An ultrastructural and immunohistochemical study of extracellular matrix in meningiomas

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Summary. Extracellular matrix of meningiomas was studied by light and electron microscopy with the aid of immunohistochemical techniques. Special attention was paid to the distribution of type I, III, IV, V collagens and laminin with a comparison between meningothelial and fibroblastic types. Connective tissue fibers and basement membrane were not found among the tumor cells in the meningothelial type, but were found in the fibroblastic type. The immunolocalizations were consistently demonstrated extracellularly, but were not within the cytoplasm. Type I, III and V collagens were usually demonstrated in the fibrous septum in the meningothelial type, while they were localized among the tumor cells in the fibroblastic type. Furthermore, type IV collagen and laminin were demonstrated within the vascular walls or around the syncytium in the meningothelial type, while they were localized among the tumor cells in the fibroblastic type. In both types the expression of type IV collagen and laminin was closely related to the distribution of basement membrane. Although meningothelial and fibroblastic meningiomas showed quite different distribution of extracellular matrices, the profile of collagen types expressed by these two basic types was essentially the same. The cellular derivation of meningiomas was discussed with particular attention to the structure of human arachnoid villi and meninges.

Key words: Meningioma, Collagen, Laminin, Immunohistochemistry, Electron microscopy

Introduction

It is widely accepted that meningiomas disclose common ultrastructural features of their cellular components regardless of histological subtypes (Kepes, 1961;

Napolitano et al., 1964; Nyström, 1965; Cervós-Navarro and Vázquez, 1969; Schwechheimer et al., 1984; Halliday et al., 1985). There is substantial evidence that meningiomas are derived from arachnoid cells (Tani et al., 1974; Schwechheimer et al., 1984) which have a propensity for producing extracellular matrix under both physiological and pathological conditions (Schultz and Pease, 1959; Kibler et al., 1961; Anderson and Kissane, 1977; Hirano, 1986). However, it remains obscure why two basic meningothelial and fibroblastic types of meningiomas show such a marked difference. In this paper, we have studied extracellular matrix of meningiomas by light and electron microscopy to clarify the difference between the two basic subtypes. Special attention was immunohistochemically paid to the distribution of type I, III, IV, V collagens and laminin. The morphological comparison of meningiomas with human arachnoid villi and meninges was done to clarify the cellular derivation of these tumors.

Materials and methods

Meningiomas

Tumor specimens were obtained at surgery from 23 subjects with 16 meningothelial and 7 fibroblastic meningiomas.

Light and Electron Microscopy

For light microscopy, tumor specimens were fixed in 3.7% buffered formalin and embedded in paraffin. Thin sections were stained with hematoxylin and eosin (H&E). For electron microscopy, small specimens were fixed in cacodylate-buffered 2.5% glutaraldehyde for 2h, postfixed in 1% osmium tetroxide for 1h and embedded in Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate followed by lead citrate and examined with a Hitachi H-600 electron microscope.

Antibodies

Rabbit antisera to bovine type I, III collagens and human type IV, V collagens were purchased from Didets, Tokyo, Japan. Rabbit antiserum to laminin was purchased from E-Y laboratories, Inc. San Meteo, USA. The crossreactivity of the antibodies to bovine type I, III collagens with corresponding human collagens was confirmed by ELISA.

Immunofluorescence Microscopy

For indirect immunofluorescence microscopy, 4-6 μm thick cryostat sections of frozen tissue were fixed with acetone for 10 min at 4° C and dried. Primary antibodies were incubated overnight at a dilution of 1:10 for anti-type I, III, V collagens and 1:50 for anti-type IV collagen or laminin in phosphate-buffered saline solution (PBS), pH 7.2. After several washings in PBS, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Cappel Laboratories Inc., Cochranville, USA), was applied as secondary antibody at a dilution of 1:10 in PBS for 2 h. After several washings in PBS, the slides were mounted in 10% glycerol in PBS and examined with an Olympus fluorescein microscope model AH2-FL. Primary antibodies were replaced with normal rabbit serum (Dakopatts, Denmark) as controls.

