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Vascular permeability changes in tumours of the peripheral nervous system *

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Summary. Vascular permeability changes were examined in 34 tumours of the peripheral nervous system by immunohistochemical demonstration of serum proteins as endogenous tracers. The blood-tumour barrier was impaired in the reticular (Antoni type B) portions of neurinomas (Schwannomas) and in cutaneous neurofibromas but was similar to the normal blood-nerve barrier in fibrillary (Antoni type A) neurinomas, in most neurofibromas, in ganglioneuromas and in anaplastic tumours. These differences in permeability are discussed in relation to aspects of pathological tumour vascularization, the histogenesis of microcystic changes, and systemic therapeutic approaches.

Key words: Peripheral nervous system tumours – Vascular permeability – Serum proteins – Immunohistochemistry – Neurofibromatosis

Introduction

The detection and grading of permeability changes by use of endogenous tracer immunohistochemistry is an established method of neuro-oncological research. In gliomas and metastases, the variability of serum protein leakage could be correlated with both tumour type and malignancy (Seitz and Wechsler 1987; Neuen-Jacob et al. 1989). In meningiomas, this method was pivotal in the definition of the microcystic variant (Schober et al. 1988). Graded permeability changes can also be expected in tumours of the peripheral nervous system. An example is the reticular portion or Antoni B pattern of neurinomas whose histological appearance is comparable to microcystic change, although its origin is controversial (Reed and Harkin 1983). Vascular permeability in peripheral nervous system tumours has, moreover, become an important issue with the development of systemic therapeutic approaches in neurofibromatosis. We have therefore examined a variety of such tumours with conventional and immunohistochemical methods. Their blood-tumour barrier was compared with the normal blood-nerve barrier as defined in biopsy material by Liebert et al. (1985) and in autopsy controls in the present study.

Materials and methods

Twenty-six tumours of the peripheral nervous system were taken from the files of the Neuropathological Institute of the University of Düsseldorf. In addition, 5 cutaneous tumours were provided by the Dermatological Clinic of the University of Düsseldorf. They were classified as follows (with localization): 11 neurinomas (cerebellopontine angle, spinal, retroperitoneal, and peripheral), 5 neurofibromas (spinal), 2 atypical plexiform neurofibromas (orbit and thigh), 6 dermal neurofibromas (including 1 specimen of cutis verticis gyrata), 2 anaplastic neurinomas (spinal), 1 anaplastic neurofibroma (retroperitoneum), 2 malignant peripheral neuroectodermal tumours (ulnar nerve and posterior mediastinum), 2 ganglioneuromas (mediastinum). Seven of these tumours had occurred in patients with neurofibromatosis type 1. One case had bilateral cerebellopontine angle lesions (neurofibromatosis type 2). Additionally, control material was studied consisting of 2 traumatic neuromas, 1 Morton's neuroma, and normal brachial plexus, sciatic nerve and median nerve from 2 adult autopsy cases without neurological disease. The tumour and control material were fixed in 4% formalin, dehydrated in a graded ethanol series and embedded in paraffin. Paraffin sections (5 μm) were cut and stained by conventional methods (H & E, Masson's trichrome stain, cresyl violet, and reticulin stain according to Tibor-Pap).

Serial paraffin sections for immunohistochemistry were mounted on glass slides coated with poly-L-lysine (Sigma, Deisenhofen, FRG). Immunoreactivity was detected using monoclonal antibodies and the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981). Polyclonal rabbit antisera were used with the peroxidase-anti-peroxidase (PAP) method (Sternberger 1986). Prior to immunostaining for immunoglobulin A, α_2-macroglobulin, β-lipoprotein and S-100, sections were incubated in 0.125% trypsin (Merck, Darmstadt, FRG) for 45 min. The following primary anti-

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Table 1. Serum proteins as endogenous tracer for the blood-tumour barrier

