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Glial Fibrillary Acidic Protein in Medulloblastomas and Other Embryonic CNS Tumours of Children

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Summary. Investigation of GFAP in 50 medulloblastomas showed a few GFAP-positive tumour cells in 5 cases only; 17 tumours were negative, and 28 showed a "pseudopositivity", i.e. GFAP-bearing cells were identified as reactive or degenerating astrocytes, intermingled with tumour elements. A high GFAP content was seen in 2 small-cell gliomas of the cerebellum, whereas 3 pineoblastomas, 2 neuroblastomas of CNS, and one medulloepithelioma were negative.

GFAP is a very good method for identificating astrocytes, but does not seem to be reliable for identifying the origin of undifferentiated tumours such as medulloblastomas. In these neoplasms glial differentiation is lacking or extremely rare, GFAP-positivity being mostly an artifact. The investigation of small tumour samples or the positivity of a single cell are inadequate data for a correct evaluation of the findings, especially taking in mind that GFAP of degenerated astrocytes can be phagocytised by cells other than glial (e.g., macrophages, epithelial and meningioma cells). The importance of carefully checking the whole structure of the tumour is stressed, GFAP positivity or negativity being not a sufficient criterion for its nosological classification.

Key words: GFAP – Medulloblastoma – Pineoblastoma – Cerebral Neuroblastoma – Medulloepithelioma – Small-cell Glioma of Cerebellum

Glial Fibrillary Acidic Protein (GFAP), the main constituent of intermediate filaments of astrocytes, is present in these cells in a water-soluble and a water-insoluble form. This protein is regarded as an astrocyte-specific component and its immunocytochemical identification can therefore be useful to define the nature of a cell and the degree of differentiation of glial elements (Bignami and Dahl 1974 and 1977). This method is therefore being used with increasing frequency in histological brain tumour diagnosis, with some contradictory results, however (for review see De Armond et al. 1980). The only facts so far established are that astrocytic tumours present a high GFAP positivity, whereas oligodendrogliomas, glioblastomas, mixed

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glial-mesenchymal tumours, ependymomas and medulloblastomas have been described as partly positive.

The exact origin and/or differentiation forms of medulloblastoma cells are still under discussion (Gullotta 1967, 1979 and 1981). Identification of GFAP in these and other "embryonic" tumours might therefore help to solve the question of their presumptive glial origin.

For two years we have been carrying out systematic investigations on GFAP in cerebrospinal tumours and other CNS diseases. We present here the results obtained in a large number of medulloblastomas and other "embryonic" tumours of children.

Material and Methods

Our investigations were carried out on 58 tumours, mostly biopsy specimens from the Neurosurgical Clinic of the University of Bonn, comprising: 50 medulloblastomas, 3 pineoblastomas, 2 neuroblastomas of CNS, 2 small-cell gliomas of cerebellum and 1 medulloepithelioma (Table 1). Specimens were fixed in formalin and embedded in paraffin. Histology was performed on slides stained with H&E, van Gieson, Nissl and Gomori for reticulin as well as special metallic impregnations for neuroblasts, pineocytes, etc.

GFAP[1] was demonstrated in 6–7 microns thick, toluol deparaffinized sections, according to the unlabeled antibody-PAP method described by Sternberger (1979). Endogenous peroxi-

Table 1. Synopsis of Cases. UN = undiffer. medull. DM = desmopl. medull. (arachnoidalsarcoma), MIX = mesodermal-neuroect. mixed tumour. M = male, F = female.

Case	Number	Age (Years)	Sex	Location		Type	GFAP
				Midline	Hemisph		
Group 1 Cerebellar medulloblastomas							
1)	160/82	32	M		×	DM	+
2)	920/81	4	M	×		Mix	
3)	1076/81	8	M	×	×	Mix	+
4)	253/80	5	F	×		DM	
5)	493/80	14	F		×	DM	
6)	907/80	10	M	×	×	UN	
7)	1031/80	37	F		×	Mix	
8)	343/79	9	F	×	×	UN	
9)	565/79	4	M	×		UN	
10)	697/79	17	M	×		UN	
11)	67/78	18	F		×	DM	
12)	120/78	11	M	×		DM	
13)	477/78	9	M	×		DM	
14)	960/78	38	M		×	DM	
15)	61/77	7	M	×		UN	
16)	108/77	16	M		×	DM	+
17)	765/77	15	M	×		DM	
18)	813/77	47	F		×	DM	
19)	883/77	29	M		×	DM	
20)	214/76	21	M		×	DM	

