

Acta Medica Okayama

Volume 39, Issue 6

1985

Article 5

DECEMBER 1985

The determination of glial fibrillary acidic protein for the diagnosis and histogenetic study of central nervous system tumors: a study of 152 cases.

Kazuo Hamaya*

Kenji Doi†

Toshio Tanaka‡

Akira Nishimoto**

*Okayama Saiseikai General Hospital,

†Okayama Saiseikai General Hospital,

‡Okayama University,

**Okayama University,

The determination of glial fibrillary acidic protein for the diagnosis and histogenetic study of central nervous system tumors: a study of 152 cases.*

Kazuo Hamaya, Kenji Doi, Toshio Tanaka, and Akira Nishimoto

Abstract

Glial fibrillary acidic protein (GFAP) was purified from human spinal cord and cerebral white matter. GFAP was localized by an immuno-peroxidase method in normal adult and fetal human brains, rat brains, and 152 central nervous system (CNS) tumors. GFAP was found in reactive and normal astrocytes, immature cells of fetal brain at the 18th to 21st gestational weeks, and normal rat astrocytes. This GFAP staining was quite specific for glial tumors, including astrocytomas, glioblastomas, astroblastomas, and ependymomas. GFAP-positive cells were also found in oligodendrogliomas and choroid plexus papillomas, and they were interpreted as being astroglial or ependymal differentiations. Stromal cells in cerebellar hemangioblastomas were negative. However, engulfed astrocytes were found at the periphery of such tumors and often adjacent to the proliferate blood vessels. In meningiomas, neurinomas, metastatic carcinomas, pituitary adenomas and other non-glial tumors, GFAP-positive cells were not identified.

KEYWORDS: glial fibrillary acidic protein, central nervous system tumors

*PMID: 4091041 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 39, (6), 453-462 (1985)

**THE DETERMINATION OF GLIAL FIBRILLARY ACIDIC
PROTEIN FOR THE DIAGNOSIS AND HISTOGENETIC
STUDY OF CENTRAL NERVOUS SYSTEM TUMORS:
A STUDY OF 152 CASES**

Kazuo HAMAYA, Kenji DOI, Toshio TANAKA* and Akira NISHIMOTO**

Anatomic Pathology, Okayama Saiseikai General Hospital, Okayama 700, *Central Pathology Unit, and **Neurosurgery,
Okayama University Medical School, Okayama 700, Japan

Received August 7, 1985

Abstract. Glial fibrillary acidic protein (GFAP) was purified from human spinal cord and cerebral white matter. GFAP was localized by an immuno-peroxidase method in normal adult and fetal human brains, rat brains, and 152 central nervous system (CNS) tumors. GFAP was found in reactive and normal astrocytes, immature cells of fetal brain at the 18th to 21st gestational weeks, and normal rat astrocytes. This GFAP staining was quite specific for glial tumors, including astrocytomas, glioblastomas, astroblastomas, and ependymomas. GFAP-positive cells were also found in oligodendrogliomas and choroid plexus papillomas, and they were interpreted as being astroglial or ependymal differentiations. Stromal cells in cerebellar hemangioblastomas were negative. However, engulfed astrocytes were found at the periphery of such tumors and often adjacent to the proliferate blood vessels. In meningiomas, neurinomas, metastatic carcinomas, pituitary adenomas and other non-glial tumors, GFAP-positive cells were not identified.

Key words : Glial fibrillary acidic protein, central nervous system tumors.

