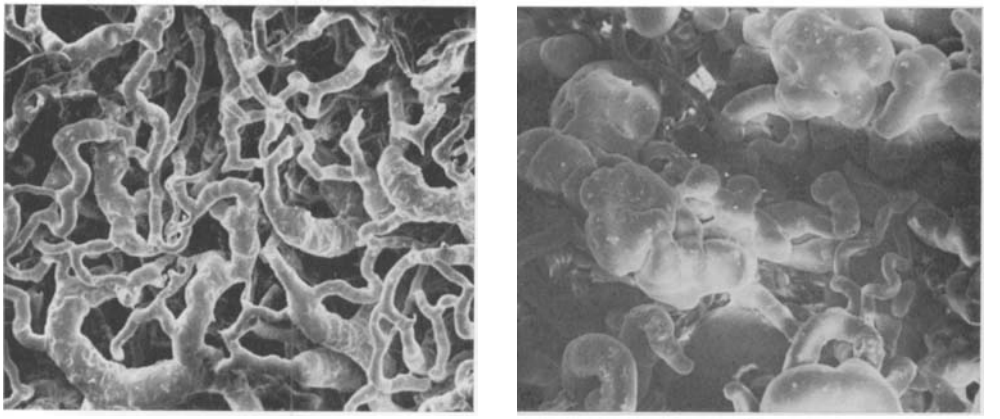


Fig. 1. Vascular cast of Ehrlich ascites carcinoma, soft radiography, SOFTEX-CSM, 15 kV.



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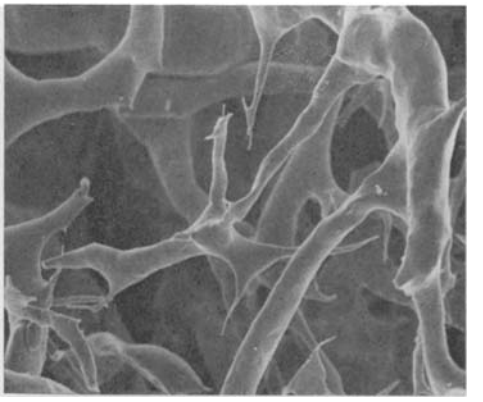


c

Fig. 2. Characteristics of the vascularity. a) Irregular vessel diameter. b) Sinusoid appearance. c) Tapering.

by microangiographic techniques (BISHTON & ROGERS 1950, ANGULO et coll. 1958, JEE & ARNOLD 1960, MARGULIS et coll., HASSLER 1964, RUBIN et coll. 1964, VOGEL 1965, HASSLER & MOVIN 1966, TIBOLDI et coll. 1968, GERARD 1970, SAEKI et coll., KAWAMURA & FUJIWARA).

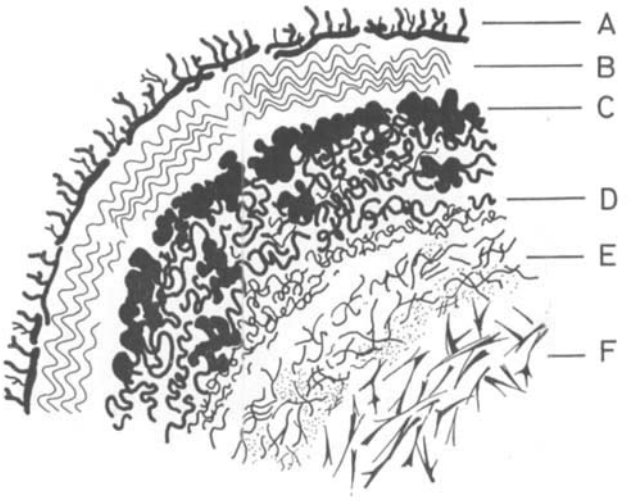


Fig. 3. Schematic drawing of tumour vascularity. A: Extruding capillaries on surface of tumour. B: Wave-like capillaries. C: Sinusoid-like capillaries. D: Tortuous capillaries. E: Disorderly running capillaries. F: Tapering capillaries in the central necrotic cavity.

Scanning electron microscopy of vascular casts has demonstrated very small details of the vascularity (BATSON 1955, MURAKAMI 1971). Recently, the radiation effect on the small blood vessels in abdominal organs using this technique was described (EGAWA & ISHIOKA 1978). Now is the vascular structure of experimental tumours reported.

Material and Methods

Experimental tumours and animals. Squamous cell carcinoma (pubic tumour), Ehrlich ascites carcinoma, ascites hepatoma 7974 and ascites hepatoma 109A were used. Into the bilateral subcutaneous tissue of the rump were injected: 10^4 to 10^5 suspended cells of squamous cell carcinoma into WH/HWT strain mice (40 males, 12–15 weeks old), about 10^6 cells of Ehrlich ascites carcinoma into ddN strain mice (30 males, 8–10 weeks old) and about 10^6 to 10^7 cells of AH7974 and AH109A, respectively, into Donryu rats (10 females in each group, 3 months old). At 5 to 15 days after transplantation, the tumours were used for the experiments. At this time the tumours measured 2.5 to 15 mm in diameter (average of length, width and thickness).