[1] GF-Antiserum raised in rabbits, was generously supplied by Drs. Doris Dahl and Amico Bignami, Dept. of Neuropathology, Harvard Medical School, Boston, Mass. (USA)

Table 1 (continued)

Case	Number	Age (Years)	Sex	Location		Type	GFAP
				Midline	Hemisph		
21)	852/76	17	M	×		UN	
22)	915/76	5	M	×		DM	
23)	62/75	29	M		×	DM	
24)	127/75	11	M	×		Mix	
25)	304/75	10	M	×	×	Mix	
26)	444/75	5	M	×		UN	
27)	505/75	24	M		×	DM	+
28)	567/75	16	F	×		DM	
29)	637/75	16	M	×		UN	
30)	682/75	9	F	×		UN	
31)	707/75	11	F	×		UN	
32)	867/75	3	M	×		Mix	
33)	320/74	26	F		×	UN	
34)	344/73	6	F		×	UN	
35)	357/73	12	M		×	Mix	
36)	419/73	5	M	×		UN	
37)	396/72	3	F		×	UN	
38)	559/72	3	M	×		Mix	
39)	736/72	9	M	×		UN	
40)	802/72	5	M	×		UN	
41)	961/72	2	F		×	DM	
42)	501/71	22	M	×		DM	
43)	271/70	15	F	×		DM	
44)	161/69	5	M	×	×	UN	
45)	175/69	9	M	×	×	UN	
46)	193/68	8	M	×	×	UN	+
47)	230/68	15	M		×	DM	
48)	269/68	27	M		×	DM	
49)	178/67	1	F		×	UN	
50)	355/67	4	M		×	UN	
Group 2							
Pineoblastomas							
1)	774/80	3	M	Pinealis			
2)	1027/80	16	M	Pinealis			
3)	89/71	8	F	Pinealis			
CNS-Neuroblastomas							
1) SN	252/71	33	F	Pons and IV. Ventricle			
2) SN	68/81	3	M	Brain stem, basal meninges			
Medulloepithelioma							
1) SN	8/72	2	M	Basal meninges with parenchyma infiltration			
Smallcell gliomas of posterior fossa							
1)	288/76	10	M	Cerebellar hemispheres			+
2)	584/72	3	M	Cerebello-pontine angle			+

dase activity was blocked by treatment of deparaffinized sections with 0.3 per cent H_2O_2 in methanol for 30 minutes. Sections were then preincubated for 10 minutes with normal swine serum[2], diluted 1:20 in phosphate buffered saline (PBS), pH 7.2. In the next step, sections were incubated with the antibody against GFAP for 12 h at 4° C, in a dilution of 1:500 in 4% albumine PBS. Other antibodies were also diluted in this buffer. Antiserum against GFAP was thoroughly washed with PBS, and sections were then incubated for 30 minutes at room temperature in a 1:20 dilution of swine antirabbit IgG[2]. Following thorough washing in PBS, sections were incubated with rabbit peroxidase-antiperoxidase (PAP)-complex[2], diluted 1:50. Final washing was made in PBS and tris-buffered saline, pH 7.6. Peroxidase reaction was visualized with 0.05% diaminobenzidine tetrahydrochloride (DAB[3]) and 0.01% H_2O_2 in trisbuffered saline.

All sections were counterstained with haematoxylin. Each staining was controlled with incubation of normal swine serum instead of the GFAP-antibody. Positivity of GFAP reaction was always tested in control slides, by visualization of Bergmann glia.

As only paraffin-embedded material was available, a control of GFAP staining in frozen sections could not be performed.

Results

A. *Group 1, Medulloblastomas.* Histologically these 50 tumours were classified according to Gullotta (1967) in a) 22 isomorphic-round celled, b) 20 small-spindle celled, and c) 8 mixed-tumours. Tumours of type a) are composed of round cells with a large nucleus and scanty cytoplasm: they correspond to classical "undifferentiated" medulloblastomas. Tumours of type b) consist of small, oval-spindle shaped cells with short polar processes, often parallely arranged in fish-like formations, building three-dimensional whorls and thus appearing round-celled in some areas. Most of these tumours (but not all) are very rich in reticulin fibers, not only in cases localised in the leptomeninges but also in intracerebellar tumours. They correspond to desmoplastic medulloblastomas (Rubinstein 1972) or arachnoidal sarcomas (Gullotta 1967). Tumours of type c) are built up by two different, tightly intermingled tissue components, and correspond to the mesodermal-neuroectodermal mixed tumours described by Gullotta 1966.

