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Vascular Structure of the C3H Mammary Carcinoma, the B16 Melanoma and the Lewis Lung Carcinoma in Syngeneic, Conventional Mice and Congenitally Athymic Mice*

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Abstract—*The vascular system of three commonly used murine experimental tumours, the C3H mammary carcinoma, the B16 melanoma and the Lewis lung carcinoma, in syngeneic (C3D2F1/Bom or C57BL/6J/Bom) and athymic (BALB/c/nu/nu/Bom) mice was studied. The main vascular characteristics of each tumour, i.e. the mean vessel diameter, the total vessel volume and the distribution of the total vessel volume among vessels with different diameters, did not change upon transplantation from conventional to athymic mice. However, the length of vessels with diameters in the range 5–15 μm was for all tumours shorter in athymic than in conventional mice. The vascular volume of the B16 melanoma per unit histologically intact tumour volume in athymic mice (0.040 ± 0.004) was considerably larger than that of five human melanoma xenografts previously studied (0.009 ± 0.001 to 0.022 ± 0.002). This difference was mainly due to occurrence of vessels with diameters in the range 55–145 μm in the B16 melanoma; vessels which were generally not observed in the human melanoma xenografts.*

INTRODUCTION

THE ARCHITECTURE of the vascular system is probably of considerable importance for the response of tumours to chemotherapy, radiotherapy and hyperthermia. Chemotherapeutic agents are partly distributed by the vascular system, and the effect of some agents may depend on the oxygen concentration in the tumour tissue [1]. The radiation response of tumours is often assumed to depend on the fraction of hypoxic cells and the reoxygenation pattern [2–4]. The response to heat treatment is reported to be influenced by temperature distribution, pH, nutritional conditions and perhaps also oxygen concentration [5–7].

Human tumour xenografts are by some authors believed to be preferable to rodent tumours in experimental cancer therapy research [8–11]. However, one characteristic property of xenografts may limit their use as a model for human cancer: the vascular system and the supporting stromal elements originate from the host while only the parenchymal tumour cells are of human origin [12]. Consequently, it is important to investigate whether the vascular system of human tumour xenografts differs from that of murine tumours as well as whether vascular changes occur when tumours are transplanted across an immunological barrier. Previously, we have studied the vascular structure of five human melanomas in athymic mice [13]. In the present communication we report on the vascular structure of three murine tumours, the C3H mammary carcinoma, the B16 melanoma and the Lewis lung carcinoma, grown in syngeneic and athymic mice. There was a dual purpose with the work: (1) to investigate whether vascular changes occur when tumour lines are transplanted from syngeneic mice to athymic mice with a different genetic background; and (2) to compare the vascular system of these murine tumours with

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that of the five human melanoma xenografts studied previously.

MATERIALS AND METHODS

Tumours and mice

The C3H mammary carcinoma was obtained from Radiumstationen, Aarhus, Denmark, and the B16 melanoma and the Lewis lung carcinoma from the National Institute of Health, Bethesda MD, U.S.A. Since then the tumours have been propagated by serial transplantation at our institute for several years. In the present experiments the C3H mammary carcinoma was grown in C3D2F1/Bom mice and the B16 melanoma and the Lewis lung carcinoma in C57BL/6J/Bom mice. The athymic mice used were BALB/c/nu/nu/Bom. Only female mice were used.

Tumour material for inoculation was obtained by dissection of large flank tumours. Macroscopic viable tumour tissue was minced with a scalpel and a pair of tweezers, and 5 μl were injected subcutaneously in the flanks of the mice. The tumours were carefully inoculated at the same site in the flanks, and the tumour volumes were about 200 mm^3 when the experiments were carried out. The tumour volumes were measured with callipers. Two perpendicular diameters (length and width) were recorded, and the tumour volumes were calculated as

$$V = 1/2 \cdot ab^2, \qquad (1)$$

where a and b are the longest and the shortest diameter, respectively.

