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Amyloid stroma in meningiomas

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Summary. Twenty-three cases of meningiomas with psammoma bodies (PBs) and 15 without PBs have been studied using histochemical, ultrastructural and immunohistochemical methods for amyloid. Amyloid was found in all cases showing PBs and in only 5 cases in the group devoid of PBs. Meningiomas may contain amyloid in their stroma.

Key words: Amyloid – Meningioma – Amyloid P-component – Elastin – Psammoma bodies

Introduction

Amyloid stroma is present in several tumours. Medullary carcinomas of the thyroid display this stromal change (Rosai 1989), which is also seen in prolactinomas (Kovacs and Horvath 1983), pheochromocytomas (Steinhoff et al. 1992), islet cells tumours of the pancreas (Bordi and Tardini 1980), calcifying odontogenic tumours (Shafer et al. 1974) basal cell carcinomas of the skin (Looi 1983), tumours of the diffuse endocrine system (Pages 1974) and sporadic cases of carcinomas at different sites (Azzopardi and Lenner 1966). In a pilot study of a series of meningiomas with psammoma bodies (PBs) we became aware that the eosinophilic substance present in this type of tumours displayed the histochemical, immunohistochemical and ultrastructural features of amyloid. This finding has not been mentioned in the literature, with the exception of a very brief passage in a monograph on meningiomas (Kepes 1982). The purpose of the present study was to evaluate the presence and distribution of amyloid in a large series of meningiomas.

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Materials and methods

Thirty-eight meningiomas from which at least two blocks were available were studied. The cases were divided into two groups, according to the presence (23 cases, group A) or absence (15 cases, group B) of PBs. Tissues had been fixed in 10% buffered formalin and processed as routine. New sections were obtained from all cases and stained with haematoxylin and eosin (H&E) and Congo red.

In addition, from 8 cases of Group A the most representative block in terms of numerical content of PBs was selected and decalcified in citric acid, according to a method recently described (Foschini and Muzzi 1992). Briefly, blocks were deparaffinized and brought to water, through a graded alcohol series. The specimens were then decalcified overnight in 7% citric acid solution. After decalcification sections were reembedded in paraffin. Serial 5-μm sections were obtained from each decalcified block and stained with H&E, von Kossa method for calcium phosphate, Weigert-van Gieson for elastic fibres, Congo red and thioflavine T for amyloid. In addition immunohistochemistry was performed according to the method described by Hsu et al. (1981a, 1981b), using the following antibodies: polyclonal anti amyloid P-component (Dakopatts, Denmark) dilution 1:600 and polyclonal anti-elastin (kindly donated by Dr. G. Biagini, Ancona, Italy), dilution 1:200; monoclonal anti-kappa light chain (Dakopatts, Denmark) 1:16000; monoclonal anti-lambda light chain (Dakopatts, Denmark) 1:24000; monoclonal anti-amyloid protein A (Dakopatts, Denmark) 1:300. In one case double immunostaining was performed, using anti-amyloid P-component and anti-elastin antisera, according to the method reported by Eusebi et al. (1986), in which B-galactosidase (blue) and avidin-biotin peroxidase (brown) are employed. Elastin antiserum was employed to verify a previous suggestion that elastic material can give a false-positive Congo red reaction (Azzopardi 1979).

For electron microscopy small fresh fragments of tissue from two cases of group A were fixed in 4% glutaraldehyde, post-fixed in 1% osmium tetroxide and Epon embedded. In an additional case from group A, after decalcification with citric acid, small fragments of formalin-fixed tissues were similarly osmium post-fixed and Epon embedded. In all three cases the immunogold technique was also employed, following a method described by Bendayan and Zollinger (1983), using the same anti-amyloid P-component and anti-elastin sera employed for light microscopy immunohistochemistry, at a working dilution of 1:10.

Results

The relevant clinical and histological data are given in Table 1.

Meningiomas of group A were mostly of meningothelial (9 cases) and transitional (8 cases) type; only 6 cases were fibroblastic. Cases in group B included 10 meningothelial and 5 fibroblastic meningiomas. All tumours were composed of typical meningiomatous cells having polygonal or spindle-shaped cytoplasm with round nuclei and frequent nuclear pseudoinclusions. In all cases bundles of eosinophilic material delineated groups of meningiomatous cells and meningothelial whorls, which eventually merged into eosinophilic plaques (Fig. 1). The dimensions and distribution of these plaques varied in each case from small round eosinophilic bodies of the same size as meningioma cells to larger plaques having a greater axis of 1 mm on average.

Congo red positivity, showing green birefringence under polarized light, was seen in all 23 cases of group A. In 20 of them Congo red-positive material (CRPM) was mainly localized in the intercellular eosinophilic material (Fig. 1). Thin strands of CRPM circumscribed occasional meningothelial whorls in 17 cases. In 16 of the 23 cases Congo red stained occasional PBs. CRPM was localized in the vessel walls of only 2 cases. In 4 cases normal dura was removed together with the tumours. It was focally stained with Congo red in 3 of these cases.