Immunoelectron Microscopy

For immunolocalization at the ultrastructural level, an indirect peroxidase-labelled antibody method (Nakane and Pierce, 1967) was used. Fresh tumor specimens were fixed in periodate-lysine-paraformaldehyde (Nakane, 1975) for 12h at 4° C and washed in PBS containing sucrose. Cryostat sections were previously incubated with normal goat serum (Dakopatts, Denmark) to block non-specific bindings. Then, they were incubated with primary antibodies to type IV collagen and laminin overnight at a dilution of 1:30 at 4° C. After several washings in PBS, horseradish peroxidase-conjugated goat anti-rabbit IgG (Dakoppats) was applied as secondary antibody at a dilution of 1:30 for 6h and washed in PBS. Subsequently, the sections were fixed in 1% glutaraldehyde in PBS, washed in PBS and immersed in Graham-Karnovsky's solution (Graham and Karnovsky, 1966). The sections were postfixed with 2% osmium tetroxide, dehydrated in graded alcohol and embedded in Epon-Araldite mixture. Ultrathin sections were examined without additional stainings with a Hitachi H-600 electron microscope. Controls were made by supplying normal rabbit serum (Dakopatts, Denmark) as primary antibodies.

Results

Histology and FITC

Meningothelial type

The interstitial tissue was sparse in the syncytium and was confined to the vascularized fibrous septum that separated the tumor into lobules (Fig. 1A). Immunohistochemically, type I, III collagens were diffusely distributed in the fibrous septum (Fig. 1B, C). The vessels were surrounded by connective tissue containing abundant type I, III collagens. Type IV, V collagens and laminin were co-localized around the syncytium and the vascular walls (Fig. 1D, E, F). The cytoplasm of meningioma cells were not stained with any of the collagen types or laminin.

Fibroblastic type

Numerous interstitial fibers were seen among interwoven bundles of spindle-shaped tumor cells (Fig. 2A). Immunohistochemically, type I, III, IV, V collagens and laminin were not demonstrated within meningioma cells, but they were in the extracellular space. Type I collagen was diffusely distributed in a thick fibrillar pattern (Fig. 2B) and type III collagen in a thin fibrillar pattern (Fig. 2C). Type V collagen was distributed in a granular or thin fibrillar pattern (Fig. 2E). Type IV collagen and laminin were co-localized in the vascular walls and around the tumor cells in a fine fibrillar pattern (Fig. 2D,F).

Electron Microscopy

Meningothelial type

Connective tissue fibers and basement membrane were usually not seen among the tumor cells within the syncytium. However, the fibrous septum contained closely packed collagen fibers, some microfibrils and elastic fibers. There were a number of elongated cells which were quite similar to dural border cells (Nabeshima et al., 1975; Schachenmayr and Friede, 1978) and which were intermingled with some cellular debris (Fig. 3). The extracellular space sometimes contained fine granular material. Basement membrane separated the syncytium from the fibrous septum or the perivascular connective tissue (Fig. 4).