17 Medulloblastomas were GFAP negative (4a, 9b, 4c), *5 were GFAP positive* (1a, 3b, 1c) and *28 were pseudopositive* (15a, 10b, 3c).

GFAP-Positivity. In 5 tumours, some small GFAP-positive cells were found intermingled with neoplastic cells and bearing morphological similarities to them (Fig. 1a). These cells were mostly scattered among tumour elements, but occasionally appeared in small clusters. Morphologically they were indistinguishable from the remaining, GFAP-negative cells: small elements with scanty cytoplasm, large hyperchromatic nucleus. Cytoplasmic processes, if recognizable, were short. Positivity was shown by a perinuclear brown rim or a small intracytoplasmic brown droplet. By comparing these

[2] Dako (Garching/München, FRG)
[3] Fluka (Neu-Ulm, FRG)

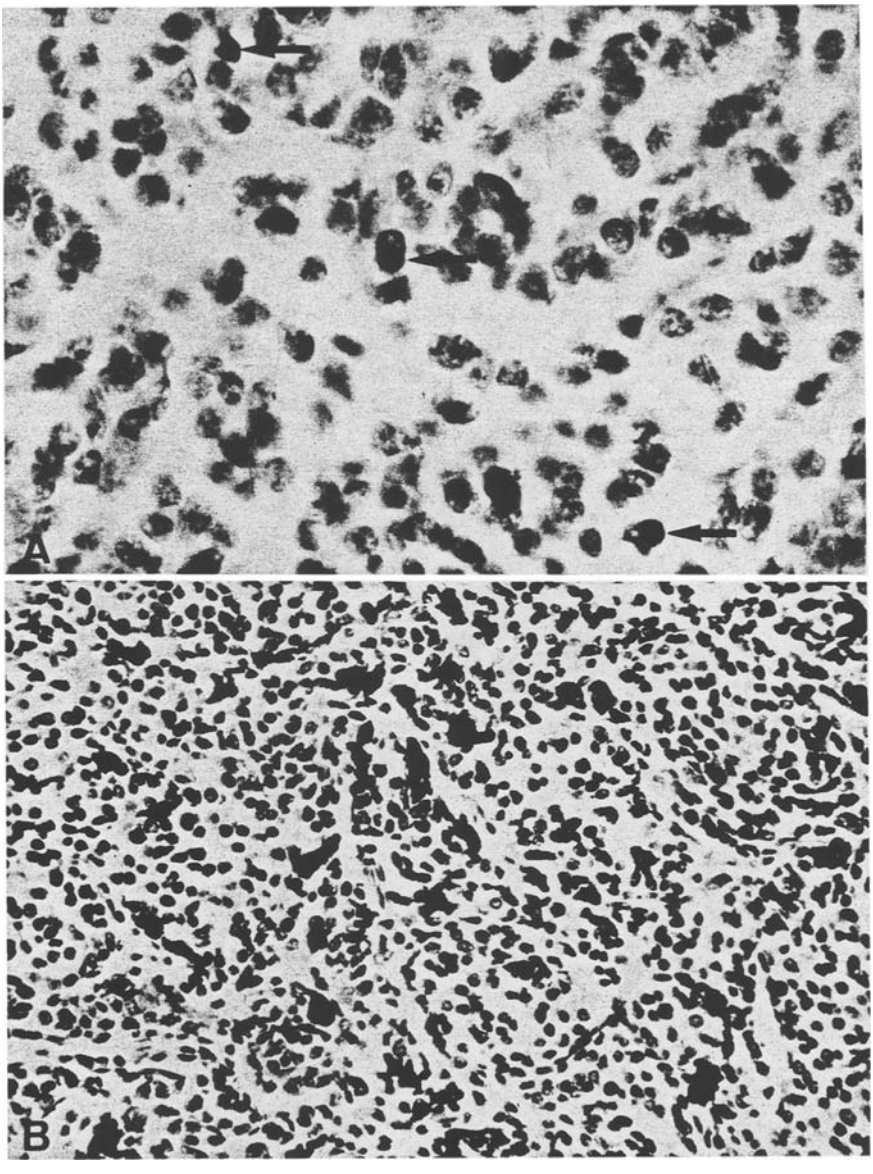

Fig. 1. A In this medulloblastoma three GFAP-positive cells (*arrows*) are evident. GFAP is present as small brown droplets in the cytoplasm. These three cells are morphologically identical to the remaining GFAP-negative tumour cells. Such a case was classified as "positive". 900:1. **B** Pseudopositive medulloblastoma. In the peripheral parts of this "desmoplastic" medulloblastoma, many strongly GFAP-positive elements are easily recognizable (in black). They are reactive astrocytes and can be distinguished from tumour cells by their shape and their location 350:1