Vascular studies

The vascular system of the tumours was filled with a contrast medium [100 ml 0.9% saline, 5 g gelatin, 50 g Pb_3O_4, 1 ml detergent (Joy/Salo), 5000 U heparin], prepared as previously described [13]. The contrast medium, kept at 40°C, was filtered through a fine-meshed nylon cloth before use. About 1 ml of the contrast medium was injected in the abdominal aorta of the mice. The blood pressure of the mice during injection was measured in the arteria carotis using the Olli Blood Pressure Meter 532 and the Gould Statham P50 Transducer. It was ensured that the injection pressure did not vary significantly from animal to animal, keeping it close to the normal blood pressure of the mice. The mice were fixed in 4% formalin for 1–2 weeks before the tumours were removed. Histological sections, 2 μm thick, were prepared from the tumours. Necrotic areas could easily be distinguished from the areas with vital tissue, i.e. histologically intact areas. Due to the contrast medium, the vessels appeared in the sections as dark circles or ellipses, depending on whether they were cut at a right or an oblique angle. The vascular parameters of the tumours were obtained by stereological analysis of the sections. The sections were examined at a magnification of $\times$ 400 by the use of a projecting light microscope and a counting frame, 20 $\times$ 20 cm in size. The vessel profiles which were projected within the counting frame were counted and classified with respect to vessel diameter. Eight diameter classes, 5–15, 15–25, 25–35, 35–45, 45–55, 55–85, 85–115 and 115–145 μm, were used. The length $[L_{VHI}(\bar{d})]$, surface $[S_{VHI}(\bar{d})]$ and volume $[V_{VHI}(\bar{d})]$ of the vessels belonging to the class with mean diameter $\bar{d}$—all per unit histologically intact tumour volume—were calculated from these measurements. The mean diameter $\bar{d}$ of the eight classes was 10, 20, 30, 40, 50, 70, 100 and 130 μm, respectively. Further details in the experimental procedure and the mathematics involved have been reported previously [13].

RESULTS

Growth curves for the three tumours in conventional and athymic mice are shown in Fig. 1. The Lewis lung carcinoma grew faster than the C3H mammary carcinoma and the B16 melanoma. The growth rate of the tumours in athymic mice was not significantly different from that in conventional mice. However, the lag period, i.e. the time from inoculation to beginning of growth, tended to be longer in athymic mice.

The vascular parameters of the tumours are summarized in Table 1. The three tumours showed individual and characteristic vascular structures. The main differences between the tumours were observed in conventional as well as in athymic mice. This is illustrated in Fig. 2, which shows histograms for $V_{VHI}(\bar{d})$. In the C3H mammary carcinoma large vessels contributed more to the total vessel volume than did the smaller ones. On the other hand, in the Lewis lung carcinoma the major part of the total vessel volume was due to the smaller vessels. The total vessel volume in the B16 melanoma was more evenly distributed among vessels with different diameters.

The similarity of the vascular structure of the tumours in conventional and athymic mice is underlined further by the data in Table 2. Neither the total vessel volume nor the mean vessel diameter for any of the tumours was significantly different in these two types of mice. It should be noted that these parameters varied considerably among the three tumours. Furthermore, the tumour volume doubling time and the necrotic

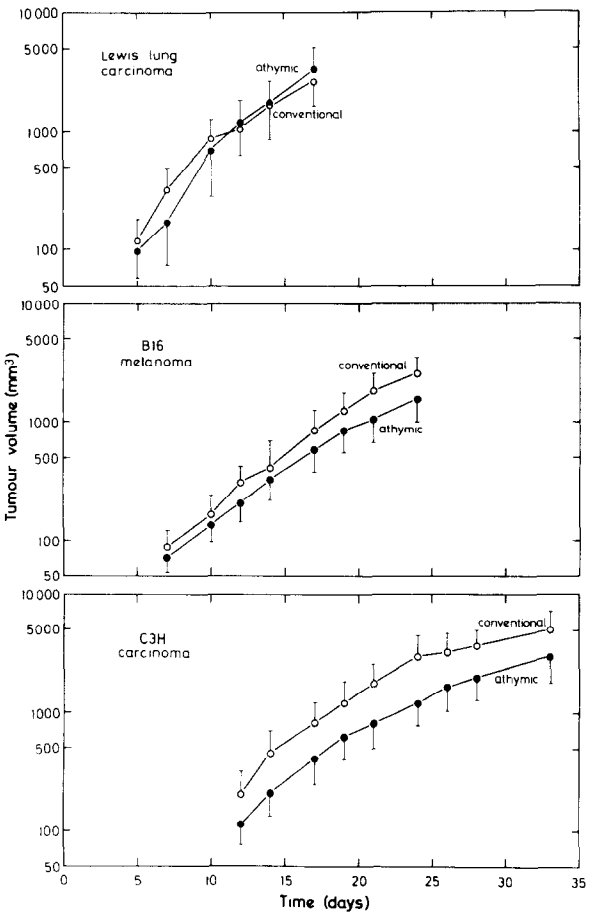

Fig. 1. Growth curves for the C3H mammary carcinoma, the B16 melanoma and the Lewis lung carcinoma in conventional and athymic mice. Each curve is based on the mean volume of 11–16 individual tumours. The vertical bars represent standard errors.