Congo red positivity was seen in only 5 of the 15 cases of group B. In these tumours CRPM was mainly localized in intercellular stroma, forming scattered small plaques through the meningiomatous tissue. Occasional thin rims of CRPM around meningothelial whorls were seen in only 1 case. No CRPM was seen in the vessel walls. Fragments of normal dura were present in 8 cases, whereas focal and weak Congo red staining was present in only 2 cases; these 2 cases did not show CRPM in meningiomatous tissue.

In the 8 cases of group A in which calcium was removed by citric acid, decalcified PBs (DPBs) displayed

Table 1. Clinical and histological data

	Meningiomas	
	Group A	Group B
Patients' age (years)	25–81 (60)	36–83 (62)
Patients' sex (M/F)	4/19	6/9
Histological tumour type	6 Fb, 9 Mn, 8 T	5 Fb, 10 Mn
Greatest diameter of tumour (cm)	1–5 (3)	3–8.5 (5.1)

Mn, meningotheliomatous; Fb, fibroblastic; T, transitional

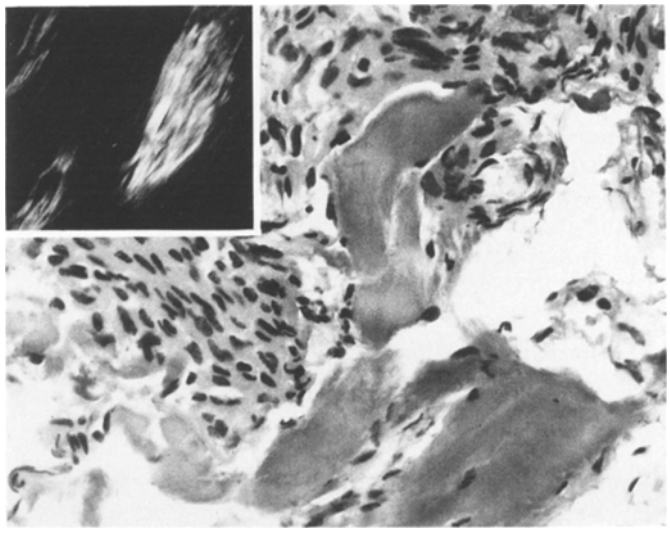

Fig. 1. Eosinophilic plaques. These are birefringent under polarized light (*inset*). H&E, ×250; inset: Congo red, ×250

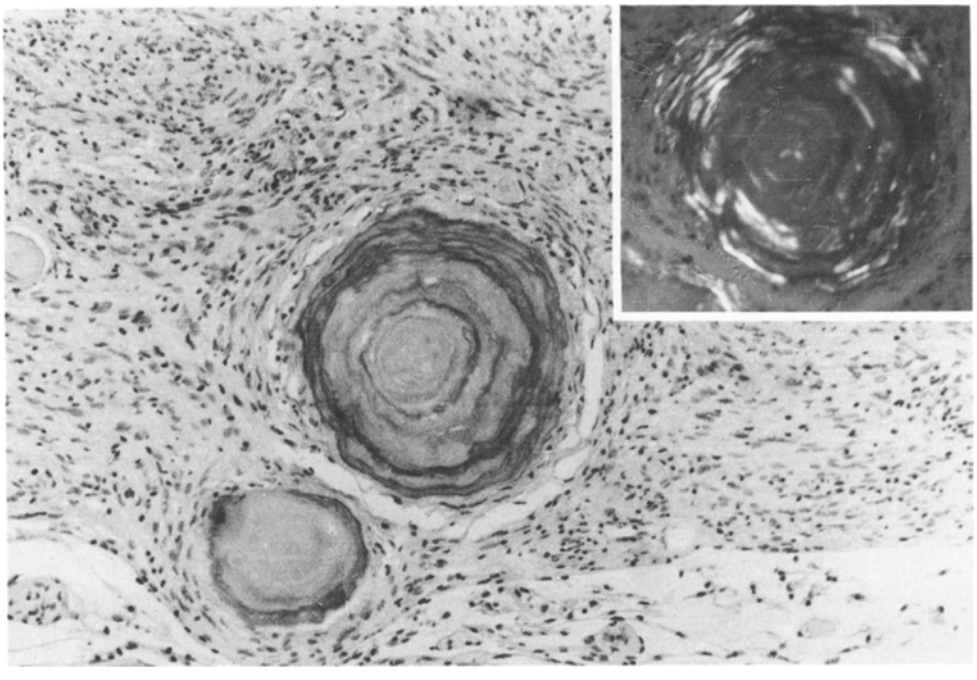

Fig. 2. Congo red positive-material in a decalcified psammoma body. It is birefringent under polarized light (*inset*). Congo red, ×125