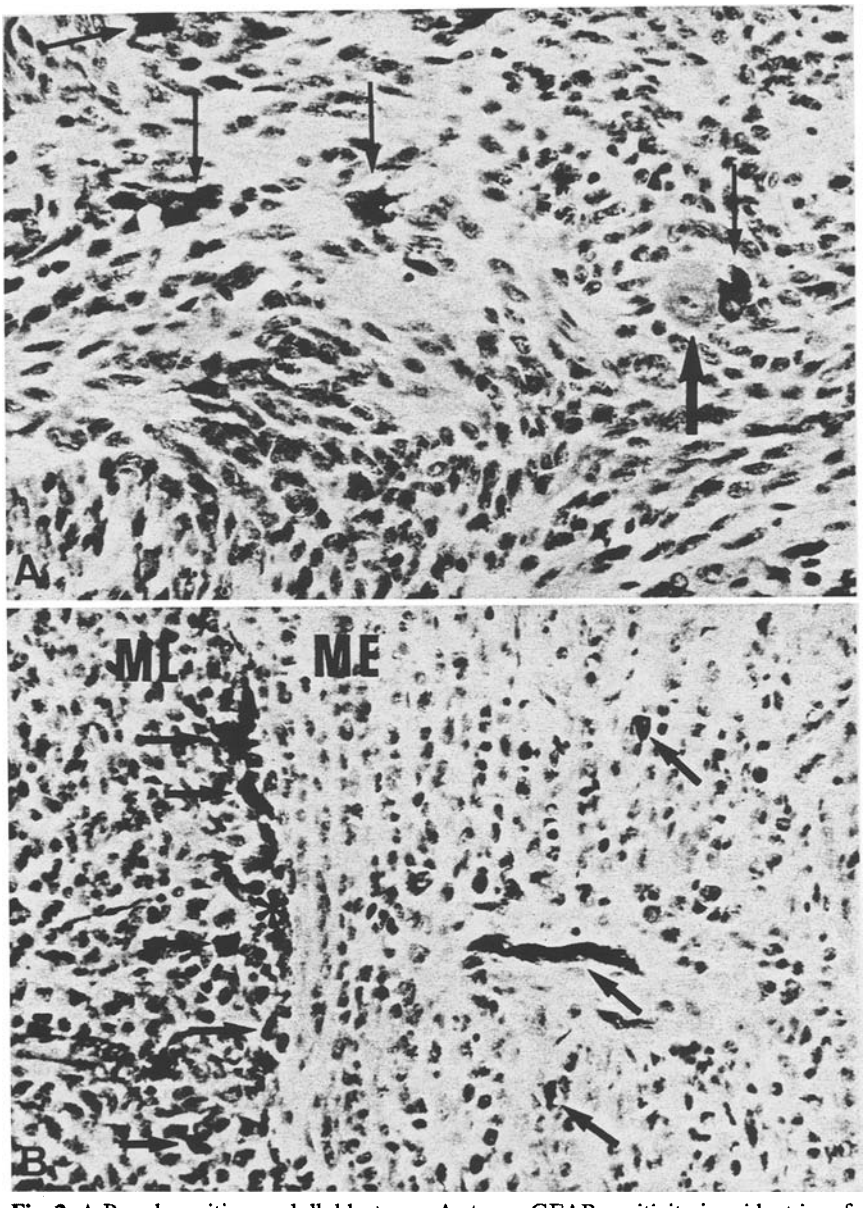

Fig. 2. **A** Pseudopositive medulloblastoma. A strong GFAP-positivity is evident in a few reactive astrocytes (*thin arrows*), intermingled with tumour cells; a Purkinje cell is recognizable (*thick arrow*). 380:1. **B** Pseudopositive "desmoplastic" medulloblastoma. Many strong GFAP-positive cells (*arrows*) are evident in the infiltrated molecular layer (*ML*), especially in proximity of the pial-glial-border (*), whereas in leptomeningeal tumour (*ME*) GFAP is evident only in two small tumour cells and in perivascular cell processes (*middle arrow*). 350:1