Fibroblastic type

The slender cytoplasmic processes of tumor cells were intermingled with narrow extracellular matrix containing collagen fibers, microfibrils, elastic fibers and fine granular material with a variable ratio (Fig. 5). Continuous basement membrane was often seen along the cell membrane abutting the extracellular matrix (Fig. 6). The cytoplasm usually contained well-developed rough endoplasmic reticulum and numerous free ribosomes. The dilated rough endoplasmic reticulum was sometimes filled with fine granular material.
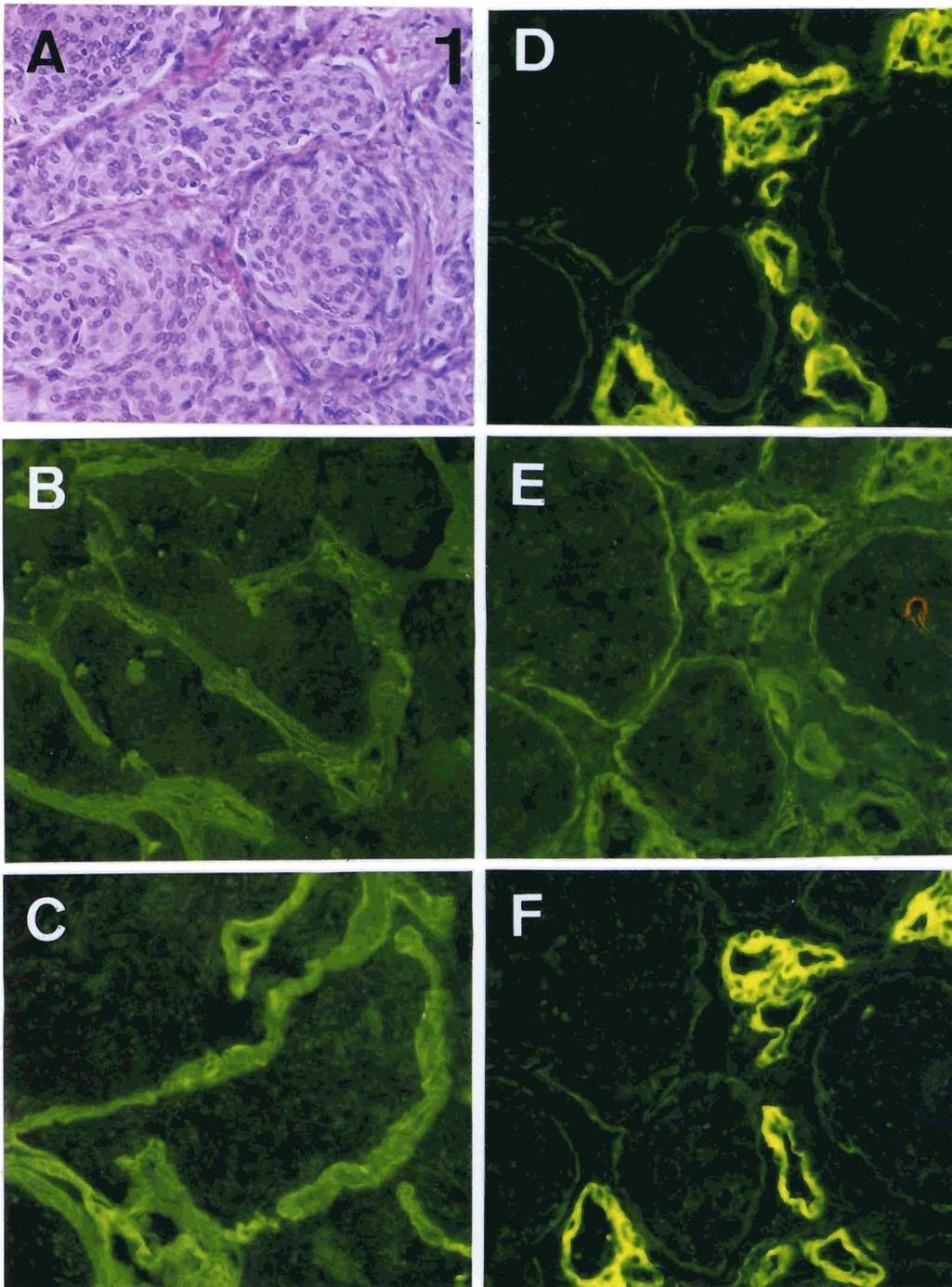
Fig. 1. Hematoxylin & Eosin (A) and immunofluorescent stainings (B-F) of the meningothelial meningioma.
A: Vascularized fibrous septum separates the tumor into lobules. × 185.
B,C: Type I (B) and III (C) collagens are localized diffusely in the fibrous septum and in the vascular walls. B, × 92, C × 185.
D,E,F: Type IV (D), Type V (E) collagens and laminin (F) are co-localized in the vascular walls and around the syncytium. × 185

Immunoelectron Microscopy

Type IV collagen and laminin showed an almost similar distribution and were closely related to the localization of basement membrane. In the meningothelial type, neither antigen was demonstrated among the tumor cells, but were localized around the syncytium being in contact with the fibrous septum or the perivascular connective tissue (Fig. 7A,B). In the fibroblastic type, they were demonstrated at the surface of tumor cells confronting interstitial fibers (Fig. 8A,B). Type IV collagen and laminin were not found within the cytoplasm of tumor cells.