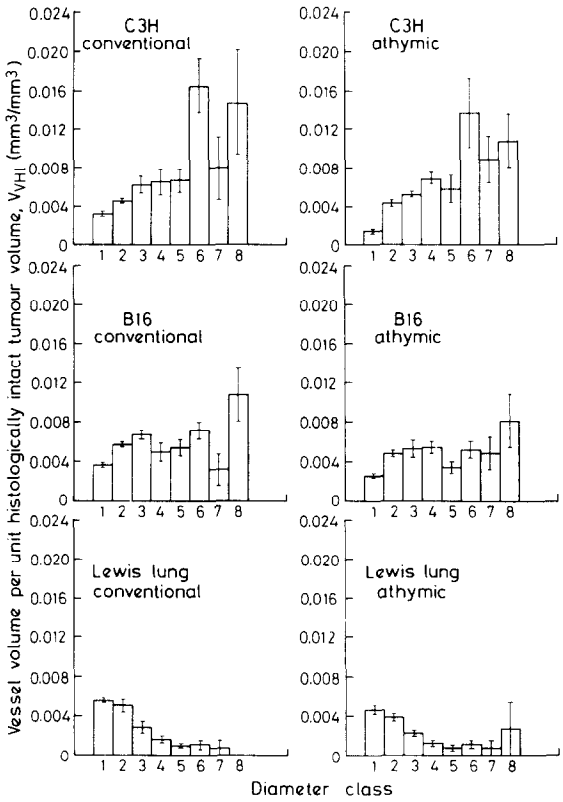

Fig. 2. Histograms for $V_{VHI}(\bar{d})$ for the C3H mammary carcinoma, the B16 melanoma and the Lewis lung carcinoma in conventional and athymic mice. Diameter classes 1–8 include vessels with diameters 5–15, 15–25, 25–35, 35–45, 45–55, 55–85, 85–115 and 115–145 μm, respectively. $V_{VHI}(\bar{d})$ was calculated as the mean of $V_{VHI}(\bar{d})$ for 4–6 individual tumours. The vertical bars represent standard errors.

fraction were similar in conventional and athymic mice. However, Table 2 indicates that the total vessel length was larger in tumours in conventional than in athymic mice. For all three tumours the differences in total vessel length were mainly due to differences in the capillary length, i.e. the length of vessels with diameters in the range 5–15 μm (Table 1).

DISCUSSION

The C3H mammary carcinoma, the B16 melanoma and the Lewis lung carcinoma grown in syngeneic hosts were shown to exhibit individual and characteristic vascular parameters. The total vessel volume per unit histologically intact tumour volume as well as the mean vessel diameter were largest in the C3H mammary carcinoma, second largest in the B16 melanoma and smallest in the Lewis lung carcinoma. Furthermore, the shape of the histograms for $V_{VHI}(\bar{d})$ was different for the three tumours (Fig. 2). When the tumours were transplanted from syngeneic hosts to athymic mice with a different genetic background, the vascular differences between the tumours were generally maintained. This observation indicates that biological characteristics of the parenchymal tumour cells, including features like the ability of the cells to synthesize and secrete tumour angiogenesis factor [15], are decisive for the architecture of the vascular network of tumours. This suggestion is in agreement with our previous observation that melanoma xenografts derived from different patients show different vascular characteristics in athymic mice [13].

However, the present work indicates that the architecture of the vascular system of tumours also may depend on the host. For all three tumours the capillary density, L_{VHI} ($\bar{d} = 10$ μm), was smaller in athymic than in conventional mice (Table 1).

