Histol Histopath (1994) 9: 535-540

Histology and Histopathology

Lectin histochemistry of human meningiomas

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Summary. The lectins Peanut agglutinin (PNA), *Canavalia ensiformis* (Con A), *Ulex europaeus*-1 (UEA-1), *Dolichos biflorus* (DBA), *Triticum vulgaris* (WGA) were studied in a series of 36 meningiomas (16 meningotheliomatous-including 3 recurrences, 7 transitional, 4 angiomatous, 2 «hemangiopericytic», 3 papillary-including 1 recurrence, 4 anaplastic-including 3 recurrences.

PNA binds to all cases of meningotheliomatous, transitional, papillary and anaplastic meningiomas (including recurrent cases) but the staining is more intense in tumor cells of anaplastic and papillary type. A semiquantitative study showed differences of PNA-reactivity in the different subtypes of meningiomas. In meningotheliomatous meningiomas PNA-positivity was encountered in numerous neoplastic cells (50%), whereas papillary and anaplastic subtypes expressed strong cytoplasmic staining of few tumor cells (< 5%). Con A shows the same pattern of reactivity described for PNA, but more weakly.

Our results suggest that PNA is a marker of differentiation in meningiomas rather than malignant transformation and can have prognostic relevance.

Key words: Lectins, Meningiomas, Tumoral differentiation markers

Introduction

Recent immunocytochemistry, cytogenetics and steroid receptor analysis studies have increased our knowledge about diagnosis, prognosis and the natural history of meningiomas; therefore it is now possible to make some changes in the present WHO classification (Zulch, 1975) of the tumors of the meninges (Jellinger, 1989; Kepes, 1989; Scheithauer, 1990). Maier et al. (1992) have defined four histological subtypes of meningiomas with clinical relevance and with different rates of recurrence: classic, atypical, anaplastic and «hemangiopericytic». Immunohistochemical findings seem to confirm the epithelial nature of meningioma cells because they almost always show positivity for vimentin, but may express cytokeratins and often also Epithelial Membrane Antigen (EMA) (Coackham et al., 1985; Kepes, 1986; Theaker et al., 1986; Schnitt and Vogel, 1986; Radley et al., 1987; Winek et al., 1989; Artlich and Schmidt, 1990; Cruz-Sánchez et al., 1992). Immunohistochemical studies have not shown any difference between the common forms of meningiomas and the angioblastic variants. Bohling et al. (1983) and later Nakamura et al. (1987) found the tumor cells to be negative for factor VIII-related antigen for the *Ulex europaeus* lectin (UEA-1). Neither has immunohistochemistry indentified any markers that may indicate the possibility of impeding recurrence of the neoplasm (Artlich and Schmidt, 1990). Therefore, the clinical prognosis of meningiomas cannot always be predicted from histological and histochemical observations; a better approach would be to supplement the morphological criteria with defined structural alterations frequently associated with neoplastic transformation, such as changes in the glycosylation process of cell-surface glycoconjugates and secreted glycoprotein molecules. In this respect, lectins, specific carbohydrate-binding proteins, can be extensively used to recognize glycoconjugate alterations which may occur in meningiomas. However, only one report has described lectin binding sites in meningiomas without information about recurrence (Kleinert and Radner, 1987).

The aim of this study was to investigate the histochemical pattern of five lectins in a series of primary meningiomas including some cases of local recurrence.

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Materials and methods

36 surgical specimens of primary meningiomas were fixed in 4% buffered formalin and embedded in paraffin; histological sections were stained with haematoxylin-eosin in order to study the microscopic morphology of the different subtypes of meningiomas, which were subdivided on the ground of the WHO classification as follows: 16 meningotheliomatous (including 3 recurrences), 7 transitional, 4 angiomatous, 2 «hemangiopericytic», 3 papillary (including 1

recurrence), 4 anaplastic (including 3 recurrences).