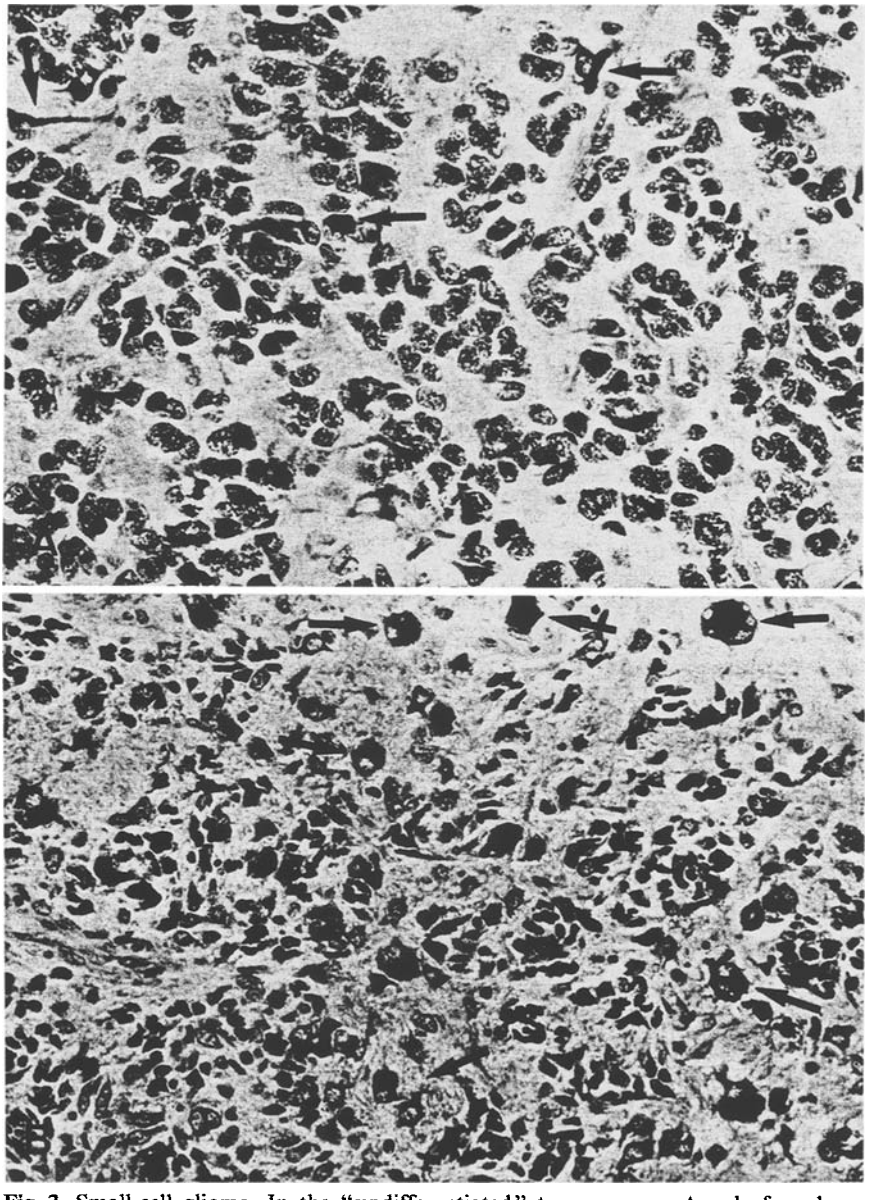

Fig. 3. Small-cell glioma. In the "undifferentiated" tumour areas **A** only few elements are GFAP-positive (*arrows*). The tumour resembles a medulloblastoma in this field, nuclei being larger however. 350:1. **B** Differentiated astrocytic area: many mono-, bi- and multinucleated astrocytes (*arrows*) are easily visualized in this tumour part. 280:1

slides with those stained by usual histological methods, no certain evidence of an astrocytic or oligodendroglial differentiation could be detected, however. Taking into account the theoretical possibility of a glial origin or differentiation of "undifferentiated" medulloblasts, we classified these cases as positive.