Since the cell proliferation kinetics in tumours may depend on the capillary density, the growth pattern may change when tumours are transplanted into immune-deficient animals. Although the growth rate of the present three tumours was similar in conventional and athymic mice, the lag period tended to be longer in the latter, and this

Table 1. Vascular parameters

Parameter*	C3H		B16		Lewis lung	
	Conv.	Athymic	Conv.	Athymic	Conv.	Athymic
$L_{VHI}(\bar{d}=10\ \mu m)$ (mm/mm³)	33.6 ± 1.5	15.3 ± 2.1	37.7 ± 2.4	26.5 ± 2.0	57.0 ± 1.8	47.9 ± 3.6
$L_{VHI}(\bar{d}=20\ \mu m)$ (mm/mm³)	13.8 ± 0.6	13.2 ± 0.9	17.1 ± 0.5	14.4 ± 1.0	15.3 ± 1.9	11.7 ± 1.2
$L_{VHI}(\bar{d}=30\ \mu m)$ (mm/mm³)	8.7 ± 1.1	7.3 ± 0.4	9.2 ± 0.7	7.3 ± 1.1	4.0 ± 0.8	3.2 ± 0.4
$L_{VHI}(\bar{d}=40\ \mu m)$ (mm/mm³)	5.2 ± 1.0	5.4 ± 0.5	3.9 ± 0.7	4.2 ± 0.5	1.3 ± 0.2	1.0 ± 0.2
$L_{VHI}(\bar{d}=50\ \mu m)$ (mm/mm³)	3.4 ± 0.6	2.9 ± 0.7	2.7 ± 0.4	1.7 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
$L_{VHI}(\bar{d}=70\ \mu m)$ (mm/mm³)	4.1 ± 0.7	3.4 ± 0.9	1.8 ± 0.2	1.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
$L_{VHI}(\bar{d}=100\ \mu m)$ (mm/mm³)	1.0 ± 0.4	1.1 ± 0.3	0.4 ± 0.2	0.6 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
$L_{VHI}(\bar{d}=130\ \mu m)$ (mm/mm³)	1.1 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0	0.2 ± 0.2
$S_{VHI}(\bar{d}=10\ \mu m)$ (mm²/mm³)	1.06 ± 0.05	0.48 ± 0.07	1.18 ± 0.08	0.83 ± 0.06	1.79 ± 0.06	1.50 ± 0.11
$S_{VHI}(\bar{d}=20\ \mu m)$ (mm²/mm³)	0.87 ± 0.04	0.83 ± 0.06	1.07 ± 0.03	0.90 ± 0.06	0.96 ± 0.12	0.74 ± 0.08
$S_{VHI}(\bar{d}=30\ \mu m)$ (mm²/mm³)	0.82 ± 0.10	0.69 ± 0.04	0.87 ± 0.07	0.69 ± 0.10	0.38 ± 0.08	0.30 ± 0.04
$S_{VHI}(\bar{d}=40\ \mu m)$ (mm²/mm³)	0.65 ± 0.13	0.68 ± 0.06	0.49 ± 0.09	0.53 ± 0.06	0.16 ± 0.03	0.13 ± 0.03
$S_{VHI}(\bar{d}=50\ \mu m)$ (mm²/mm³)	0.53 ± 0.09	0.46 ± 0.11	0.42 ± 0.06	0.27 ± 0.05	0.08 ± 0.02	0.06 ± 0.02
$S_{VHI}(\bar{d}=70\ \mu m)$ (mm²/mm³)	0.90 ± 0.15	0.75 ± 0.20	0.40 ± 0.04	0.29 ± 0.04	0.07 ± 0.02	0.07 ± 0.02
$S_{VHI}(\bar{d}=100\ \mu m)$ (mm²/mm³)	0.31 ± 0.13	0.35 ± 0.09	0.13 ± 0.06	0.19 ± 0.06	0.03 ± 0.03	0.03 ± 0.03
$S_{VHI}(\bar{d}=130\ \mu m)$ (mm²/mm³)	0.45 ± 0.16	0.33 ± 0.08	0.33 ± 0.08	0.25 ± 0.08	0	0.08 ± 0.08
$V_{VHI}(\bar{d}=10\ \mu m)$ (mm³/mm³)	0.0033 ± 0.0001	0.0015 ± 0.0002	0.0037 ± 0.0002	0.0026 ± 0.0002	0.0056 ± 0.0002	0.0047 ± 0.0004
$V_{VHI}(\bar{d}=20\ \mu m)$ (mm³/mm³)	0.0046 ± 0.0002	0.0044 ± 0.0003	0.0057 ± 0.0002	0.0048 ± 0.0003	0.0051 ± 0.0006	0.0039 ± 0.0004
$V_{VHI}(\bar{d}=30\ \mu m)$ (mm³/mm³)	0.0063 ± 0.0008	0.0053 ± 0.0003	0.0067 ± 0.0005	0.0053 ± 0.0008	0.0029 ± 0.0006	0.0023 ± 0.0003
$V_{VHI}(\bar{d}=40\ \mu m)$ (mm³/mm³)	0.0066 ± 0.0013	0.0069 ± 0.0006	0.0050 ± 0.0009	0.0054 ± 0.0006	0.0017 ± 0.0003	0.0013 ± 0.0003
$V_{VHI}(\bar{d}=50\ \mu m)$ (mm³/mm³)	0.0067 ± 0.0012	0.0058 ± 0.0014	0.0054 ± 0.0008	0.0034 ± 0.0006	0.0010 ± 0.0002	0.0008 ± 0.0002
$V_{VHI}(\bar{d}=70\ \mu m)$ (mm³/mm³)	0.0165 ± 0.0028	0.0137 ± 0.0036	0.0072 ± 0.0008	0.0052 ± 0.0008	0.0012 ± 0.0004	0.0012 ± 0.0004
$V_{VHI}(\bar{d}=100\ \mu m)$ (mm³/mm³)	0.0080 ± 0.0032	0.0088 ± 0.0024	0.0032 ± 0.0016	0.0048 ± 0.0016	0.0008 ± 0.0008	0.0008 ± 0.0008
$V_{VHI}(\bar{d}=130\ \mu m)$ (mm³/mm³)	0.0148 ± 0.0054	0.0108 ± 0.0027	0.0108 ± 0.0027	0.0081 ± 0.0027	0	0.0027 ± 0.0027