The lectins used are listed in Table 1. To detect lectin-binding sites 5 µm-thick sections were incubated with the following technique: 1) 3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase; 2) three rinses in distilled water; 3) phosphate-buffered saline peroxidase-conjugated (HRP) lectins (listed in Table 1) for 16-18 hrs in a moist chamber at 4 °C; 5) three changes in PBS for 5 min each; 6) 3,3'-diamino-benzidine tetrahydrochloride (DAB) (Sigma) and 0.03% hydrogen peroxide for 10 min in the dark at room temperature (Weir et al., 1974); 7) several rinses in distilled water. A solution of the same lectin concentration containing sugar hapten (0.2M) to inhibit lectin binding was used on adjacent control sections.

In parallel sections, before the incubation with PNA, sialic acid was removed with neuramindase from *Vibrio cholerae* (Calbiochem, Behring) 1 U/ml for 30 min in a moist chamber at room temperature.

The degree of lectin binding sites to neoplastic elements was evaluated semiquantitatively as negative, i.e., no staining (-), and positive when <5% (+), 5-50% (++), and > 50% (+++) of tumor cells stained; the sections were scored blindly.

Results

The percentage of tumor cells stained with all lectins used is summarized in Table 2.

All cases of meningotheliomatous, transitional, papillary and anaplastic meningiomas (including recurrent cases) were stained with PNA, and before as well as after treatment with neuraminidase (Fig. 1a,b); the staining was more intense in tumor cells of anaplastic and papillary subtypes (Figs. 2, 3a); in this latter form the PNA- reactivity was mainly localized as diffuse surface membrane pattern (Fig. 3a). With Con A the same pattern of reactivity described for PNA was