Glial fibrillary acidic protein (GFAP) was firstly isolated from old fibril-rich plaques of a human case of multiple sclerosis by Eng *et al.* (1) in 1971 and was verified to crossreact with GFAP of other mammalian species (2). This protein appears to be related to astrocytic glial filaments of 8 to 10 nm in diameter. It is extremely susceptible to in situ proteolysis, resulting in a characteristic disc electrophoretic pattern of multiple, closely spaced bands, which suggests that small fragments are successively cleaved from the original polypeptide chain (54,000 daltons) (3). Recently, aspartic and glutamic acids, alanine, leucine and arginine were found to be the major components of this polypeptide (4). For the last ten years, GFAP has been used as a specific antigenic "marker" of normal or altered glial cells. An immunohistochemical technique using anti-GFAP antibody has been quite reliable for the identification of glial fibrils in brain tumors and other pathological conditions. However, there is no single interpretation of the results of immunohistochemical studies of GFAP in some CNS tumors including ependymomas, choroid plexus papillomas, oligodendrogliomas and cerebellar heman-

glioblastomas. The presence of GFAP in brain tumors is useful for the evaluation of the histogenesis as well as for making a pathological diagnosis.

MATERIALS AND METHODS

GFAP was isolated from human spinal cord and cerebral white matter. The cord tissue was obtained from 12 patients autopsied materials of less than 5 h after their death. The normal white matter, approx. 100g in weight, was obtained from a 78 year-old female 2h post mortem. GFAP was isolated using hydroxylapatite as described by Bignami and Dahl (2, 5). Seventy-four mg of GFAP was obtained from the spinal cord, and 42mg from the cerebral white matter. The molecular weight of GFAP was in the range of 45,000 to 51,000 daltons showing three closely spaced bands by sodium dodecyl sulfate, polyacrylamide gel electrophoresis (Fig. 1). Separate antibodies were obtained by immunizing the rabbits with GFAP derived from either cerebral white matter or spinal cord. These two antisera produced one common main precipitation line in Ouchterlony's immuno-diffusion test against GFAP of brain and spinal cord origin (Fig. 2).

Immunohistochemical localization of GFAP by Sternberger's peroxidase-anti-peroxidase (PAP) method (6) was performed on formaldehyde-fixed paraffin-embedded tissues of normal human brain of adults and fetuses, spinal cord, rat brain and 152 CNS tumors. Some ambiguous cases were examined by electron microscopy to confirm the presence of glial filaments.

RESULTS

Normal brain and spinal cord of human adult and fetus and rat brain. In adult human brain and spinal cord, GFAP was found in normal astrocytes in the white matter and showed strongly positive staining of the subpial astrocytes. By examining fetal brains of various gestation periods, granular or banded aggregates of GFAP were found at the ventral subventricular zone at the roof of the lateral ventricle during the 18th to 21st weeks of gestation. Subpial astrocytes were also positive at this stage. Some of the ependymal cells on the floor of the lateral ventricle, as well as subependymal immature round cells, were positive for GFAP (Fig. 3).

Normal astrocytes of the rat brain were also positive for the anti-human GFAP antibody.

CNS tumors. CNS tumors were divided into three groups according to the staining pattern.

Group I: GFAP was observed in astrocytomas (40 cases), glioblastomas (21 cases), ependymomas, including one myxopapillary type (10 cases), subependymomas (2 cases) and an astroblastoma (one case).

In low grade astrocytomas, fibrillary (Fig. 4), protoplasmic (Fig. 5) and gemistocytic tumor cells (Fig. 6) were strongly positive for GFAP. Elongated fine fibrils of piloid astrocytomas were also positive. In high grade astrocytomas, GFAP was stained less than in adjacent reactive astrocytes. Undifferentiated small glial cells were practically negative even at their cell processes. Most of the Rosenthal fibers had a thin positive margin (Fig. 7), although some remained unstained.

Giant cells in glioblastomas were generally positive (Fig. 8). Some of the

more bizarre and larger cells were negative. Perivascular tumor cells were positive at their foot processes. Tumor cells around the necrosis, showing pseudopalisading and small immature cells, were usually negative.

In some ependymomas, numerous multipolar fibers encased the negative tumor cells (Fig. 9). Fine long processes around the blood vessels were positive. Ependymal rosettes were weakly stained. The myxopapillary type was not much different from other ordinary tumors. One case of ependymoblastoma which developed in the cervical spinal cord of a 2 year-old girl was negative for GFAP.