Preparation of the corrosion cast and scanning electron microscopy. The techniques used have previously been described by EGAWA & ISHIOKA. The radiographic appearance of the vascular cast of a tumour is illustrated in Fig. 1.

Results

The vascular structure of the four tumours was very similar.

In contrast to normal tissues the tumour vessels were irregular with sinusoid-like structures and tapering (Fig. 2).

Fig. 4. Extruding capillaries on surface of tumour mass.

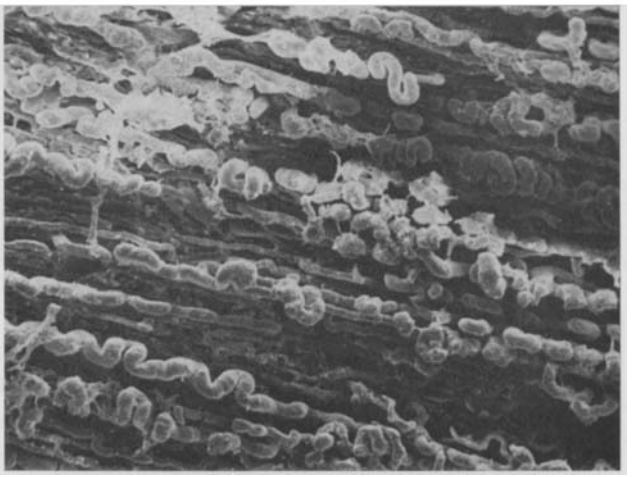
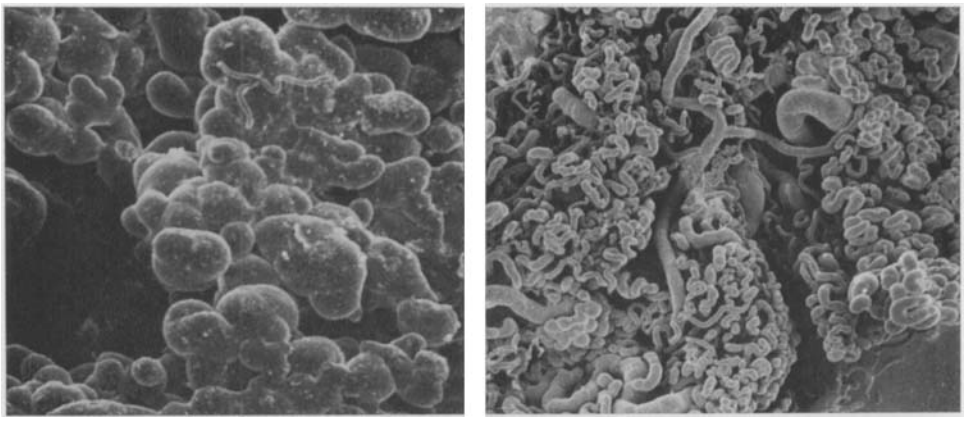


Fig. 5. Wave-like capillaries.

The vascularity was complicated. The appearances of the capillary network had a similar topographic position within the tumours (schematically illustrated in Fig. 3). Even in small tumours extruding capillaries on the surface of the tumour mass branched off from larger vessels. The terminals of the extruding capillaries were not connected with other exterior vessels. It appeared as if the club-like capillaries invaded radially from the tumour surface into the surrounding tissue (A in Fig. 3, Fig. 4). Blood vessels with a wave-like appearance running parallel to each other were lying deeper in the mass (B in Fig. 3, Fig. 5). In the next inner layer, sinusoid-like and tortuous vessels were observed (C and D in Fig. 3, Fig. 6). In a further layer located near the central necrotic cavity, the capillaries ran in a disordered fashion. The vessels were irregular, regardless if they were considered to be degenerated or



a b

Fig. 6. a) Sinusoid-like, b) tortuous capillaries.

destructed. Leakage of the resin was often observed in this layer (E in Fig. 3, Fig. 7). In the central necrotic cavity, tapering capillaries existed (F in Fig. 3, Fig. 8).

Tumours with a diameter of 5 to 15 mm had a similar vascular appearance. In tumours with a diameter less than 3 mm the tapering blood vessels found in and around the necrotic cavity of larger tumours were not observed. These small tumours mainly contained tortuous and sinusoid-like vessels.

A correlation between the tumour volume and the weight of its vascular cast per volume appears in Fig. 9. When the tumour volume (length × width × thickness) was less than about 125 mm^3, the density of the vascular cast was inversely proportional to the tumour volume; when it was more than 125 mm^3, the density was not correlated to the tumour volume. These findings suggest that development of necrosis is correlated to changes in the vascular network.

Discussion

For demonstration of very small details of the vascularity of experimental tumours, the resin cast technique is superior to methods using micromanipulation (BATSON, MURAKAMI, EGAWA & ISHIOKA). Extruding club-like, parallel wave-like, tortuous, sinusoid-like, disorderly running and tapering capillaries were found to be characteristic for the vessels in the four types of experimental solid tumours. These fundamental vascular characteristics were observed in tumours with a diameter between 5 and 15 mm. MARGULIS et coll. using an angiographic technique found that the basic vascular appearances were similar, regardless of the tumour size.