*Mean ± S.E.

Table 2. Biological characteristics

Parameter*	C3H		B16		Lewis lung	
	Conv.	Athymic	Conv.	Athymic	Conv.	Athymic
T_d (days)†	2.5	3.0	3.2	3.2	1.5	1.4
N (%)‡	43 ± 3	48 ± 2	42 ± 3	44 ± 4	49 ± 2	40 ± 2
$\bar{D}$ (μm)§	25.1 ± 1.7	29.3 ± 2.4	21.1 ± 1.1	22.2 ± 1.5	14.1 ± 0.8	14.3 ± 1.2
L_{VHI} (mm/mm^3)‖	71 ± 3	49 ± 3	74 ± 3	57 ± 3	79 ± 3	65 ± 4
S_{VHI} (mm^2/mm^3)‖	5.6 ± 0.4	4.6 ± 0.3	4.9 ± 0.2	4.0 ± 0.2	3.5 ± 0.2	2.9 ± 0.2
V_{VHI} (mm^3/mm^3)‖	0.067 ± 0.008	0.057 ± 0.006	0.048 ± 0.004	0.040 ± 0.004	0.018 ± 0.002	0.018 ± 0.003

*Mean ± S.E.

†Tumour volume-doubling time (T_d) in the volume range 200-400 mm^3 was determined as previously described [14].

‡The volume fraction of necrosis (N) was determined as previously described [13].

§Mean vessel diameter ($\bar{D}$) was calculated as:

$$\bar{D} = \frac{\sum_{i=1}^{8} \bar{d}_i \, L_{VHI}(\bar{d}_i)}{\sum_{i=1}^{8} L_{VHI}(\bar{d}_i)} .$$

‖Total vessel length (L_{VHI}), surface (S_{VHI}) and volume (V_{VHI}) per unit histologically intact tumour volume were calculated as:

$$L_{VHI} = \sum_{i=1}^{8} L_{VHI}(\bar{d}_i); \quad S_{VHI} = \sum_{i=1}^{8} S_{VHI}(\bar{d}_i); \quad V_{VHI} = \sum_{i=1}^{8} V_{VHI}(\bar{d}_i).$$

may well be due to reduced angiogenesis. Others have also shown that the growth pattern may change when tumours are transplanted across an immunological barrier. Fu and Steel [16] transplanted a rat mammary tumour from syngeneic rats to immune-suppressed mice and observed that the first-passage tumours in mice showed irregular and generally slow growth. Similarly, human tumours transplanted into congenitally athymic or immune-suppressed mice often have a long lag period and grow slowly in their first passage, but then grow faster and more regularly in subsequent passages [17-20]. The reasons for these changes in growth pattern are not understood, but it should not be excluded that they are somehow related to changes in the angiogenesis and thus to the capillary density.