Subependymomas had strongly positive coarse fibers, as do fibrillary astrocytomas (Fig. 10), and a small number of cells with darkly stained abundant cytoplasm resembling gemistocytic cells.

One case of astroblastoma we examined was of a rather immature type composed of round immature cells around the capillaries. Positive globular staining was identified in the perikarya (Fig. 11). The presence of glial filaments was confirmed by electron microscopy. However, perivascular foot processes were not stained.

Group II; GFAP was not observed in tumor cells with positive entrapped astrocytes or astroglial differentiation in hemangioblastomas (20 cases), oligodendrogliomas (9 cases), choroid plexus papillomas (5 cases), and medulloblastomas (6 cases).

Ten of 20 hemangioblastoma cases had GFAP-positive astrocytes entrapped within the tumor. These positive cells were predominantly near the proliferate blood vessels, and at the periphery of the tumor (Fig. 12). They all had fibers stained positively with phospho-tungstic acid hematoxylin (PTAH) stain. All stromal cells were negative.

Of 9 oligodendrogliomas, 3 had GFAP at the perikarya (Fig. 13), resembling protoplasmic astrocytomas, but not at the cytoplasmic processes. All were of the differentiated type and not mixed with astrocytoma cells.

Only one of 5 choroid plexus papillomas had GFAP in the elongated cytoplasm which extended toward the center of the tumor (Fig. 14). The presence of glial filaments at the processes was confirmed by electron microscopy.

Positive astrocytes without cellular atypism was scattered in small areas of medulloblastomas. However, elongated tumor cells suggestive of spongioblastic differentiation were negative for GFAP.

Group III; GFAP was not detected in meningiomas (11 cases), neurilemmomas (5 cases), malignant lymphomas (4 cases), pituitary adenomas (3 cases), metastatic carcinomas, arterio-venous malformations, teratomas and germinomas (2 cases each), nor in one case each of chordoma, craniopharyngioma, malignant melanoma, malignant fibrous histiocytoma, neuroblastoma, ganglioneuroma and retinoblastoma.

DISCUSSION

Among low grade astrocytomas, gemistocytic, protoplasmic, fibrillary, and

piloid types had positive staining for GFAP. Van der Meulen (7) reported that all tumor cells of grade 1 and 2 astrocytomas were GFAP positive and that those of grade 4 were all negative. Duffy (8) stressed that three groups of astrocytomas, stellate-shaped, gemistocytic and piloid astrocytomas were always positive because of their wealth of filaments. Abundant GFAP in gemistocytic astrocytomas is supported by the study of Hoshino (9) in which a low rate of proliferation was observed using tritiated thymidine *in vitro*. Tumor cells of high grade astrocytomas were often negative because of cell immaturity. The positive correlation of GFAP and cell maturation was supported by quantitative analysis (10). Peripheral staining of the Rosenthal fibers was reported by Velasco (11). This is consistent with the electron microscopic findings of glial filaments surrounded the central amorphous material (12).

In glioblastomas, giant cells as well as gemistocytic or spindle cells were GFAP positive. Some giant cells with bizarre nuclei were weakly positive, possibly due to abnormal mitotic states (endomitosis) (8). Small-sized tumor cells or those composing pseudo-palisades were usually negative, because of undifferentiation.

As for ependymomas, the reported results are variable (7, 11, 13, 14). In our material, prominent perivascular processes and multipolar fibers were characteristically positive with GFAP as reported by Velasco (11), Deck (13) and Eng (14). Also, the ependymal cells of fetal brain had positive fibers extending toward the parenchyma. These results possibly reflect the greater tendency of neoplastic and fetal ependymal cells toward gliofibrillogenesis than epithelial differentiation. This idea is in accordance with the development of astroglial filaments in cultured ependymoma cells (15).