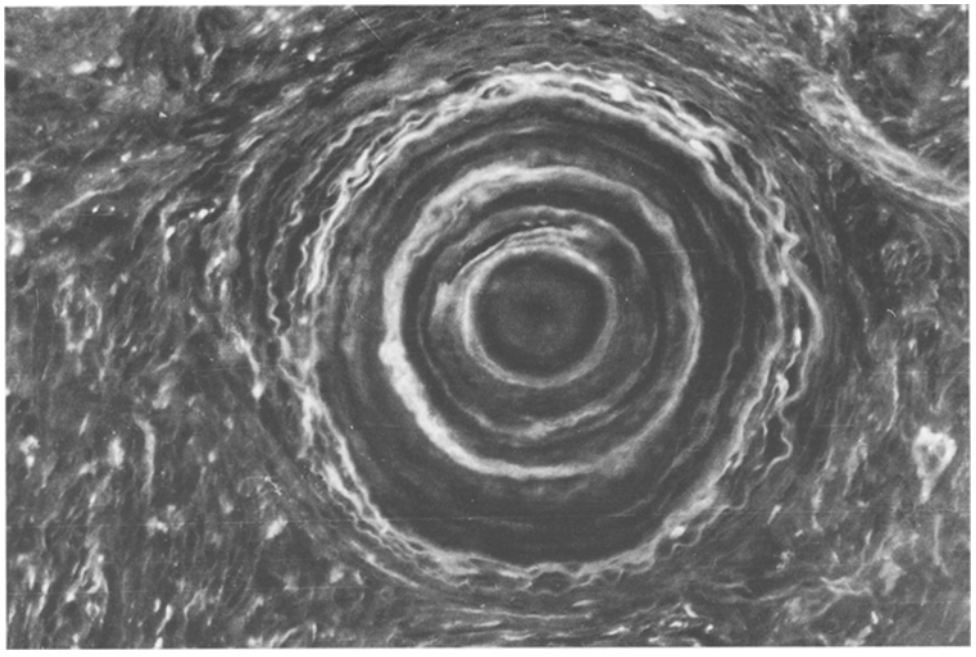

Fig. 3. The same DPB appears thioflavine T stained. Small intercellular deposits. Thioflavine T, ×250

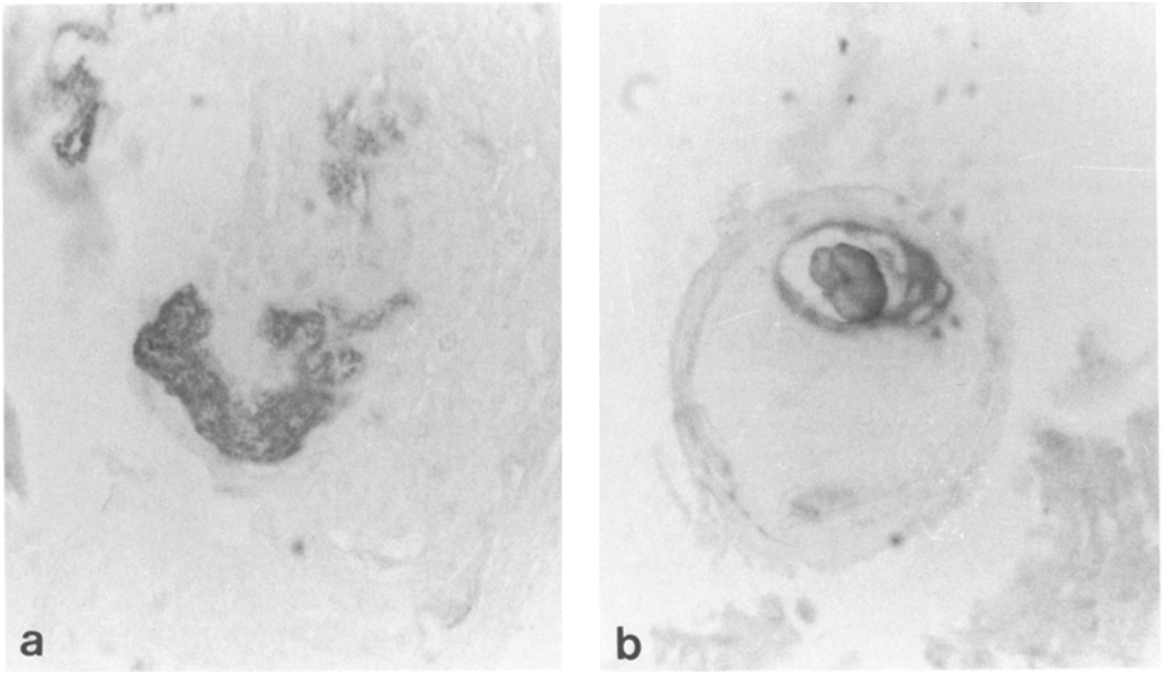

Fig. 4. a A small plaque of material immunoreactive with amyloid P-component is present among meningiomatous cells. **b** Positivity is also seen in DPBs. (Streptavidin biotin peroxidase, ×250, not counterstained

a structure composed of concentric weakly basophilic lamellae; amorphous eosinophilic material was intermixed with these lamellae. No von Kossa-positive DPBs were observed. In all 8 cases a variable number of scattered DPBs showed Congo red positivity (Fig. 2). Such positivity was localized in the amorphous eosinophilic material, while basophilic lamellae were negative. CRPM was also seen in the intercellular stroma and around occasional meningothelial whorls. Virtually identical positivity was found, in 7 cases, with thioflavine T (Fig. 3). Antibody against amyloid P-component