Serum protein	Molecular weight [kDa]	Hydro-dynamic radius [nm]	Serum concentration [g/l]
Albumin	69,000	3.58	35.0–55.0
Immunoglobulin A	170,000	5.34	0.8– 2.8
Alpha$_2$-macroglobulin	820,000	9.35	1.5– 4.5
Immunoglobulin M	900,000	12.10	0.6– 1.7
Beta-lipoprotein	2,500,000	12.40	4.0–14.0

bodies were used: polyclonal rabbit anti-albumin (Dako, Hamburg, FRG) diluted 1:15000, polyclonal rabbit anti-immunoglobulin A (Dako) diluted 1:1000, polyclonal rabbit and anti-α_2-macroglobulin (Dako) diluted 1:2000, monoclonal mouse anti-immunoglobulin M (Dako, clone R1/69) diluted 1:50, polyclonal rabbit anti-β-lipoprotein (Dako) diluted 1:1000. The molecular weight and the hydrodynamic radius of the serum proteins are listed in Table 1. Peroxidase activity was visualized by freshly prepared 0.05% 3,3-diaminobenzidinetetrachloride (Sigma) plus 0.01% hydrogen peroxide in phosphate-buffered saline. All sections were counterstained with haematoxylin. Control sections were prepared by omitting primary antisera or applying non-immune mouse or rabbit serum.

Results

All peripheral nerves examined showed a similar permeability behaviour irrespective of their anatomical localization. Albumin and immunoglobulin A were detectable in the epineural connective tissue, the perineurium and in endoneural structures sparing the nerve sheaths. The expression of α_2-macroglobulin and serum proteins of higher molecular weight was demonstrated in the epineurium and in outer cell layers in the perineurium only, whereas inner perineurial cell layers as well as the endoneurium were negative (Fig. 1).

The traumatic neuromas and the case of Morton's neuroma demonstrated the same permeability behaviour as the control material of normal nerves, showing immunoreactivity for albumin and immunoglobulin A, while lacking reactivity for serum proteins of higher molecular weight.

Striking differences in vascular permeability and intratumour oedema were observed in the more common tumour types. Five plexiform neurofibromas of typical histological appearance, including one of the tactile corpuscle type, were free of endoneurial extravascular serum proteins of higher molecular weight than immunoglobulin A (Fig. 2). Strong immunoreactivity was only observed for the smaller serum proteins albumin and

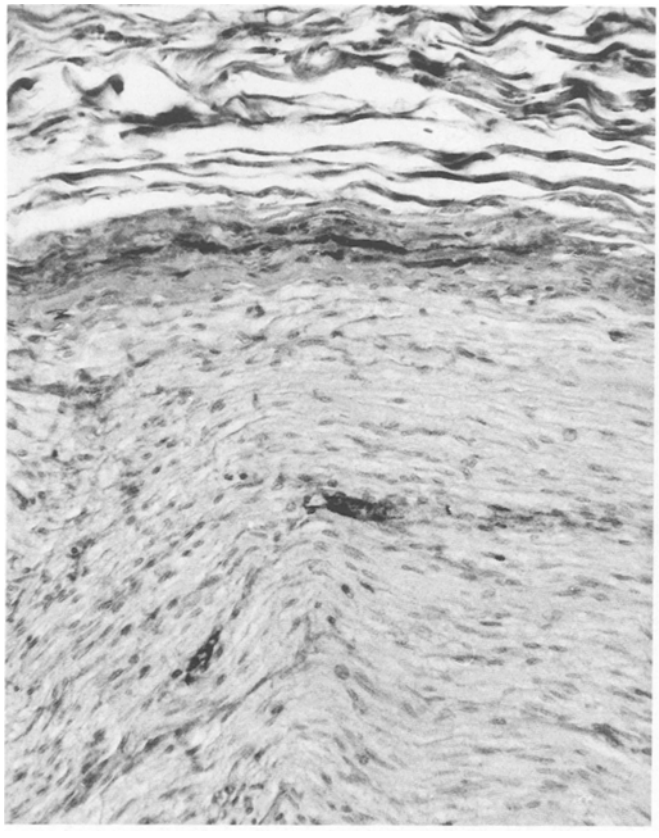


Fig. 1. Immunohistochemical demonstration of the blood-nerve barrier in the normal brachial plexus. The reaction product is confined to the epineurium (*top*), the outer layers of the perineurium, and the lumen of endoneurial blood vessels. IgM, counterstained with haematoxylin, ×180