Discussion

Although meningiomas are customarily divided into a number of separately designated variants (Cushing and Eisenhardt, 1962; Zülch, 1979), they are represented by two basic subtypes: meningothelial and fibroblastic. It
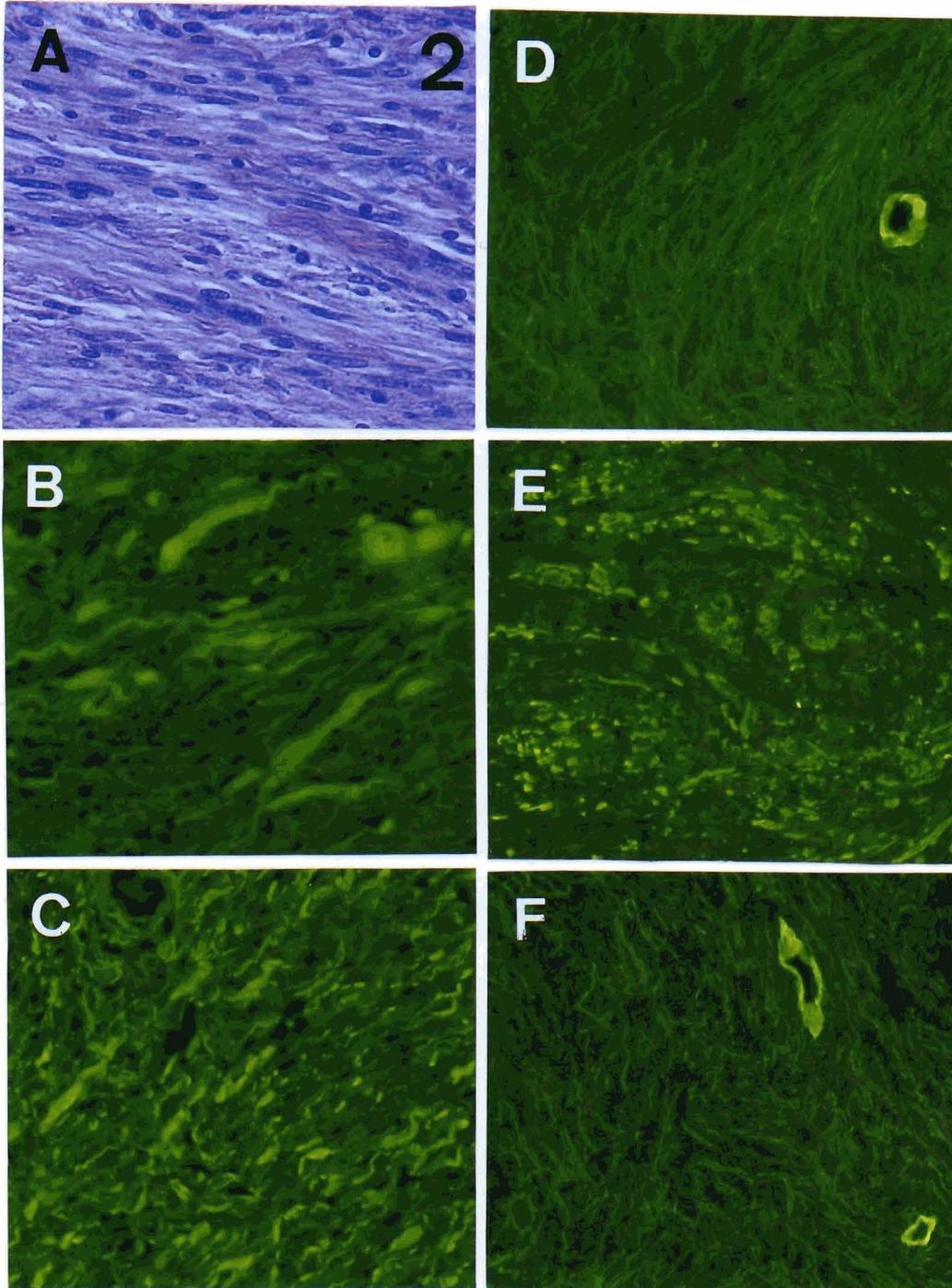
Fig. 2. Hematoxylin & Eosin (A) and immunofluorescent stainings (B-F) of the fibroblastic meningioma. **A:** Fusiform tumor cells are arranged in tiers with intervening interstitial fibers. × 185. **B:** Type I collagen shows diffuse fibrillar pattern. × 185. **C:** Type III collagen shows thin fibrillar pattern. × 185. **D,F:** Type IV collagen (D) and laminin (F) show diffuse fine fibrillar pattern. Vascular walls are stained. × 185. **E:** Type V collagen shows granular or thin fibrillar pattern. × 185

has been demonstrated that both types show common ultrastructural features of their cellular components such as intermediate filaments, interdigitations and desmosomes (Kepes, 1961; Napolitano et al., 1964; Nyström, 1965; Cervós-Navarro and Vázquez, 1969; Schwechheimer et al., 1984; Halliday et al., 1985). However, in this study a striking contrast of the extracellular matrices was ultrastructurally demonstrated between meningothelial and fibroblastic types. By immunohistochemical analysis, Bellon et al.