GFAP-Pseudopositivity. In 28 tumours, GFAP was detected in cells that were evidently reactive or degenerative in origin, intermingled with tumour cells (Fig. 1b). These preexistent glial elements were recognised by their cellular shape and their strong GFAP-positivity (typical for reactive astrocytes, as demonstrated in reactive gliosis of infarcts, traumas, metastases etc.), but mainly *on the basis of their topographical distribution within the tumour:* precise examination of the architectural arrangement of the tumour and the spreading pattern of neoplastic cells by low magnifications and with different stains, allowed us in all these cases, to distinguish "pure" tumour part (GFAP negative) from pseudopositive areas, i.e. infiltrated cerebellar tissue. Occasionally, the presence of single Purkinje cells among neoplastic elements was further support in the identification of these areas (Fig. 2a). A very strong positivity was often detected in perivascular cytoplasmic processes and in some variably long cellular processes running among tumour cells, but without any evidence of their belonging to a neoplastic element. In both instances, as confirmed by the global study of many slides, we were dealing with astrocytic cell processes, the bodies of which were lying at a different level to that of the GFAP slide. Five tumours were classified as pseudopositive after a careful investigation of many additional tumour samples, in part after a new orientation of paraffin block. On first screening these cases had been classified as positive, given the similarities of GFAP bearing cells with tumour elements. Finally, some strong GFAP positive elements, obviously astrocytic in origin were also detected in a few leptomeningeal ("desmoplastic") tumours: these cells were generally located in peripheral parts of the tumour, that is in proximity to the pial-glial border (Fig. 1b and 2b).

B. *Group 2*. In tumours of this group (3 pineoblastomas, 2 primary neuroblastomas of cerebrum, 1 medulloepithelioma, 2 small-cell gliomas of cerebellum) GFAP-reaction was negative, only reactive astrocytes of infiltrated nervous tissue being strong positive. A *true positivity* was detected only in the cells of the *two small-cell gliomas* (Fig. 3), tumours which may bear considerable resemblance to a medulloblastoma, being indistinguishable from them in small specimens, but showing in larger sections clear evidence of an astrocytic differentiation. This was already evident in both cases from the examination of different tumour samples by usual histological stainings.

Discussion

Investigations on GFAP in medulloblastomas have been carried out by few authors, with different results. Delpech et al. (1978) reported a GFAP positivity in one of two cases. Deck et al. (1978) found some GFAP positive cells in reticulin-free areas of a desmoplastic medulloblastoma. Astrocytes could not be identified however, even in retrospect, in sections stained by H&E, and PTAH. Van der Meulen et al. (1978) examined 6 medulloblastomas, which were all negative. Velasco et al. (1980) described areas of distinct astrocytic differentiation in 3 tumours: the cells depicted in their fig. 14

do show, however, a very strong GFAP positivity, quite unusual for "differentiating" medulloblastoma cells and similar, in our experience, to the small reactive astrocytes often detectable in many tumours and different lesions of cerebellum and brain stem. Tascos et al. (1982) found no GFAP positivity in 3 medulloblastomas, one medulloepithelioma, one neuroblastoma and one pineoblastoma.

Two papers specifically dealing with GFAP in medulloblastomas are those of Mannoji et al. (1981) and Palmer et al. (1981). Mannoji et al. (1981) disclosed GFAP positive cells in 16 out of 25 medulloblastomas, the greatest number of these cells being considered to be reactive astrocytes by the authors. Only in three cases, scattered among neoplastic elements and morphologically identical to them, were GFAP positive cells detected. In these three cases, GFAP positivity was considered indicative of astrocytic differentiation. Cells building up rosettes of Homer Wright type were negative.

Palmer et al. (1981) investigated 13 tumours, histologically classified as medulloblastomas with astrocytic, ependymal, neuronal and small-cell "subtypes". In one case features of glioblastoma were also present. A strong GFAP positivity was detected in 6 astrocytic, 1 ependymal and 1 neuronal "subtype". Of the remaining small cell subtypes, 2 were negative, 2 mildly positive and one strongly positive: no pictures of these five cases are presented. In the author's opinion these results confirm the pluripotential differentation capability of medulloblastoma cells. Palmer et al. (1981) therefore propose a classification of medulloblastomas, in effect suggesting what already had been proposed 50 years ago by Bailey and Cushing (1925). The possibility that GFAP positive cells detected in their tumours might be, at least in part, non-neoplastic glial elements, is neither discussed not mentioned. In our opinion, their results can be explained on the basis of a heterogeneous material, which includes not only "true" medulloblastomas, but also different tumours, glial in origin, with small-cell areas and classified therefore as medulloblastoma. Such small-cell gliomas are mostly supratentorial (Cappricci and Gullotta 1981); in the posterior fossa they are infrequent but occasionally occur as demonstrated by our two cases, which were GFAP-positive. These tumours must be distinguished from medulloblastomas, being really glial in origin and showing clear-cut evidence of a glial differentiation with common histological stainings.