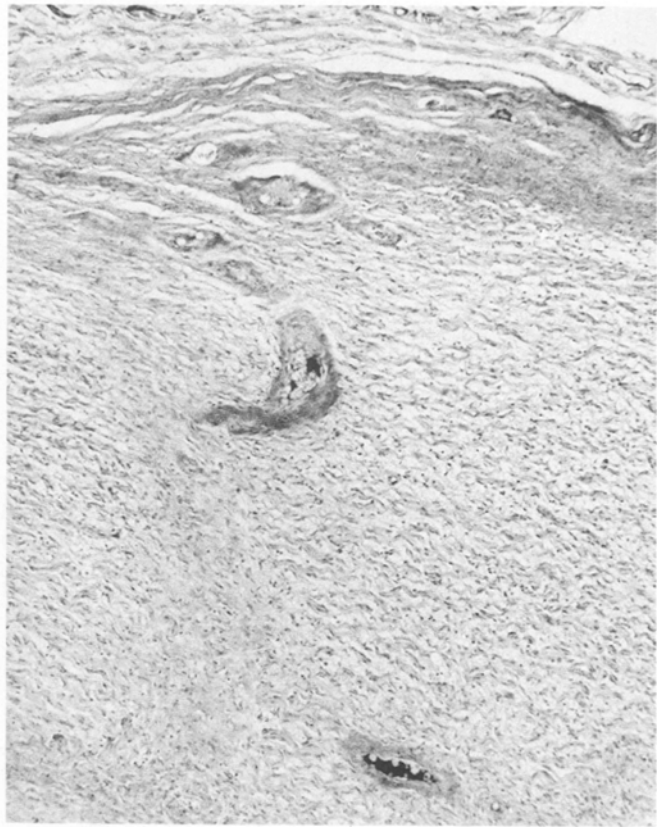


Fig. 2. The staining pattern of a neurofibroma resembles the normal blood-nerve barrier. IgM, counterstained with haematoxylin, ×75