Kepes (16) suggested the presence of astrocytic elements in stromal cells of cerebellar hemangioblastomas. Our study also showed GFAP-positive cells in 10 of 20 samples. These cells were, however, interpreted as being entrapped astrocytes, not stromal cells. GFAP-positive cells always had PTAH-positive fibers. Electron microscopic study of stromal cells did not reveal glial filaments. The engulfment of astrocytes was supported by their being located at the periphery of the tumor and always adjacent to proliferate blood vessels. Engulfment is probably enhanced when tumors are large even though this type of tumor is primarily cystic and located at the midline. In our examples, tumors positive for GFAP were larger in size and often deviated from the midline. The possibility of non-fibrillary GFAP being phagocytized by the stromal cells is unlikely, because such phagocytic activity has not been verified in stromal cells. Even macrophages have not been proved to be able to phagocytize this protein.

With the easy availability of this astrocytic marker, the origin of oligodendrogliomas has been questioned. This type of tumor is generally agreed as being GFAP negative (8, 11, 14). However, our 3 cases of pure oligodendroglioma were positive for GFAP at the perikarya, resembling miniature protoplasmic astrocytomas. This result supports the quantitative analysis by Rasmussen (17) of high content of GFAP in oligodendrogliomas. Van der Meulen (7) reported that oligodendroblastomas

were positive for GFAP, while mature oligodendrogliomas remained negative. They suggested the possibility of astrocytic "transition" of oligodendrogloma cells. Meneses (18) found 15 examples of GFAP-positive oligodendrogliomas out of 19 cases. These positive cells were few in number and scattered within the tumor, unlike mixed gliomas. Such ambiguous cases will have to be examined by specific markers of oligodendrogliomas including leu-7 as recently suggested by Motoi (19).

GFAP was found in a choroid plexus papilloma of a 2-year-old girl. The positive cell processes were confirmed as having glial filaments by PTAH stain and electron microscopy. Rubinstein (20) reported 9 examples of GFAP-positive choroid plexus papillomas out of 22 cases, including 4 with malignant changes. This is possibly an expression of ependymal differentiation of choroid plexus papillomas. It is surprising that such benign tumor cells have divergent differentiating potentials. The normal choroid plexus of fetal brain was not stained with anti-GFAP.

GFAP was not stained in meningioma, neurilemmoma, malignant lymphoma, pituitary adenoma, metastatic carcinoma, arterio-venous malformation, chordoma, craniopharyngioma, germinoma, malignant melanoma, malignant fibrous histiocytoma, neuroblastoma, ganglioneuroma and retinoblastoma tissue except for reactive astrocytes adjacent to the tumor.

From these results, GFAP is a specific marker of glial cells, especially astrocytes. The PAP method for GFAP detection is superior to PTAH staining as far as specificity is concerned. GFAP can be detected even in smooth homogenous and non-fibrillar conditions. PTAH stain becomes positive only in the fibrillary form. False negative and false positive results are often experienced with PTAH stain, i.e., smooth muscle fibrils and extracellular proteins such as fibrins are stained and often mistakenly interpreted as glial fibrils.

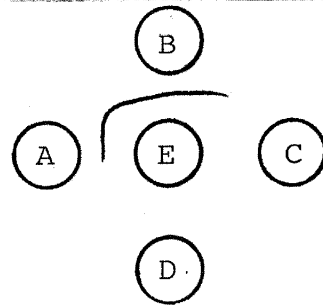
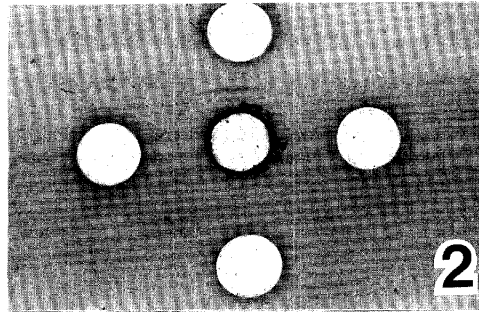
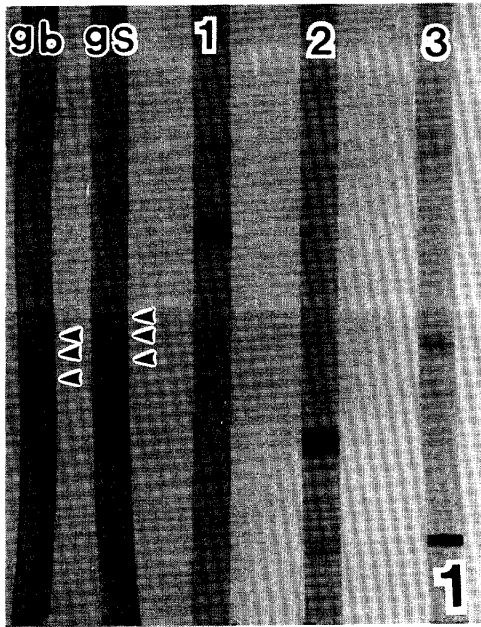
This paper was partly presented at the 22nd and 23rd meetings of the Japanese Neuropathology Society (1981, 1982) and the 70th meeting of the Japanese Pathological Society (1981).