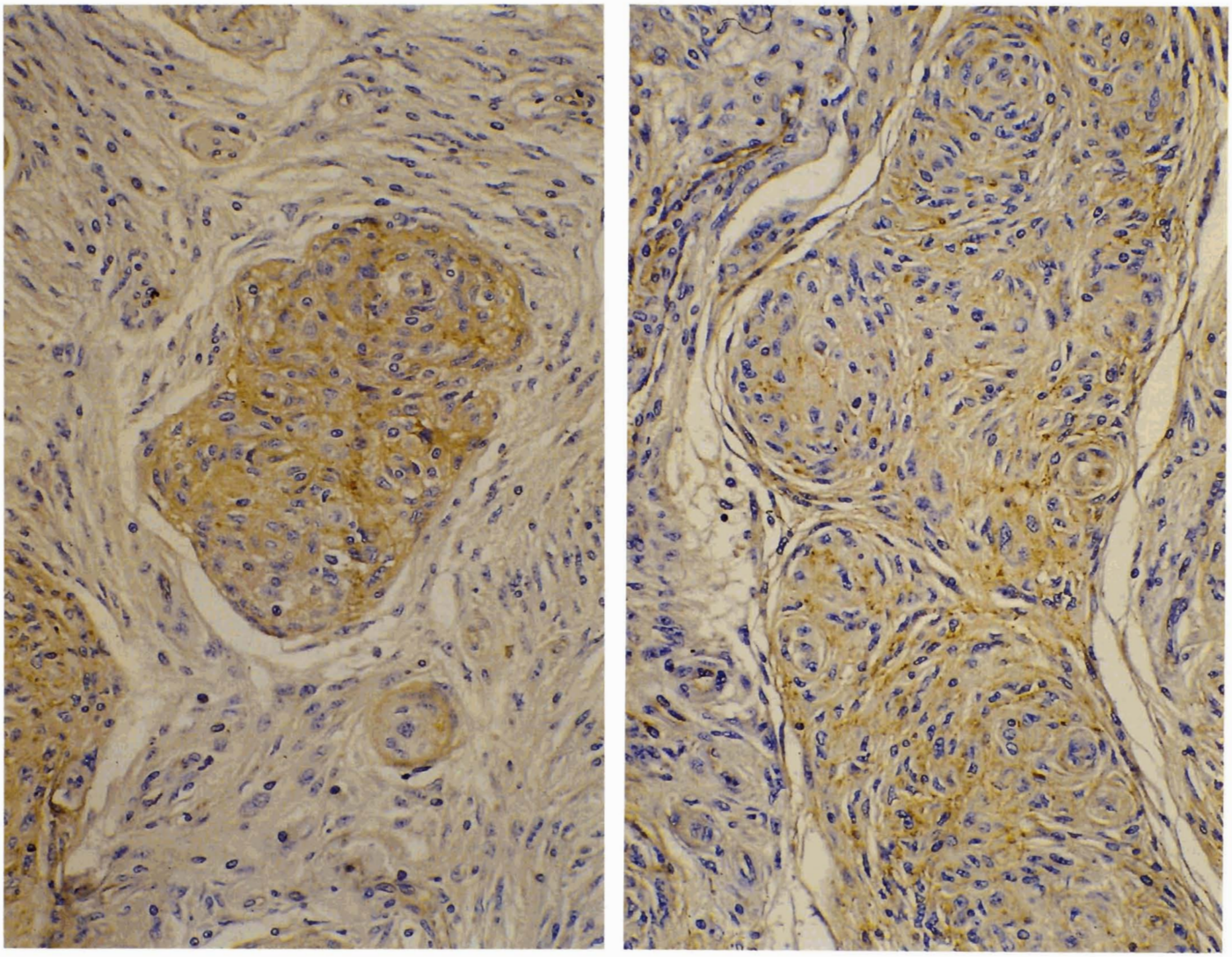

Fig. 1. Meningotheliomatous meningioma. Cells arranged in islands are reactive for both PNA **(a)** and Con A **(b)**.

Table 1. Sugar specificities, inhibitory carbohydrates and dilutions of lectins used.

LECTIN	SUGAR SPECIFICITY	INHIBITOR	DILUTION
Arachis hypogaea (PNA)	Galactosyl-ß-(1-3)-N-Acetyl-D-Galactosamine	D-Galactose	16 µg/ml
Canavalia ensiformis (Con A)	D-Glucose, D-Mannose	D-Glucose, D-Mannose	16 µg/ml
Ulex europaeus (UEA-1)	L-Fucose	L-Fucose	2 µg/ml
Dolichos biflorus (DBA)	α-N-Acetyl-D-Galactosamine	α-N-Acetyl-D-Galactosamine	8 µg/ml
Triticum vulgaris (WGA)	N-Acetyl-D-Glucosamine, N-Acetyl-neuraminic acid	N-Acetyl-D-Glucosamine	4 µg/ml

encountered, weaker; Con A, however, did not stain the angiomatous type. In a case of meningioma with foci of glandular metaplasia secretory meningioma from the classification of Scheithauer, 1990) PNA and Con A clearly stained the cytoplasm of the metaplastic cells that border the glandular structures (Fig. 3b). Positivity for both PNA and Con A was found around and within psammoma bodies. In the angiomatous meningioma, the endothelial cells were reactive with UEA-1; this pattern was related to the large amount of blood vessels typical of this variant, whereas the tumor cells themselves were negative for UEA-1 (Fig. 4). In hemangiopericytic forms no staining was encountered with any lectin utilized. Both WGA and DBA were negative in all meningiomas examined.