The contradictory results reported in the papers quoted above, can mostly be explained by the well known fact that surgical medulloblastoma samples usually consist of infiltrated cerebellar tissue, since the largest part of this tumour is very soft (especially in children) and is therefore removed by suction. In small pieces of tissue it is very difficult or impossible to determine which of the cells or structures are neoplastic or not (Gullotta 1967).

The investigation of these samples with cell specific metallic methods or with antigenic markers can therefore give false positive results, due to preexistent, non-neoplastic elements. This is especially the case in rapidly infiltrating tumours, such as medulloblastomas, capable of engulfing glial and neuronal elements without destroying them. An accurate investigation

of *whole* tumour samples, with different histological methods and at low magnifications is therefore very helpful in distinguishing "true" tumour areas from infiltrated parts. The GFAP positivity of a single cell or of few elements in small tissue specimens is in fact inadequate for a correct evaluation of the findings. This is more evident if we consider that many histopathologists classify *tout court* as "medulloblastoma" every posterior fossa tumour of childhood, just because clusters of small, "undifferentiated" cells are present. Many of these "medulloblastomas" are in reality ependymomas, piloid astrocytomas or small-cell gliomas (see our two cases quoted above). The exact identification of all these oncotypes is only possible is sufficient material is examined.

Our results confirm those of Mannoji et al. and seem to demonstrate that in *few* medulloblastomas *few* GFAP positive cells are really present. According to the theoretical premisses of GFAP identification, this would indicate the beginning of a glial (astrocytic) differentation in tumour cells. It remains to be explained why no further evidence of cellular maturation, with development of clearly recognizable glial cells, is seen. Areas "suggestive" of astrocytic or oligodendroglial differentiation have been reported in only a few medulloblastomas, a fact that sharply contrasts with the high frequency of these tumours (in more than 150 of our own cases we have never seen these areas). Critical analysis of these tumours discloses that in about the half of them, the presumed "differentiation" areas consist of infiltrated cerebellar tissue. Furthermore, established differentiation of in vitro-cultured medulloblastomas into astrocytic or oligodendroglial cells has not been observed (Kersting 1968; Gullotta and Kost 1980). It is remarkable that areas "suggestive" of astrocytic differentiation (Van der Meulen et al. 1978) as well as "spindle-shaped tumour cells, which might be interpreted as spongioblastic" (Mannoji et al. 1981), were GFAP negative.

The demonstration of GFAP positive cells in leptomeningeal "desmoplastic" medulloblastoma is very interesting, giving support to the theory of astroglial differentiation. It must not be forgotten however, that glial cells of molecular layers (in both cerebrum and cerebellum), as a consequence of chronic local irritation with breakage of pial membrane, react with intraleptomeningeal proliferation and fibrillogenesis. Beautiful examples of this have already been reported 1922 by Spielmeyer (his Fig. 221) and 1930 by Roussy et al. (their Fig. 19–22). Under this aspect it has to be stressed that in our cases the few strongly positive cells detected in "desmoplastic" medulloblastomas were mostly located in the peripheral part of the tumours, near the pial-glial-border. In some chronic spinal cord disorders outgrowth of glial bundles into spinal nerve roots is well documented. This finding is particularly frequent in infantile spinal muscular atrophy (Werdnig-Hoffmann's disease), probably because the proliferative activity of astrocytes in infancy is greater than that occurring in adults (Ghatak and Nochlin 1982).

Furthermore the frequent occurrence of heterotopic leptomeningeal glia-cell clusters, casually detected by routine brains examinations, has to be