(1985) could demonstrate a thin extracellular deposition of type IV collagen in the transitional type. McComb and Bigner (1985) could demonstrate a fibrillar distribution of laminin in the fibroblastic and transitional types but not in the meningothelial type. Rutka et al. (1986) have recently shown that cultured meningioma cells express type I, III procollagens, type IV collagen and laminin regardless of the histological subtypes. We could demonstrate type IV collagen and laminin around the syncytium in the meningothelial type. These two antigens

Fig. 3. The fibrous septum of the meningothelial meningioma contains closely packed collagen fibers (asterisks), microfibrils (arrows) are intermingled with elongated cells quite similar to dural border cells (Nabeshima et al., 1975; Schachenmayr and Friede, 1978). × 9,000

distribution of type I, III and V collagens. In the fibroblastic type, abundant type I, III and V collagens were found among the tumor cells, while in the meningothelial type, they were found in the fibrous septum and around the syncytium.

Meningiomas are believed to derive from a single type of cell called arachnoid cells (Tani et al., 1974; Schwechheimer et al., 1984). Accordingly, it remains obscure why two basic types of meningiomas have disclosed such marked differences. There is substantial evidence that meningiomas derive from meninges or arachnoid villi (Cushing, 1922; Bailey and Bucy, 1931; Wolman, 1952; Shabo and Maxwell, 1968). Schachnmayr and Friede (1978) ultrastructurally studied the dura-arachnoid interface of human meninges. In the dural border layer, a small number of collagen fibers and microfibrils were found extracellularly, while in the arachnoid barrier layer, there were almost no interstitial fibers. Basement membrane was only seen at the subarachnoid surface of arachnoid barrier cells. Dural border cells had no basement membrane. Recently, Yamashima (1988a,b,c) and Kida et al. (1988) demonstrated ultrastructurally that human arachnoid villi were usually encompassed by both the arachnoid cell layer and the fibrous capsule which reflected from the arachnoid barrier layer or the dural border layer of the surrounding meninges, respectively. In the fibrous capsule, there were tiers of dural border cells which were intermingled with collagen fibers

corresponded to the ultrastructural localization of basement membrane, which was confirmed by immunoelectron microscopy. Furthermore, meningothelial and fibroblastic types showed a marked contrast in the

and elastic fibers and were connected by a small number of cell junctions. The thickened portion of the arachnoid cell layer formed the cap cell cluster which was characterized by a paucity of interstitial fibers. Core arachnoid cells formed a trabecular network and were surrounded by bundles of collagen fibers, microfibrils and elastic fibers. Basement membrane discontinuously covered core arachnoid cells and the innermost surface of the arachnoid cell layer.
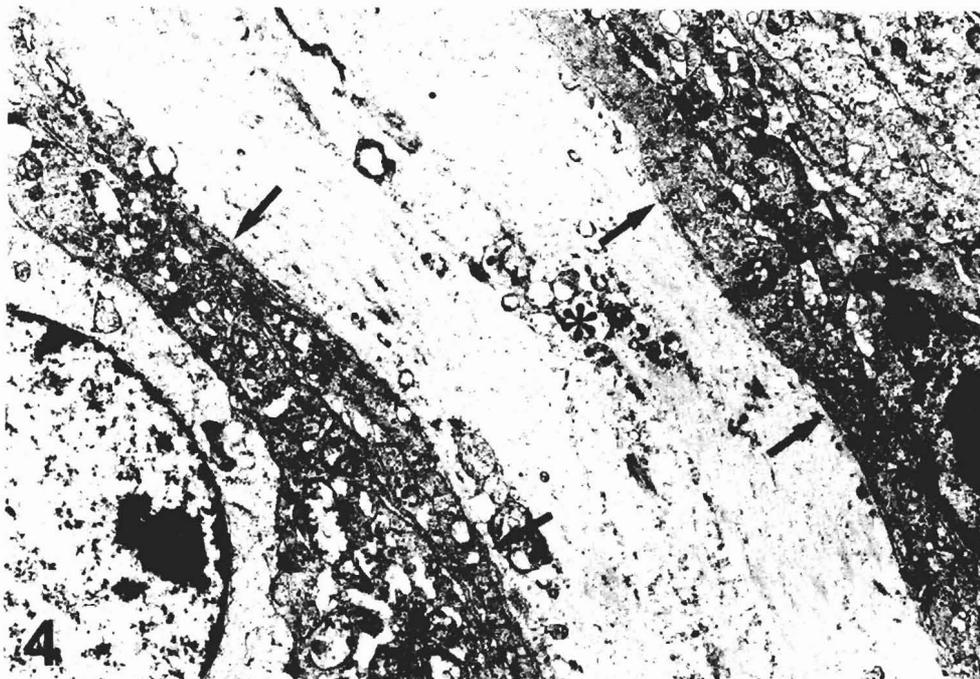