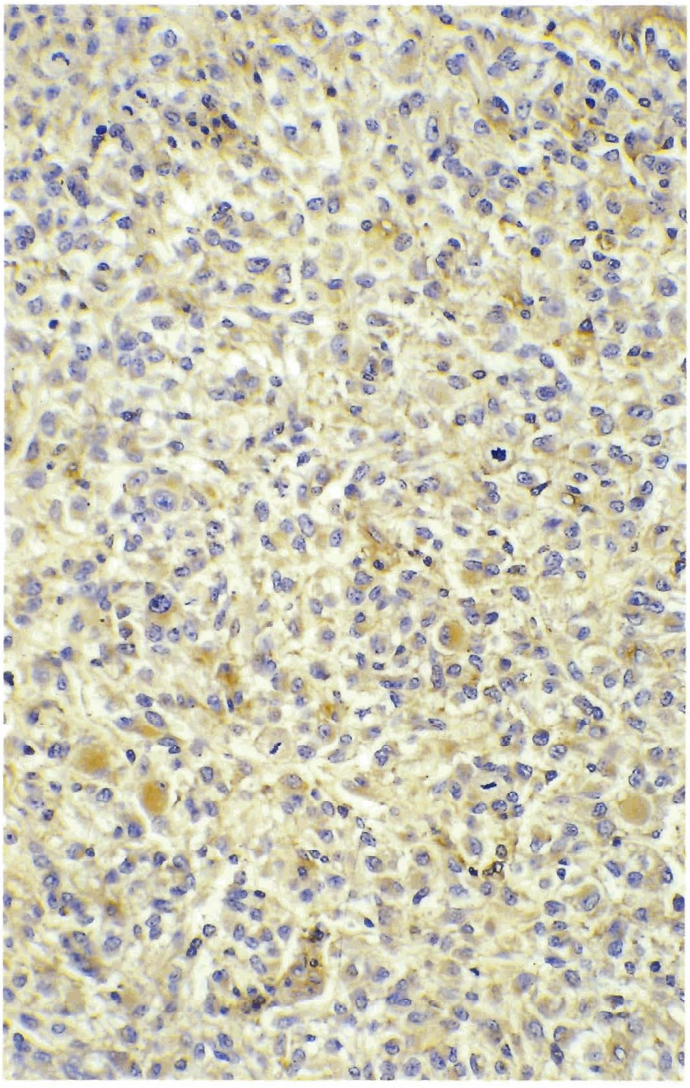

Fig. 2. Anaplastic meningioma. Some neoplastic elements with a strong cytoplasmic positivity for PNA.

Discussion

The results of the present study on different subtypes of meningiomas show a co-expression of glyco-conjugates in neoplastic cells. In particular meningo-theliomatous, transitional, angioblastic, papillary and anaplastic meningiomas express either galactosyl residues, not masked by sialic acid (staining with PNA and PNA/S) or mannosidic and glucosidic residues (staining with Con A). Our data about PNA and Con A are well correlated with a report by other authors (Kleinert and Radner, 1987), in which lectin binding to tumor cells of meningotheliomatous and anaplastic meningiomas were reported, using labelled Con A, PNA as well as VFA (*Vicia faba*) and LAA (*Laburnum alpinum*) lectins. In the present study, semiquantitative differences of PNA-reactivity have been encountered between the different subtypes of meningiomas; in fact the menigotheliomatous meningiomas showed numerous tumor cells (> 50%) with a diffuse granular PNA-positivity within the cytoplasm, whereas papillary and anaplastic subtypes expressed strong staining in cytoplasmic membrane of few tumor cells (< 5%). The PNA reactivity represents the detection of nascent oligosaccharides and may be related to incomplete glycoprotein synthesis. This was described in human foetal colon (Coopman et al., 1987; Forard et al., 1987)

Table 2. Lectin binding to neoplastic cells of meningiomas.

LECTIN	MENINGIOMA SUBTYPES					
	MTH	TST	PAP	ANA	ANG	HPC
PNA, PNA/S	+++	++	+	+	+	
Con A	+++	++	+	+	+	
UEA-1	-	-	-	-	-	
DBA						
WGA						

-: negative; +: < 5% of tumor cells stained positively; ++: 5-10% of tumor cells stained positively; +++: > 50% of tumor cells stained positively; MTH: meningotheliomatous meningioma; TST: transitional meningioma; PAP: papillary meningioma; ANA: anaplastic meningioma; ANG: angiomatous meningioma; HPC: hemangiopericytic meningioma.