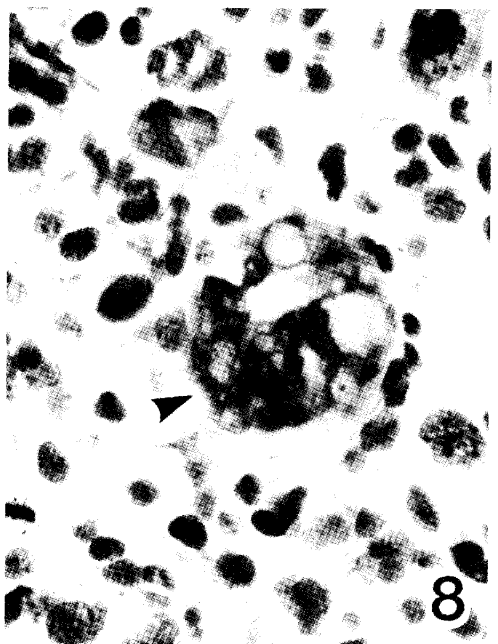
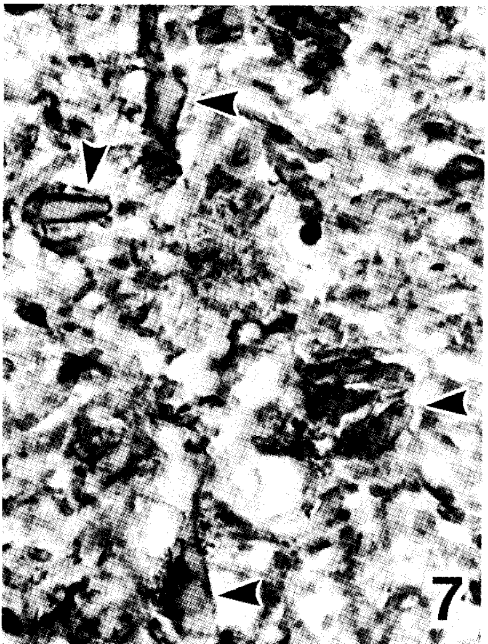
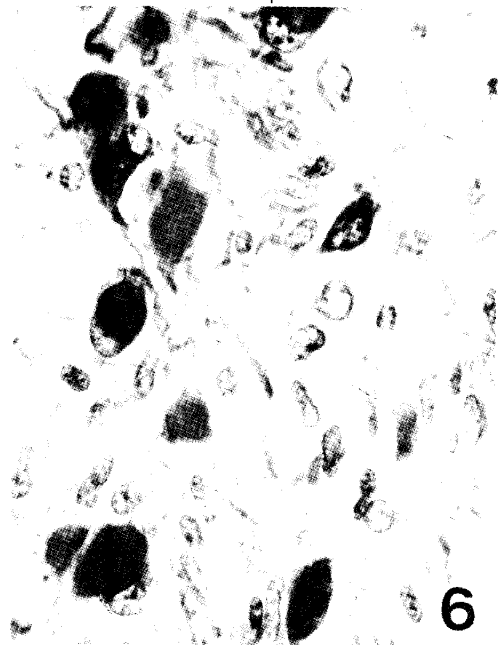
Acknowledgements ; We thank Dr. H. Sonobe, Department of Clinical Laboratory Science, Kochi Medical School Hospital for allowing us to examine fetal brain, Miss M. Yabuki and Miss N. Hara for their technical assistance.