Table 2. Comparison of results in decalcified psammoma bodies (DPBs), meningothelial whorls (MWs) and meningiomatous tissue (MT)

Stain	Staining		
	DPBs	MWs	MT
Congo red	8/8	5/8	6/8
Thioflavine T	7/8	2/8	6/8
Anti-amyloid P-component	6/8	3/8	7/8
Anti-elastin	5/8	3/8	5/8

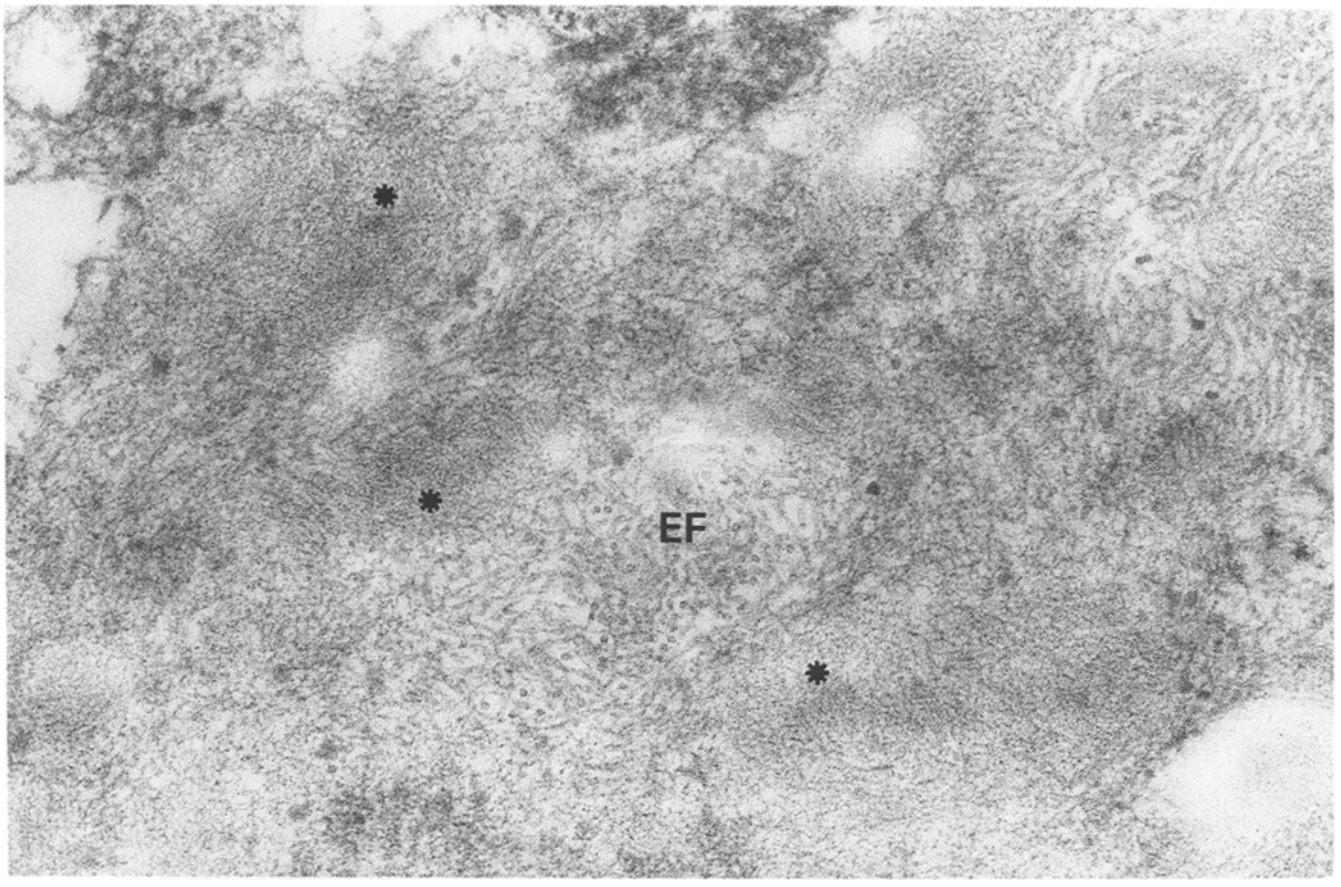

Fig. 5. Focal aggregates of amyloid fibrils (*) associated with elastic microfibrils (*EF*) located in intercellular stroma. × 71 940