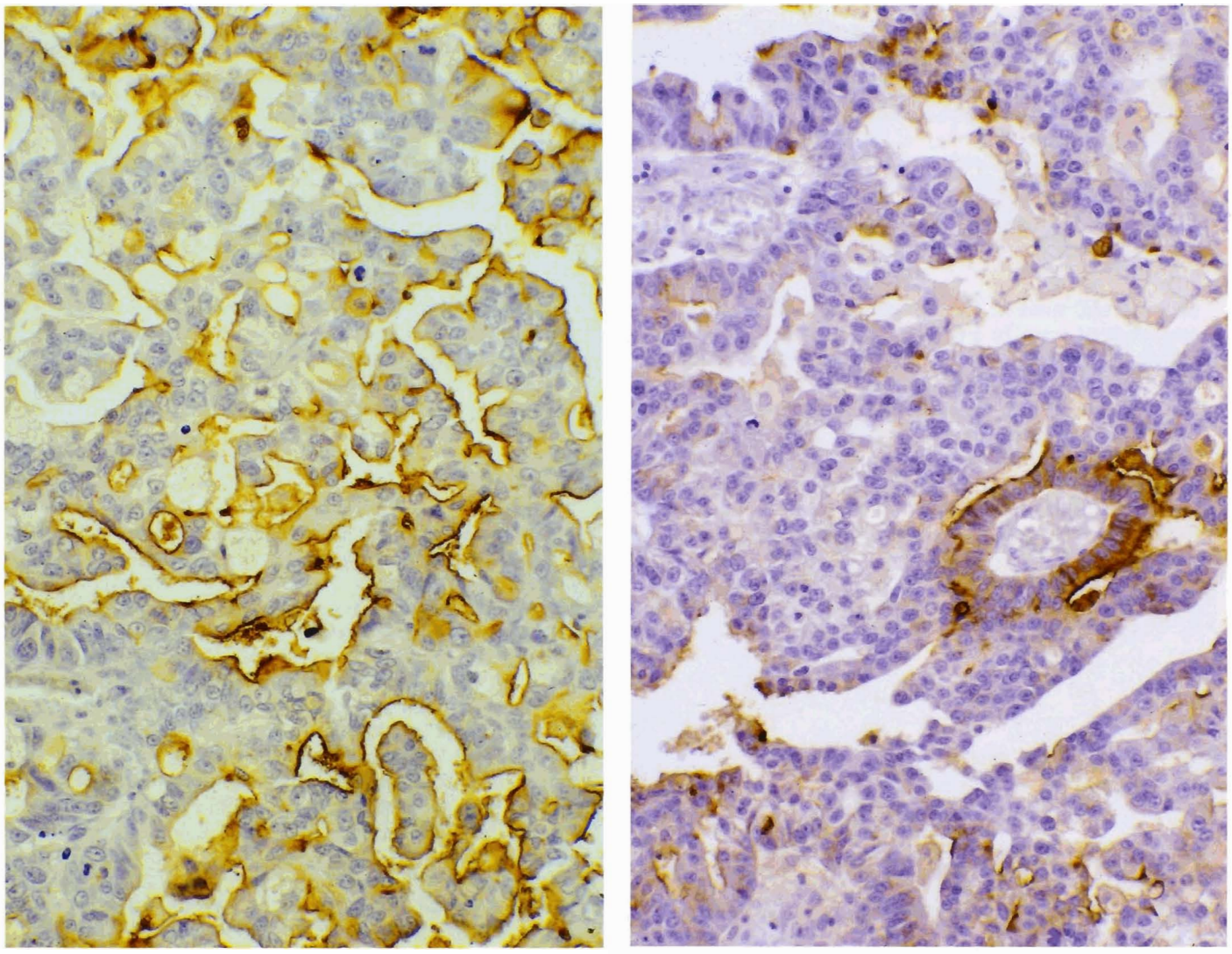

Fig. 3. Papillary meningioma. Strong PNA-reactivity is localized in plasma membrane of neoplastic cells (**a**). Some areas of glandular metaplasia also showed cytoplasmic positivity (**b**).

in large bowel carcinoma (Cooper, 1982), and in intestinal metaplasia of the urinary bladder with foci of dysplastic epithelium (Barresi and Marafioti, 1990).