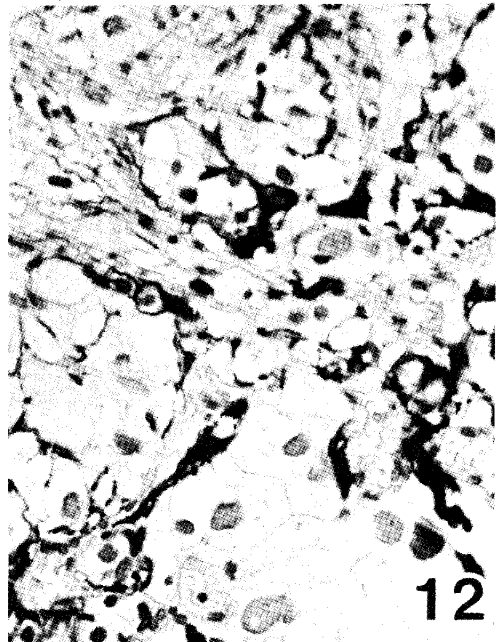
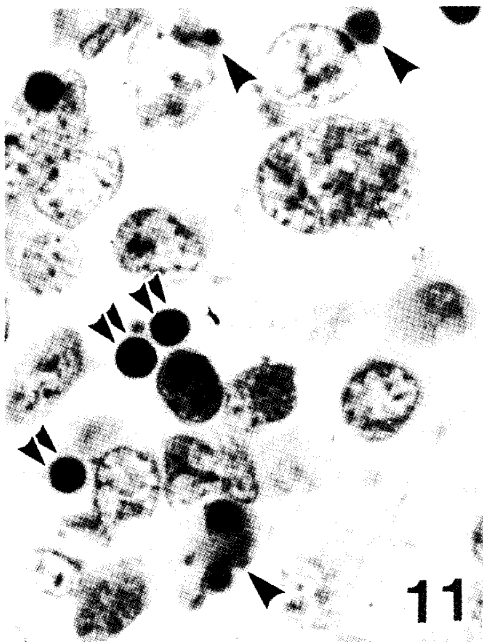
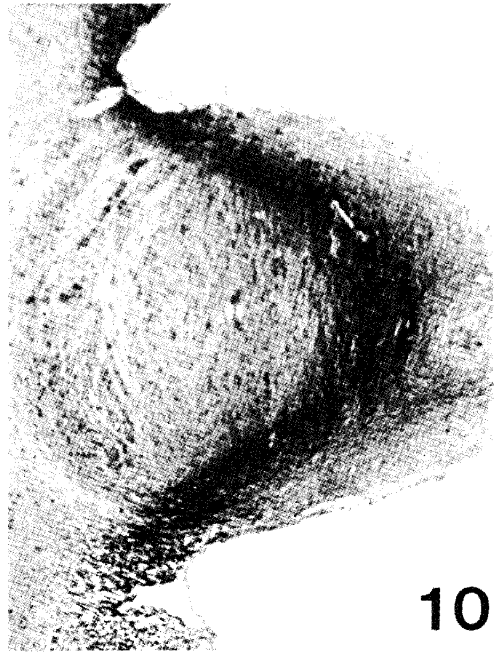
REFERENCES

1. Eng, L.F., Vanderhaeghen, J.J., Bignami, A. and Gerstl, B.: An acidic protein isolated from fibrous astrocytes. *Brain Res.* **28**, 351-354, 1971.
2. Bignami, A., Eng, L.F., Dahl, D. and Uyeda, C.T.: Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Res.* **43**, 429-435, 1972.
3. Dahl, D. and Bignami, A.: Immunogenic properties of the glial fibrillary acidic protein. *Brain Res.* **116**, 150-157, 1976.
4. Rubinstein, L.J.: *Tumors of the Central Nervous System*, Supplement. Armed Forces Institute of Pathology, Washington D.C. p. 21, 1982.