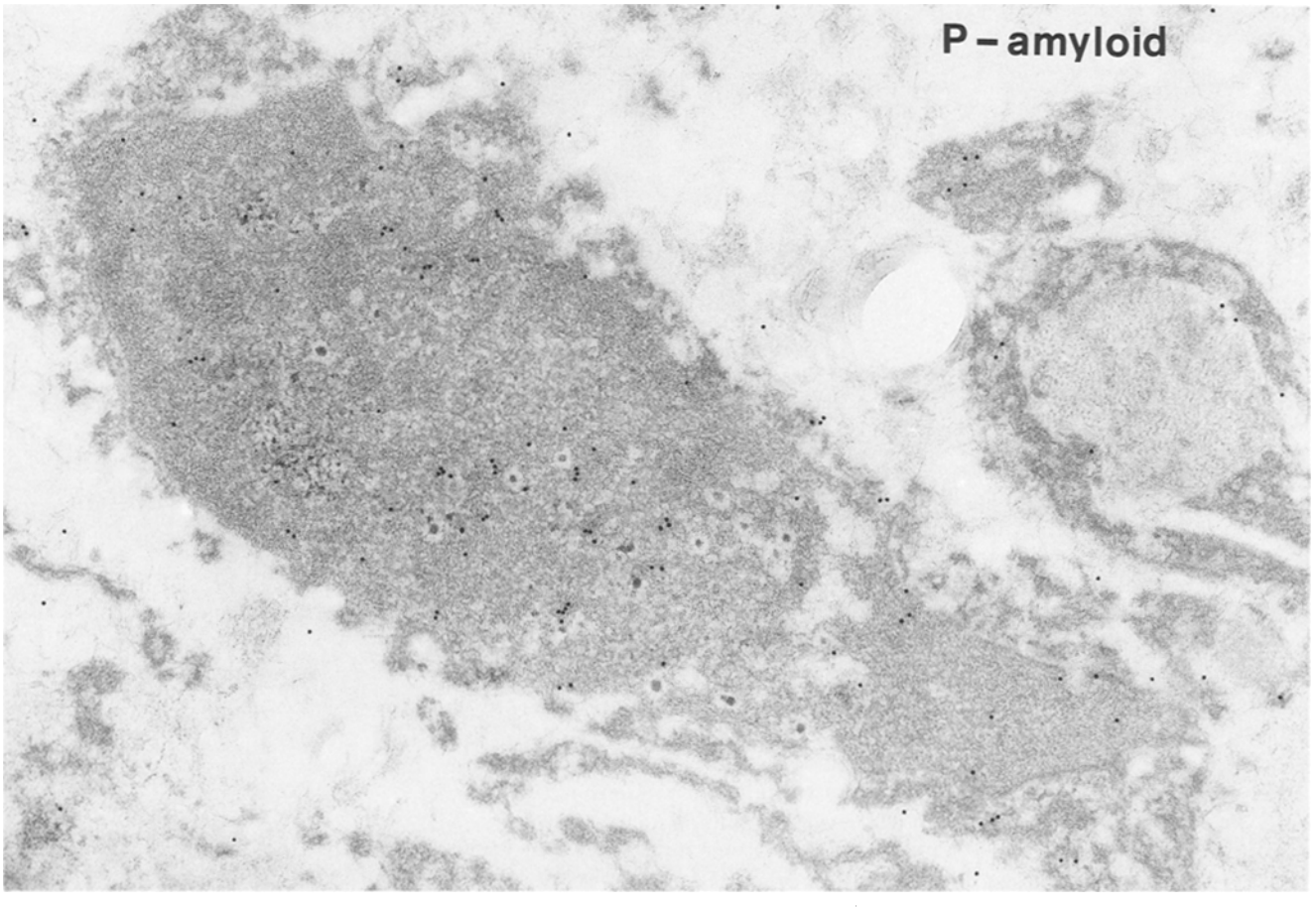

Fig. 6. Selective deposition of colloid gold particles over an amyloid aggregate after immunostaining with antiserum against amyloid P-component. × 31 200