The extruding club-like capillaries on the surface of tumours with a diameter of less than 3 mm seem to be newly produced capillaries. The presence of these capillaries could be closely related to the tumour growth and also to the invasion of the

Fig. 7. Disorderly running capillaries.

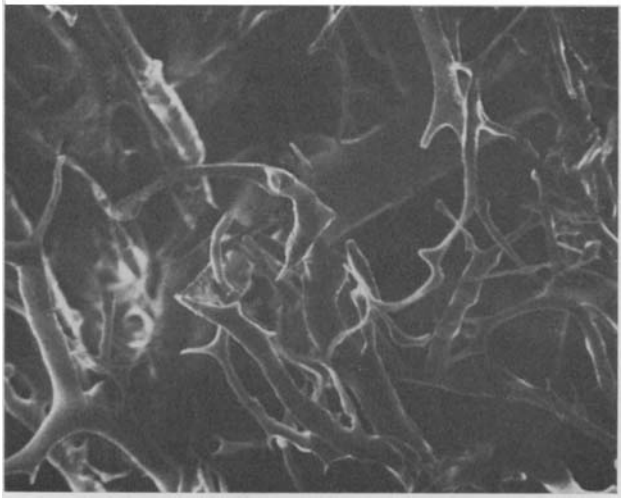


Fig. 8. Tapering capillaries in the central necrotic cavity.

tumour into surrounding tissue. The tapering capillaries occurring in and around necrotic cavities seem to be regressively changed vessels. They may originally have been tortuous vessels. As the necrosis increases, their diameter will become more and more irregular, probably ending in a tapering shape. With further degeneration of the capillaries a necrotic cavity is formed.

When the volume of the tumour was less than about 125 mm^3 (diameter about 5 mm) the density of the vascular cast was inversely proportional to the tumour volume (Fig. 9). Thus, the vascularity of small tumours was more dense and it became more and more sparse with the growth of the tumour. This may lead to a local necrosis in the tumour. When the tumour volume was more than 125 mm^3, the density of the cast became almost constant, which may be related to the development of

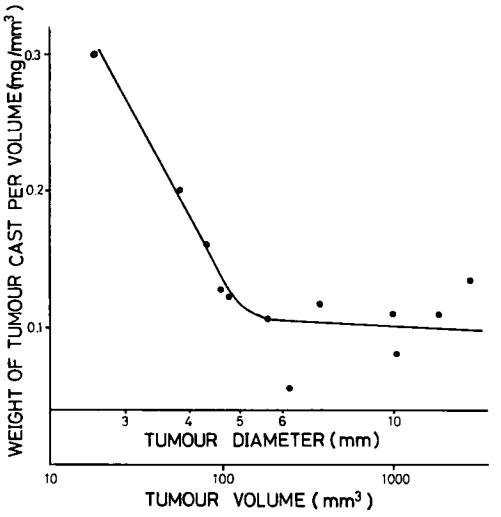


Fig. 9. Correlation between tumour volume and weight of the vascular cast per volume unit.

the necrotic cavity. This surmise fits well with the fact that typical tapering capillaries appeared in tumours with a diameter of more than 5 mm. The relation between vascular volume, vessel diameter, vessel length, surface area and necrotic tissue volume in C_3H/Bi mammary carcinoma during tumour growth, determined by a morphometric method, was reported by HILMAS & GILLETTE (1974, 1975). Their results showed that the vascular volume did not change significantly during tumour growth but retained a relatively constant proportion of the total viable tumour volume. However, the necrotic tissue volume increased suddenly when a certain tumour volume was reached and then increased only slowly. Similar results were obtained by the resin cast method.

A thorough analysis of the effects of radiation and chemotherapeutic agents on the capillary system of tumours is important and is at present going on, using the capillary cast method. The results will be reported later.

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SUMMARY

The vascular structure of experimental tumours was investigated by a resin cast technique. Six characteristic types of capillaries were found: club-like, wave-like, tortuous, sinusoid-like, disorderly running and tapering. The vascular structure was correlated to the tumour growth.

ZUSAMMENFASSUNG

Die vaskuläre Struktur von experimentellen Tumoren wurde mit der Harz-Guss-Technik untersucht. Sechs charakteristische Typen von Kapillaren wurden gefunden: Keulenähnliche, wellenähnliche, gewundene, sinusoidähnliche, unregelmässig verlaufende und spitzzulaufende. Die vaskuläre Struktur war zum Tumor-Wachstum korreliert.

RÉSUMÉ

La structure vasculaire de tumeurs expérimentales a été étudiée par une technique de moulage avec une résine. Les auteurs ont trouvé six types caractéristiques de capillaires : en massue, ondulé, tortueux, sinusoïdal, à trajet désordonné et effilé. La structure vasculaire a été correlée à la croissance tumorale.

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