Fig. 4. The fibrous septum of the meningothelial meningioma contains a number of interstitial fibers and some cellular debris (asterisk). Basement membrane (arrows) is seen between the fibrous septum and tumor cells. × 9,000
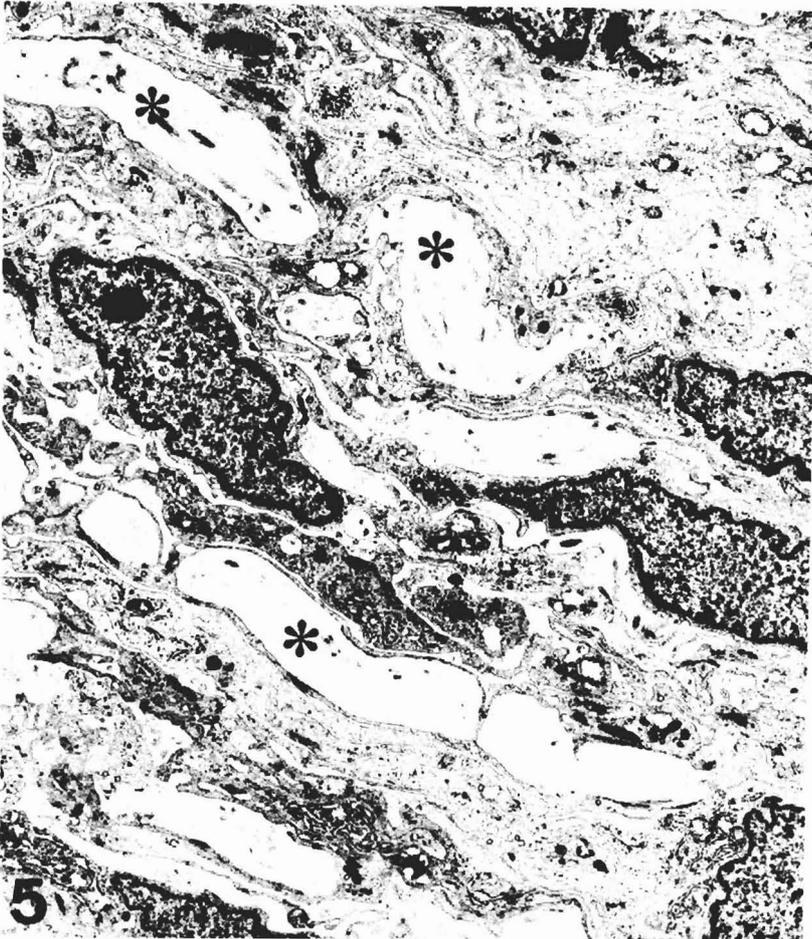
Fig. 5. Flattened cells are arranged in tiers and intermingled with connective tissue fibers (asterisks) in the fibroblastic meningioma. × 4,500

In summary, the profile of collagen types expressed by meningothelial and fibroblastic types is essentially the same, although these two basic types showed quite different distribution of extracellular matrices. A number of transitional cell forms found within the dura-arachnoid interface in human arachnoid villi and meninges are conceivably reflected in the histological varieties of meningiomas.