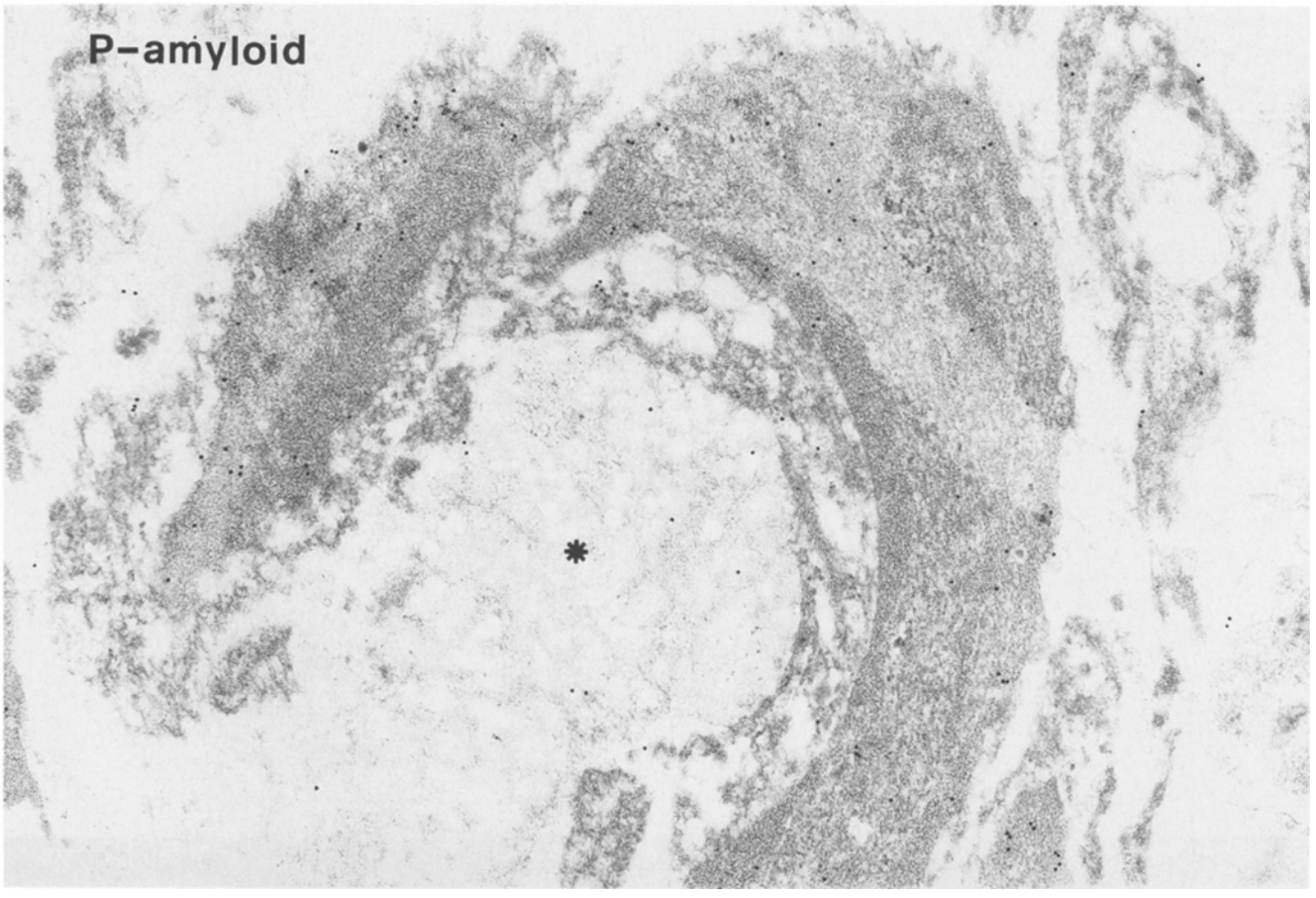

Fig. 7. Amyloid P-component immunogold localization over amyloid aggregates with absence of deposition on elastic fibres (*). × 31 200

stained the amorphous eosinophilic material of scattered DPBs in 6 cases. In addition, it stained material surrounding meningothelial whorls in 3 cases and intercellular material in 7 cases (Fig. 4). Amyloid P-component was localized immunohistochemically in the same areas which were positive with thioflavine T and Congo red, as revealed by examination of serial sections (Table 2).

Basophilic lamellae of DPBs stained with Weigert-van Gieson method and with anti-elastin serum in 5 cases. Thin bundles of material with the same tinctorial characteristics were seen in the intercellular stroma of the remaining meningiomatous tissue, occasionally underlying the structure of meningothelial whorls. In the case in which double immunostaining for elastin and amyloid P-component was performed, it was evident that these two substances, although spatially very close, never overlapped. Immunostaining for amyloid protein A and kappa and lambda light chains gave negative results.

By electron microscopy we found bundles of collagen fibres and, more frequently, elastic fibres in the intercellular material. Focal aggregates of amyloid fibrils dispersed in a network pattern were occasionally found in 2 cases, usually associated with bundles of elastic fibres (Fig. 5). These fibrils were never seen in PBs, in which, however, the ultrastructural preservation of fine details was suboptimal due to technical artefacts related to either sectioning of calcified bodies or the effect of the decalcifying treatment. With the immunogold technique a distinct deposition of gold particles over amyloid aggregates was seen with antiserum against amyloid P-component in 2 cases. In contrast, elastic fibres were constantly nonreactive (Figs. 6, 7). Amyloid aggregates were nonreactive after immunogold staining with anti-elastin serum, which heavily reacted with elastic fibres (Fig. 8). In tissues decalcified with citric acid the only ultrastructural immunoreactivity obtained was with elastin antiserum.

Discussion

Although Congo red is considered to be the best method for revealing amyloid (Elghetany and Saleem 1988), it can stain the connective (Elghetany and Saleem 1988) and elastic (Carstens et al. 1972) tissues non-specifically. The use of thioflavine T has been suggested as a further demonstration of the existence of amyloid, but in recent years antisera against the amyloid P-component have become available (Glenner 1980a). Amyloid P-component is a glycoprotein which is constantly present in all types of amyloid deposits, even if in a small proportion (10%; Glenner 1980a). In addition, amyloid consists ultrastructurally of a meshwork of thin, non-branching fibrils which have a very typical appearance (Ghadially 1982).