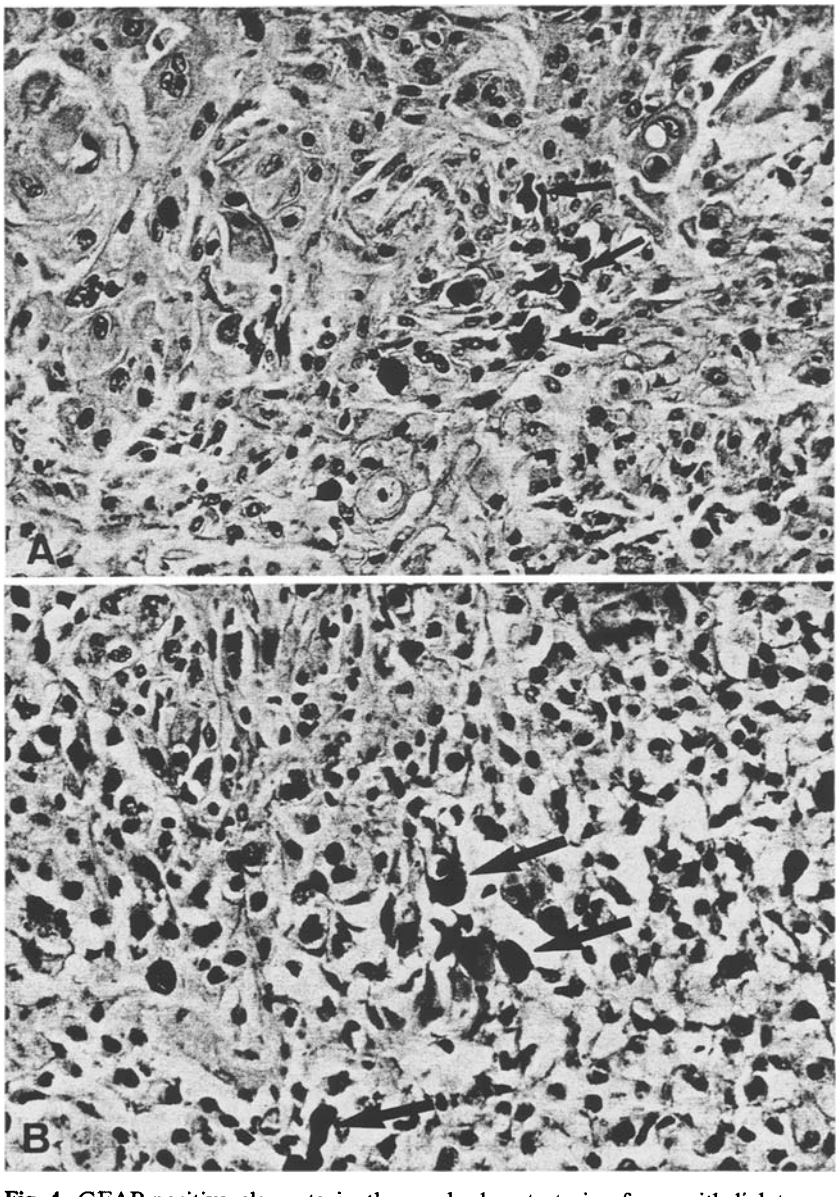

Fig. 4. GFAP-positive elements in the cerebral metastasis of an epithelial tumour **A**, and in a malignant meningioma **B**. These positive cells might be reactive astrocytes engulfed by and intermingled with tumour cells, but their morphology suggests that they are true tumour cells, which have phagocytised GFAP derived from degenerated astrocytes. **A** = 200:1, **B** = 350:1

considered. These glial islands are very frequent in the posterior fossa, they are apparently devoid of any clinical significance, but can sometimes give rise to a primary leptomeningeal gliomatosis (Cooper and Kernohan 1951; Szepan and Gullotta 1974).

Finally, given the high infiltrative capability of medulloblastoma cells, intermingled with reactive and degenerating glia cells, it is also very probable that GFAP detected in "typical" medulloblastoma elements is in reality not a cell product, but a secondary phenomenon, due to phagocytosis. The demonstration of GFAP in macrophages, in some cells of malignant meningiomas and of an epithelial metastasis (Fig. 4), confirm the possible occurrence of a false-positivity, i.e. a false "glial-specificity". This point has been mentioned by De Armond et al. (1980, Addendum), who do not exclude that stromal cells (and this means – in our opinion – every kind of cell, especially if mesodermal in origin) "can take up extracellular (i.e. water-soluble form of) GFAP derived from neighbouring reactive astrocytes" (De Armond et al.). After Deck and Rubinstein (1980) this hypothesis implies that the presence of GFAP in the cytoplasm of a cell does not always prove that the cell is glial. In other words this could mean that in human neurooncology such highly specific cytological methods might in effect complicate the problems concerning cellular differentiation – and not help to solve them (Gullotta 1981).

In conclusion the results of our investigations and critical analysis of those presented by others can be summarized as follows:

1) Glial differentiation in medulloblastomas is absent or is extremely rare; GFAP positivity is mostly an artifact. For precise interpretation of immunocytochemical results analysis of the whole tumour, of its architectural arrangements, of its growth pattern and of further histological variables are of paramount importance. The positivity of a single cell, or the investigation of only a few small tumour samples, are inadequate for a correct interpretation of the findings;

2) this immunocytochemical method is useful in distinguishing medulloblastomas from small-cell gliomas and, in part, ependymomas. In these cases however, large tissue samples have to be examined;

3) in our material, no correlations between presence or quantity of GFAP and biological behaviour of the tumour, i.e. course of the disease, could be demonstrated.

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