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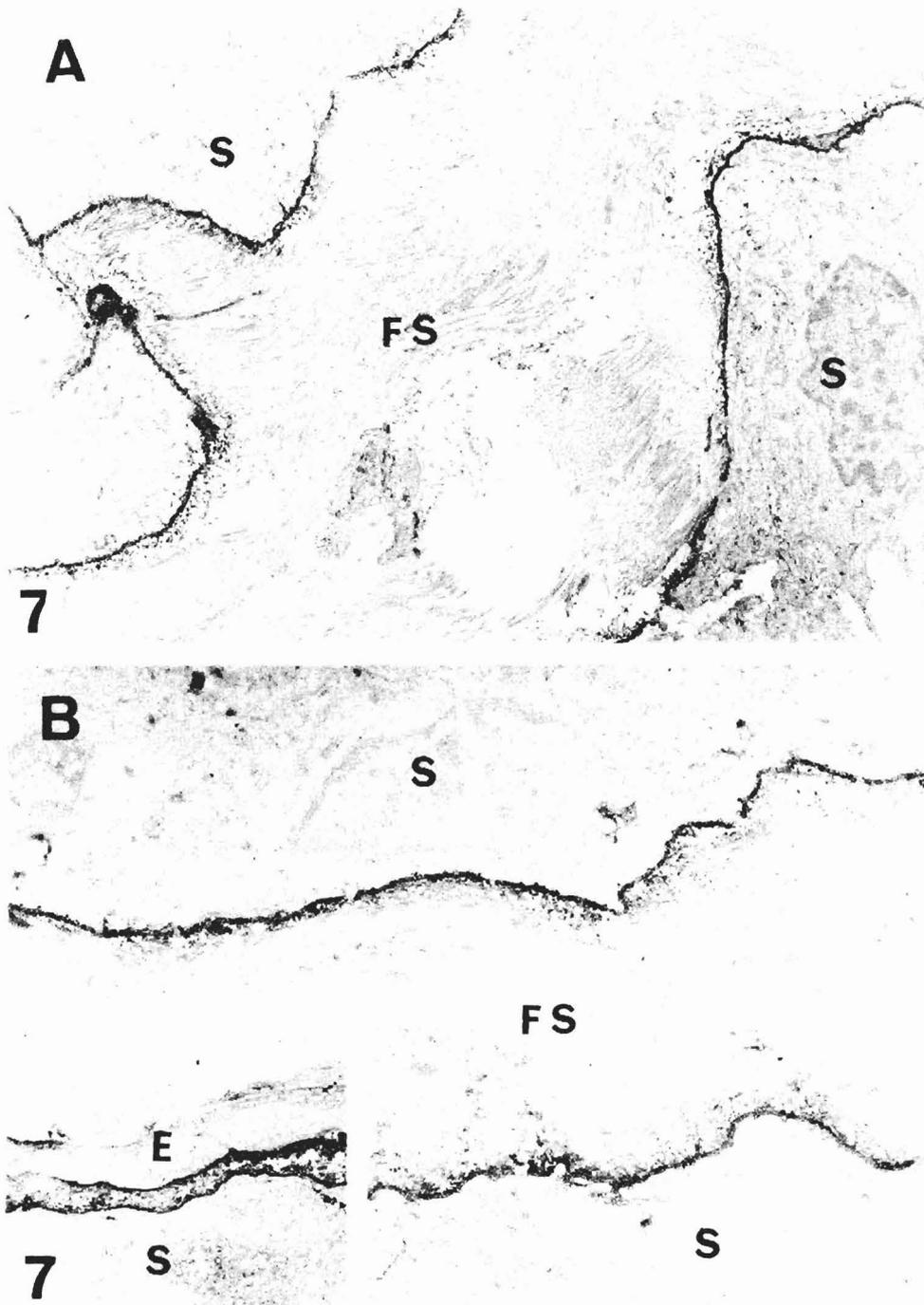
Fig. 6. Basement membrane (arrows) is seen at the surface of tumor cells confronting the extracellular matrix in the fibroblastic meningioma. Microfibrils are also seen (asterisks). × 22,500

A

S

FS

S

7

B

S

FS

E

7 S

S

Fig. 7. Immunoelectron microscopy of the meningothelial meningioma. Type IV collagen **(A)** and laminin **(B)** are co-localized along the outermost portion of the syncytium, being in contact with the fibrous septum. A, $\times$ 3,750. B, $\times$ 6,000 (Insert: Laminin is also present at the surface of endothelial and tumor cells. $\times$ 3,000). S, syncytium: FS, fibrous septum; E, endothelial cells.