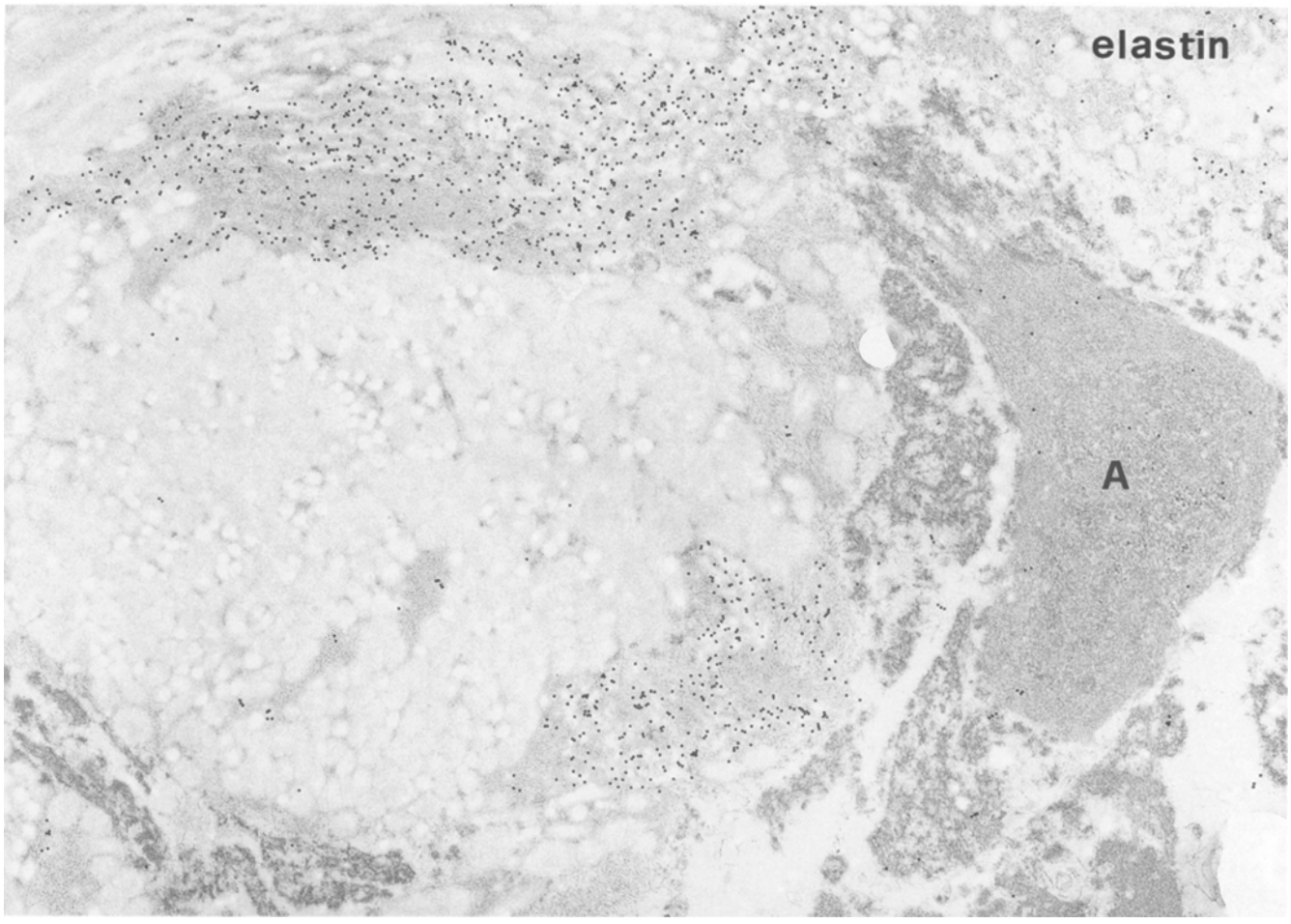

Fig. 8. Heavy immunogold staining of elastic fibres with anti-elastin antiserum, with virtually no staining of an amyloid aggregate (*A*). ×19140

In the present study, CRPM was demonstrated in all 23 cases of group A (meningiomas with PBs), as well as in 5 of 15 cases of group B (meningiomas without PBs). The positive cases included all 8 selected cases in which characterization of CRPM was also assessed after appropriate tissue decalcification. CRPM stained with thioflavine T in 7 of these cases. In these same cases CRPM was also immunoreactive with amyloid P-component, and in 2 of the 3 cases studied ultrastructurally, typical amyloid filaments were present. Finally, using an immunoelectron microscopic technique, gold particles bound to these filaments when an antiserum against amyloid P-component was used. Altogether, the results of the different techniques provide strong evidence for the existence of amyloid in meningiomas. The distribution of amyloid appeared to be vaguely similar to that of elastin. However, in the present cases the two substances were physically separated, as no immunocytochemical cross-reaction between elastic fibres and amyloid deposits was seen on either light or electron microscopy when the respective specific antisera were used. Congophilia was constantly seen in meningiomas with PBs. In contrast, it was only occasionally found in meningiomas lacking PBs, with only 5 cases showing very limited areas containing CRPM.