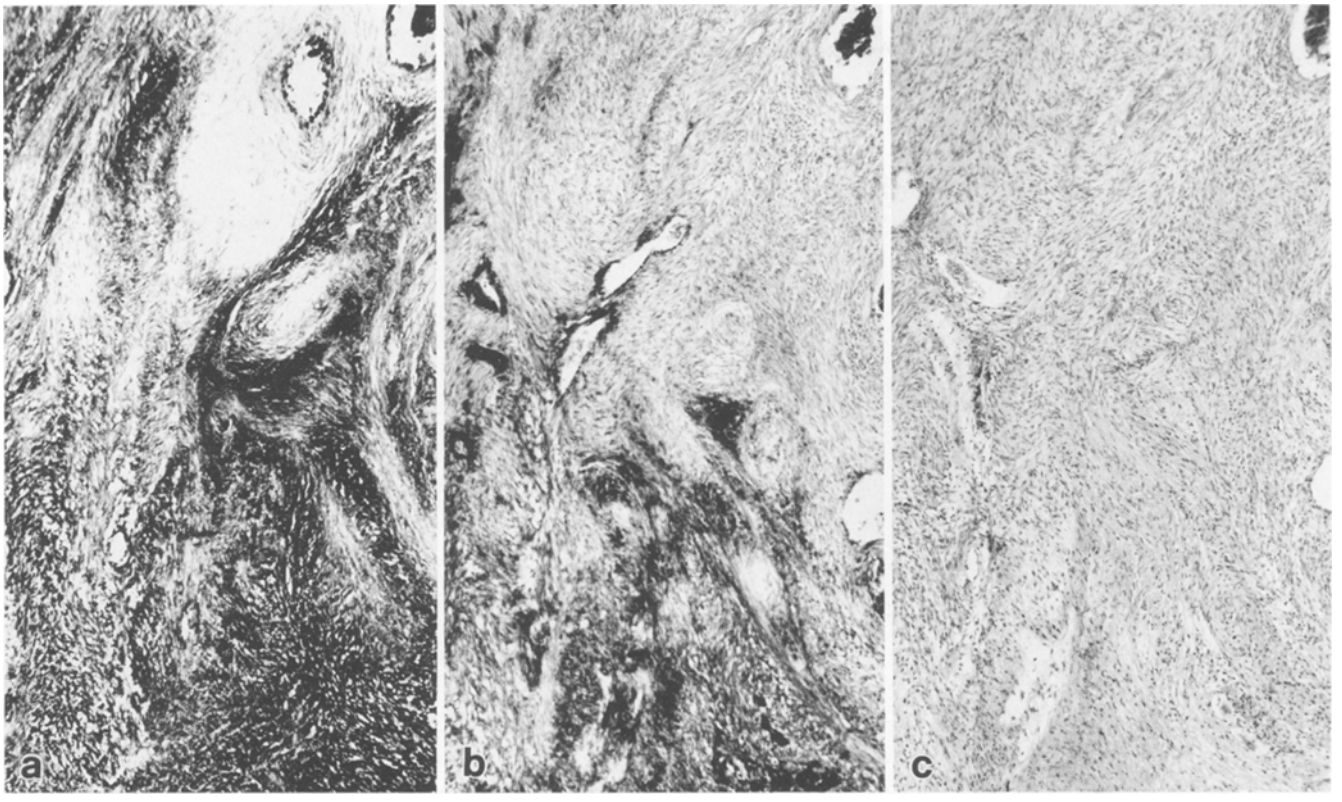


Fig. 3. Grading of the blood-tumour barrier in a neurinoma by the ubiquitous staining for albumin (**a**), the partial staining for IgM in the reticular tissue areas selectively (**b**), and the absent or exclusively intravascular staining for β-lipoprotein (**c**). Serial sections, counterstained with haematoxylin, $\times 60$

immunoglobulin A, a pattern that closely resembled the normal blood-nerve barrier. Exceptions were 2 neurofibromas of atypical histological structure. One was a neurofibroma of the orbit in a 6-year-old boy, recurring twice with eventual diffuse involvement of the subcutaneous tissue. These areas and the smaller nerve branches involved showed some permeability for α_2-macroglobulin and immunoglobulin M, while the typical plexiform tumour regions did not differ from other neurofibromas. The other atypical neurofibroma showed an unusual internal structure with multiple onion bulb differentiations developing into micro-neurinomas. This case, which will be reported in more detail separately, revealed distinct immunoreactivity for α_2-macroglobulin and immunoglobulin M in the endoneurial space but not in the onion bulbs or micro-neurinomas. The cutaneous neurofibromas displayed a higher level of permeability than the plexiform ones. Although the immunoreaction was distinctly weaker than in the adjacent normal tissue, serum proteins of higher molecular weight were demonstrated within the tumour tissue.

All 11 benign neurinomas showed marked permeability changes irrespective of their intracranial, spinal or peripheral localization. They were strongly immunoreactive for albumin, immunoglobulin A, and in a considerable proportion also for α_2-macroglobulin and immunoglobulin M. These changes were exclusively present in the reticular parts, while the fibrillary parts showed permeability for the low molecular serum proteins only.

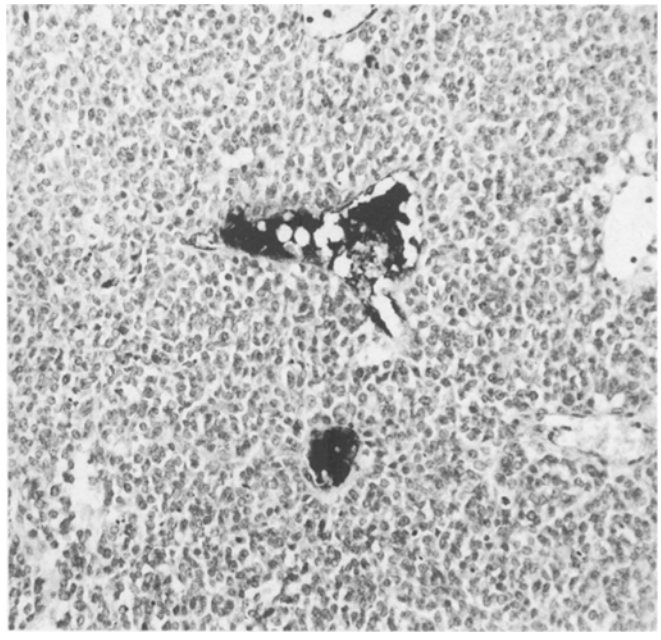
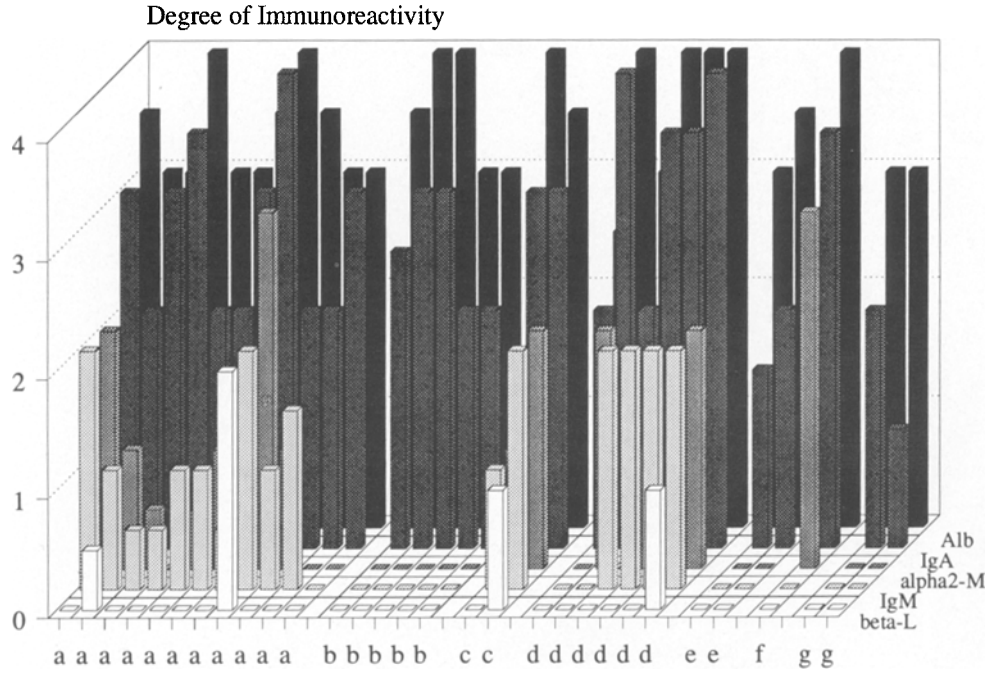