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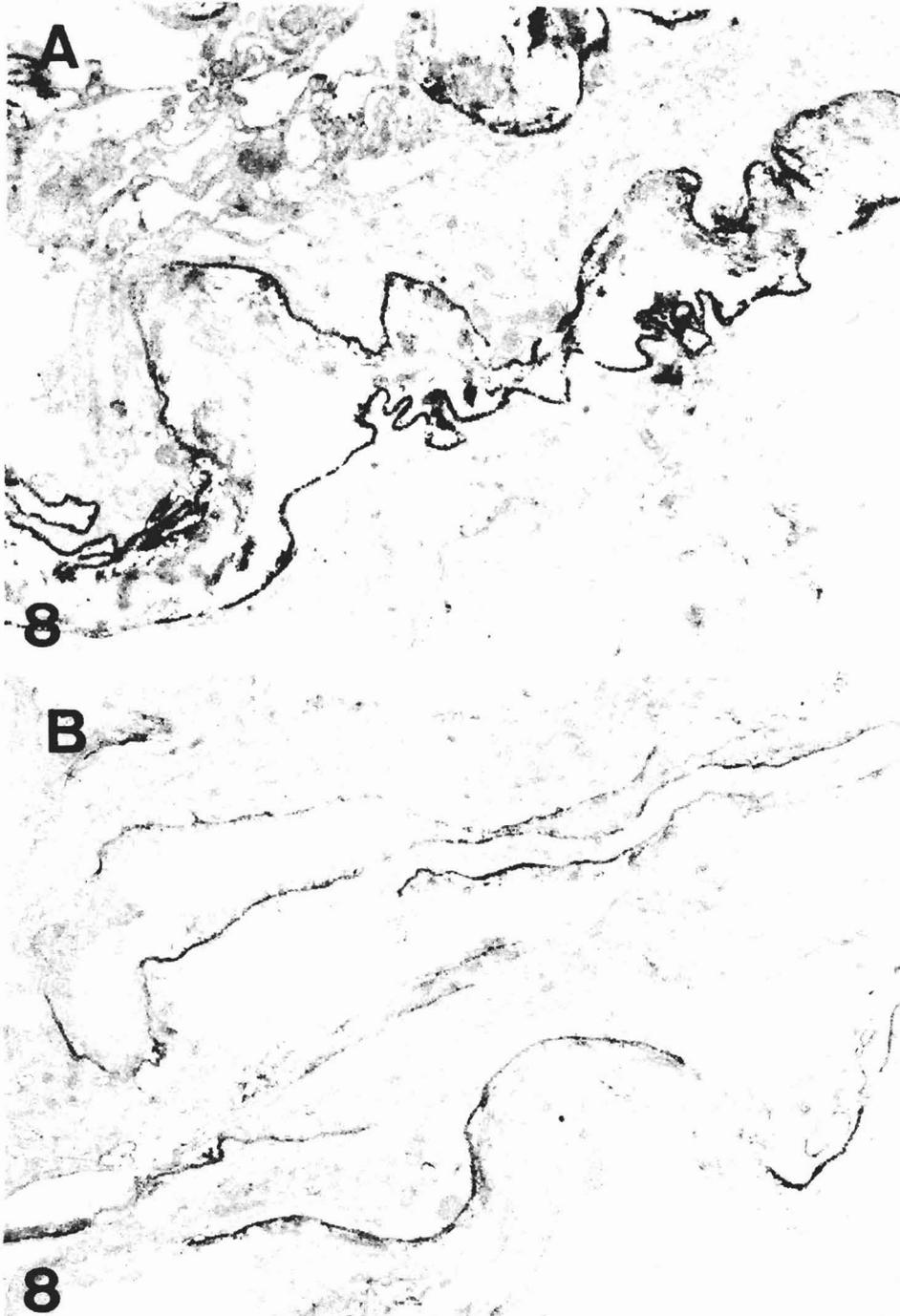
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Fig. 8. Immunoelectron microscopy of the fibroblastic meningioma. Type IV collagen **(A)** and laminin **(B)** are co-localized at the surface of tumor cells. A, × 6,000, B, × 5,250