Since amyloid is most frequently present in meningiomas with PBs, it may have a role in favouring deposition of calcium salts. Evidence of such a role of amyloid in calcium deposition has already been provided in the calcifying odontogenic tumour of Pindborg, in which calcification are seen on globules of amyloid (Shafer et al. 1974).

The reason for and the mechanism of amyloid formation in meningiomas remains unclear. Masson (1968) noticed hyaline substance in the meninges as well as around PBs of meningiomas and concluded that these changes could be the results of an aging process. Among the several forms of amyloid deposition in brain, congophilic angiopathy is characterized by amyloid replacement of the tunica media of leptomeningeal arterial walls (Tomlinson 1992). This condition has been reported in "normal" brains of aged persons (Glenner 1980b), and it appears that the incidence of angiopathy increases with age (Glenner 1980b). Small congophilic nodules were present in 5 cases when the dura was removed together with the tumour. Although no perivascular localization was seen, 4 of the 5 patients concerned were older than 60 years, the exception being a 27-year-old man. In view of this latter patient and the presence of amyloid in 3 cases of meningioma in patients younger

than 60 years, it seems difficult to relate the presence of amyloid in meningiomas to a process of aging.

In conclusion, amyloid was found in 23 cases of meningiomas with PBs and in 5 of 15 cases of meningiomas without PBs. These findings add meningioma to the long list of neoplasms containing amyloid in the stroma.

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References

Azzopardi JG (1979) Elastosis and other connective tissue changes. In: Azzopardi JG (ed) Problems in breast pathology. (Major problems in pathology, vol 11). Saunders, London, p 380

Azzopardi JG, Lenner T (1966) Systemic amyloidosis and malignant disease. J Clin Pathol 19:539–548

Bendayan M, Zollinger M (1983) Ultrastructural localization of antigenic sites on osmium fixed tissue applying the protein A-gold technique. J Histochem Cytochem 31:101–109

Bordi C, Tardini A (1980) Electron microscopy of islet cell tumors. In: Fenoglio CM, Wolff M (eds) Progress in surgical pathology, vol 1. Masson, New York, pp 135–155

Carstens PHB, Huvos AG, Foote FW Jr, Ashikari R (1972) Tubular carcinoma of the breast: a clinicopathologic study of 35 cases. Am J Clin Pathol 58:231–238

Elghetany MT, Saleem A (1988) Methods for staining amyloid in tissues: a review. Stain Technol 63:201–212

Eusebi V, Rilke F, Ceccarelli C, Fedeli F, Schiaffino S, Bussolati G (1986) Fetal heavy chain skeletal myosin. An oncofetal antigen expressed by rhabdomyosarcoma. Am J Surg Pathol 10:680–686

Foschini MP, Muzzi L (1992) Decalcification with citric acid alone: description of a method. Biotechnic and Histochemistry (in press)

Note added in proof

Since the submission of the manuscript we become aware of an histochemical study on meningiomas in which it was suggested the presence of amyloid (Archivio Italiano Anatomia Istologia Patologica 32, 470–509, 1958).

Ghadially FN (1982) Ultrastructural pathology of the cell and matrix. Butterworth, London, pp 918–921

Glenner GG (1980a) Amyloid deposits and amyloidosis. I. The beta-fibrilloses. N Engl J Med 302:1283–1292

Glenner GG (1980b) Amyloid deposits and amyloidosis. II. The beta-fibrilloses. N Engl J Med 302:1333–1349

Hsu SM, Raine L, Fanger H (1981a) A comparative study of the PAP method and avidin-biotin-complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 75:734–738

Hsu SM, Raine L, Fanger H (1981b) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedure. J Histochem Cytochem 29:577–580

Kepes JJ (1982) Light-microscopic features of meningiomas. In: Sternberg SS (ed) Meningiomas: biology, pathology and differential diagnosis. (Masson monographs in diagnostic pathology, vol 4) Masson, New York, p 71

Kovacs K, Horvath E (1983) Adenomas with prolactin production. In: Hartman and Sobin (eds) Tumors of the pituitary gland. Armed Forces Institute of Pathology, Washington DC, p 110

Looi LM (1983) Localized amyloidosis in basal cell carcinoma. A pathologic study. Cancer 52:1833–1836

Masson P (1968) Les meningiomes. In: Tumeurs humaines, 2nd edn. Maloine, Paris, pp 977–990

Pages A (1974) Tumeurs et amylose. Arch Anat Path 22:85–92

Rosai J (1989) Thyroid gland. In: Rosai J (ed) Ackerman's surgical pathology, 7th edn. Mosby, St. Louis, pp 426–431

Shafer WG, Hine MK, Levy BM (1974) Cyst and tumors of odontogenic origin. In: Shafer WG, Hine MK, Levy BM (eds) A textbook of oral pathology, 3rd edn. Saunders, Philadelphia, pp 258–261

Steinhoff MM, Wells SA Jr, DeSchryver-Kecskemeti K (1992) Stromal amyloid in pheochromocytomas. Hum Pathol 23:33–36

Tomlinson BE (1992) Ageing and dementias. In: Adams JH, Duchen LW (eds) Greenfield's neuropathology, 4th edn. Wiley, New York, pp 1315–1317