Fig. 4. Malignant peripheral neuroectodermal tumour with lack of vascular permeability for serum proteins of higher molecular weight. IgM, counterstained with haematoxylin, $\times 240$



a : Neurinoma
b : Neurofibroma
c : Atypical Neurofibroma
d : Cutaneous neurofibroma
e : Malignant neurinoma
f : Malignant neurofibroma
g : Malignant peripheral neuroectodermal tumor

Alb : Albumin (69 kD)
IgA : Immunoglobulin A (150 kD)
alpha2-M : alpha2-Macroglobulin (820 kD)
IgM : Immunoglobulin M (900 kD)
beta-L : beta-Lipoprotein (2500 kD)

Fig. 5. Semi-quantitative synopsis of the permeability changes in peripheral nervous system tumours. Four degrees of immunoreactivity are distinguished: 0, no extravascular staining; 1, weak and focal staining; 2, moderate staining; 3, marked staining; 4, strong and ubiquitous staining. Vascular permeability changes of a higher degree are only present in neurinomas and in atypical and cutaneous neurofibromas

A graded permeability with sparing of the denser fibrillary tumour areas for serum proteins of increasing molecular weight was well demonstrated (Fig. 3). Immunoreactivity for β-lipoprotein was present in the reticular parts of 2 neurinomas only. One of them was a tumour with marked regressive changes; the other had high vascular density. A preferential perivascular localization of serum proteins, especially in the vicinity of blood vessels of pathological structure, was seen in all tumours. Such blood vessels within the reticular areas were thin-walled and usually dilated, corresponding to ectatic venules or capillaries. In some vessels the vascular wall showed a stronger immunoreaction than the perivascular tissue. Intramural serum protein staining was also seen in some of the blood vessels with hyaline changes usually encountered within the reticular areas.

Two malignant neurinomas and 2 malignant peripheral neuroectodermal tumours showed very little permeability change. The staining pattern of these anaplastic tumours of high cellularity (WHO grade III), to our surprise, resembled that of tumours retaining the normal blood-nerve barrier (Fig. 4).

A synopsis of the differential staining of serum proteins in the different types of peripheral nervous system tumours is given in Fig. 5. The semi-quantitative assessment was reached by comparing the degree of tumour tissue staining with the degree of intravascular immunoreactivity whenever possible in a given section. The degree of intravascular immunoreactivity should serve as a baseline, since there is a wide individual variation of serum protein concentrations (Table 1).

Discussion

The results of this study indicate that in tumours of the peripheral nervous system, vascular permeability changes of a higher degree are confined to neurinomas and to cutaneous or diffuse neurofibromas. Other tumour types as a rule do not contain serum proteins of high molecular weight such as α_2-macroglobulin, immunoglobulin M, and β-lipoprotein. They thus display a permeability that closely resembles the normal blood-nerve barrier as demonstrated in this and in previous studies (Liebert et al. 1985).