Since the response of tumours to treatment with chemotherapeutic agents, radiation or hyperthermia may depend on the capillary density, the present work suggests that the therapeutic response may change when tumours are transplanted across an immunological barrier. Although it has been clearly demonstrated that the response to therapy of tumours may change during heterotransplantation [21], there is increasing evidence that the response to radiotherapy and chemotherapy of human tumour xenografts correlates with clinical responsiveness [22-25]. Nevertheless, the present data on the capillary density indicate that one should be very cautious when extrapolating data from human tumour xenografts to clinical practise.

In order to evaluate the usefulness of human tumour xenografts versus that of murine tumours in experimental cancer therapy it will be of interest to know to what extent the vascular system of such xenografts resembles that of murine tumours. Previously we have studied the vascular system of five human melanoma xenografts using the same experimental procedure as in the present work [13]. The total vessel volume per unit histologically intact tumour volume varied among the melanomas from 0.009 $\pm$ 0.001 to 0.022 $\pm$ 0.002. The vascular volume of the Lewis lung carcinoma in athymic mice was within that range, whereas the other two murine tumours showed significantly larger vascular volumes. The histogram for $V_{VHI}(\bar{d})$ of the B16 melanoma in athymic mice is compared with that of the human melanoma xenograft having the largest vascular volume, the V.N. melanoma, in Fig. 3. The main difference between the tumours is that the B16 melanoma has a considerable number of vessels with diameters in the range 55-145 μm, whereas in the V.N. as well as in the other four human melanomas studied, vessels with diameters larger than 55 μm were generally not observed. The number of vessels with diameters in the range 55-145 μm was even larger in the C3H mammary carcinoma than in the B16 melanoma. Since the murine tumours and the human melanomas were growing in the same site in the flanks of athymic mice and were of the same size, the observed differences in the vascular system can probably not be attributed to differences in experimental procedure.

Further studies have to be carried out to investigate whether generally there are large differences between the vasculature of human tumour xenografts and murine tumours of the same histological type, as suggested in Fig. 3. Nevertheless, Fig. 3 indicates that although the vascular system of human tumour xenografts is of murine origin, and although the vascular system to some extent may change when tumours are transplanted across an immunological barrier, the vascular system of some human tumour xenografts, e.g. melanomas, may be more representative for tumours in man than is that of murine tumours of corresponding histological type.

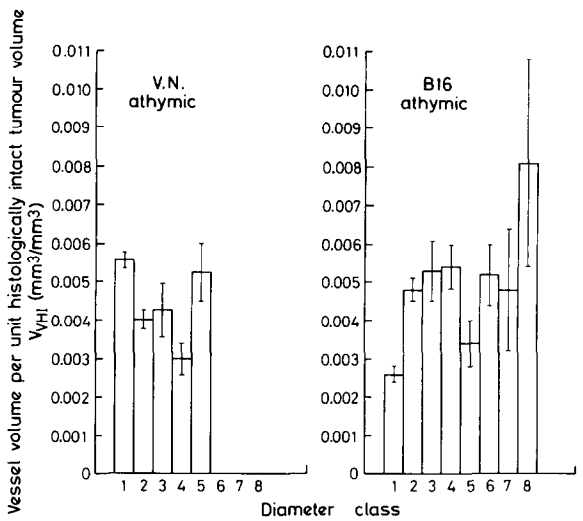

Fig. 3. Histograms for $V_{VHI}(\bar{d})$ for the V.N. human melanoma and the B16 murine melanoma in athymic mice. Diameter classes 1-8 include vessels with diameters 5-15, 15-25, 25-35, 35-45, 45-55, 55-85, 85-115 and 115-145 μm, respectively. $V_{VHI}(\bar{d})$ was calculated as the mean of $V_{VHI}(\bar{d})$ for 4-5 individual tumours. The vertical bars represent standard errors.

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