We presume that in meningiomas, PNA-binding sites increased parallel to the degree of tumor differentiation. The anaplastic forms had fewer tumor positive cells than well-differentiated subtypes. These suggestions are agreement with similar observations obtained with PNA on the anaplastic forms of oligodendrogliomas (Bardosi et al., 1988; Figols et al., 1991). Therefore, our results suggest that PNA is a marker of differentiation in meningiomas rather than malignant transformation. We hypothesize that the increased amount of PNA-binding sites in well-differentiated meningiomas leads to a low proliferation rate, whereas less-differentiated ones escape this expression with an enhanced replicating capacity.

Moreover, it is noteworthy that our cases of anaplastic meningiomas are recurrent tumors with primitive meningotheliomatous histological pattern, suggesting thus a clonal selection of anaplastic cell population, lacking PNA-reactivity. In conclusion, PNA reactive pattern in meningiomas could have prognostic relevance even if follow-up studies will be needed to define the effectiveness.

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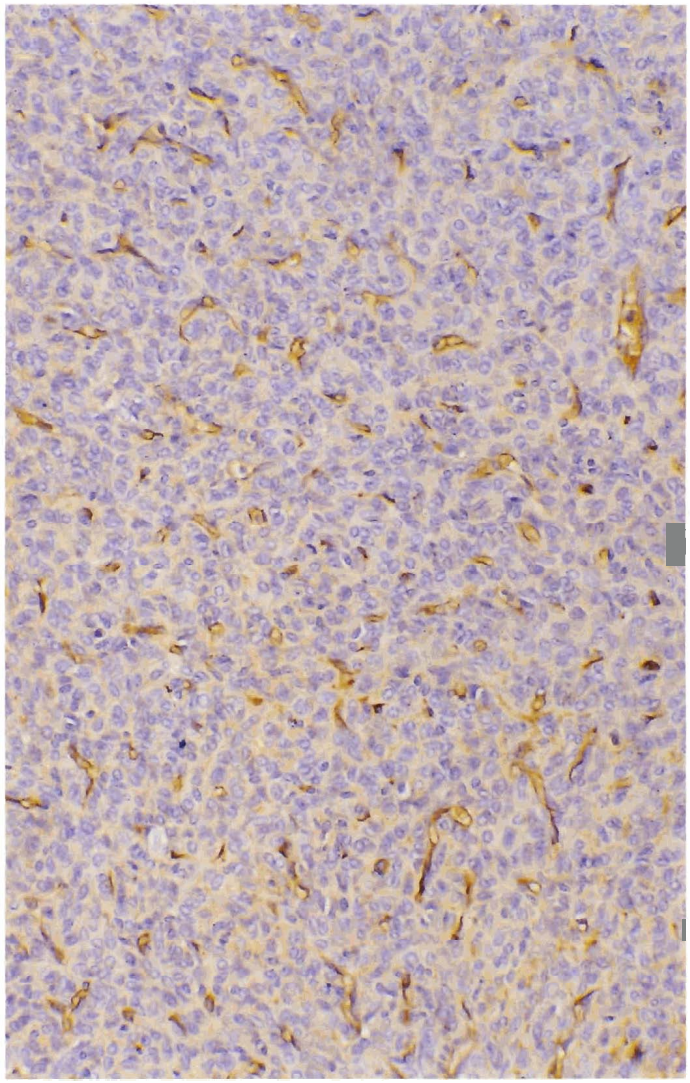

Fig. 4. Hemangiopericytic meningioma. UEA-1 selectively stains endothelial cells.

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Accepted April 11, 1994