Such differences in the degree of blood-tumour-barrier breakdown at first appear surprising but are comparable in various aspects to those observed in meningiomas (Schober et al. 1988). In these tumours, serum protein exudation is confined to one type, the meningotheliomatous meningioma, or to meningotheliomatous portions within mixed tumours. Both in meningiomas and neurinomas, the associated tissue changes are characteristically microcystic but can coalesce to form large tumour cysts. There are rare examples of meningiomas as well as of neurinomas in which the loose tissue structure is attributable to mucinous change rather than to extravasation of serum components. The permeability changes in both types of tumour are usually associated with a highly pathological tumour vascularization including hyaline changes of the vascular walls.

In neurinomas, the heterogenous immunohistochemical distribution of serum proteins is a reflection of their heterogeneous tissue structure. The reticular or Antoni

type B pattern has been interpreted as a "lytic" phase with subsequent attraction of histiocytes and lymphocytes (Reed and Harkin 1983). Serum extravasation, in addition to accumulation of lipids (Ratzenhofer 1940), may be contributory to the loose tissue structure. However, the origin of Antoni type B tissue as the result of degenerative changes in the other tissue type is highly debatable (Krücke 1974; Urich 1984), and morphological studies in a single stage of evolution cannot solve this question.

In discussing the pathogenesis of the described permeability changes, one has to take into account the factors that determine the vascular-extravascular exchange, including the transport parameters, the surface area for exchange, and the transluminal concentration and pressure gradients (Jain 1987). The concentration gradients are reflected by the degrees of extra- und intravascular staining intensity which have been evaluated on a semiquantitative basis. The surface area, when judged from the histological appearance of the tumour vessels in areas of high permeability, seems to be increased. The transport parameters are mainly governed by the structure of the vessel wall, in particular the capillar endothelium. Neurinomas have been extensively studied in this respect (Hirano et al. 1972; Long 1973; Kasantikul et al. 1979), and their vascular fine structure with endothelial fenestrations and absence of interendothelial tight junctions is compatible with the passage of probes up to 3000 nm large (Majno 1978). All the tracers listed in Table 1 fall into this range, but differences in transport rates may also depend on their molecular charge, hydrophobicity, configuration, and non-specific binding (Clauss and Jain 1990).

The fine structural endothelial changes in neurinomas have not been examined in relation to the two different tissue types, but a preponderance of pathological vascular alterations in the reticular areas has been suggested by light microscopical observations (Müller 1968) and was confirmed in the present study. The well-known high protein content in the cerebrospinal fluid of patients with intracranial neurinomas has been explained by these vascular changes and interpreted as a result of transudation of serum proteins (Merritt 1935). This interpretation is corroborated by the immunohistochemical demonstration of extravascular serum proteins in tumour areas where such vascular changes prevail.

In neurofibromas, the loose tissue structure ordinarily is of "mucoid" quality (Krücke 1939) and like to the focal subperineurial broadening of normal peripheral nerve, is apparently not related to permeability changes. The blood-tumour barrier may be porous, however, in exceptional cases that are atypical in regard to either the diffuse tissue spread or to the internal tumour structure. Dermal neurofibromas are also included in this group. They are neoplasms of the diffuse, non-encapsulated type by definition, and they presumably originate from the terminal nerve segments in which the perineurial barrier is incomplete. The observed changes in dermal neurofibromas can thus be explained by interstitial diffusion of proteins into the tumour rather than by an increased permeability of the tumour vessels.

The lack of permeability changes in malignant peripheral neuroectodermal tumours is comparable with that found in medulloblastomas, tumours of similar cell density (Figge et al. 1991). The compact structure of these malignant tumours may contribute to the preservation of the barrier. Systemic therapeutic approaches with use of endogenous proteins as carriers should take low permeability into account. Our observations are in line with several clinical studies of larger series of peripheral tumours which report no effects of chemotherapy on survival rates (Ariel 1983; Ducatman et al. 1986; Hruban et al. 1990).

Preoperative neuroradiological studies have shown differences in contrast enhancement between the various peripheral nervous system tumour types that seem to correlate well with our immunohistochemical studies of the blood-tumour barrier (Chui et al. 1988). The molecular weight of the gadolinium-DTPA complex, however, amounts to 938 daltons only and thus is several dimensions lower than the endogenous tracers used in our study. To confirm the correlation of the immunohistochemical serum protein staining with contrast enhancement, which may be of clinical importance, it appears advisable in future cases to perform a concomitant neuroradiological and neuropathological examination on the same tumours.

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