5. Bignami, A. and Dahl, D.: Astrocyte-specific protein and neuroglial differentiation. An immunofluorescence study with antibodies to the glial fibrillary acidic protein. *J. Comp. Neurol.* **153**, 27-38, 1974.
6. Sternberger, L.A., Hardy, P.H.Jr., Cuculis, J.J. and Meyer, H.G.: The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish-peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem.* **18**, 315-333, 1970.
7. Van der Meulen, J.D.M., Houthhof, H.J. and Ebels, E.J.: Glial fibrillary acidic protein in human gliomas. *Neuropathol. Appl. Neurobiol.* **4**, 177-190, 1978.
8. Duffy, P.E., Huang, Y.Y., Rapport, M.M. and Graf, L.: Glial fibrillary acidic protein in giant cell tumors of brain and other gliomas. *Acta Neuropathol.* **52**, 51-57, 1980.
9. Hoshino, T., Wilson, C.B. and Ellis, W.G.: Gemistocytic astrocytes in gliomas. *J. Neuropathol. Exp. Neurol.* **34**, 263-281, 1975.
10. Jacque, C.M., Kujas, M., Porean, A., Raoul, M., Collier, P., Racadot, J. and Baumann, N.: GFA and S-100 protein levels is an index for malignancy in human gliomas and neurinomas. *J. Natl. Cancer Inst.* **62**, 479-483, 1979.
11. Velasco, M.E., Dahl, D., Rosessmann, U. and Gambetti, P.: Immunohistochemical localization of glial fibrillary acidic protein in human neoplasms. *Cancer* **45**, 484-494, 1980.
12. Rubinstein, L.J.: *Tumors of the Central Nervous System*. Armed Forces Institute of Pathology, Washington, D.C., pp. 97-98, 1972.
13. Deck, J.H., Eng, L.F., Bigbeem, J. and Woodcock, S.M.: The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors. *Acta Neuropathol.* **42**, 183-196, 1978.
14. Eng, L.F. and Rubinstein, L.J.: Contribution of immunohistochemistry to diagnostic problems of human cerebral tumors. *J. Histochem. Cytochem.* **26**, 513-522, 1978.
15. Uraa-Jensen, J., Herman, M.M., Rubinstein, L.J. and Bignami, A.: *In vitro* characteristics of a fourth ventricle ependymoma maintained in organ culture system; light and electron microscopy observations. *Neuropathol. Appl. Neurobiol.* **2**, 349-364, 1976.
16. Kepes, J., Rengachary, S.S. and Lee, S.H.: Astrocytes in hemangioblastomas of the central nervous system and their relationship to stromal cells. *Acta Neuropathol.* **47**, 99-104, 1979.
17. Rasmussen, S., Bock, E., Warecka, K. and Althage, G.: Quantitation of glial fibrillary acidic protein in human brain tumors. *Br. J. Cancer* **41**, 113-116, 1980.
18. Meneces, A.C.O., Kepes, J.J. and Sternberger, L.H.: Astrocytic differentiation of neoplastic oligodendrocytes. *J. Neuropathol. Exp. Neurol.* **41**, 368, 1982.
19. Motoi, M., Yoshino, T., Hayashi, T., Nose, S., Horie, Y. and Ogawa, K.: Immunohistochemical studies on human brain tumors using anti-leu 7 monoclonal antibody in paraffin-embedded specimens. *Acta Neuropathol.* **66**, 75-77, 1985.
20. Rubinstein, L.J. and Brucher, J.M.: Focal ependymal differentiation in choroid plexus papillomas. An immunoperoxidase study. *Acta Neuropathol.* **53**, 29-33, 1981.







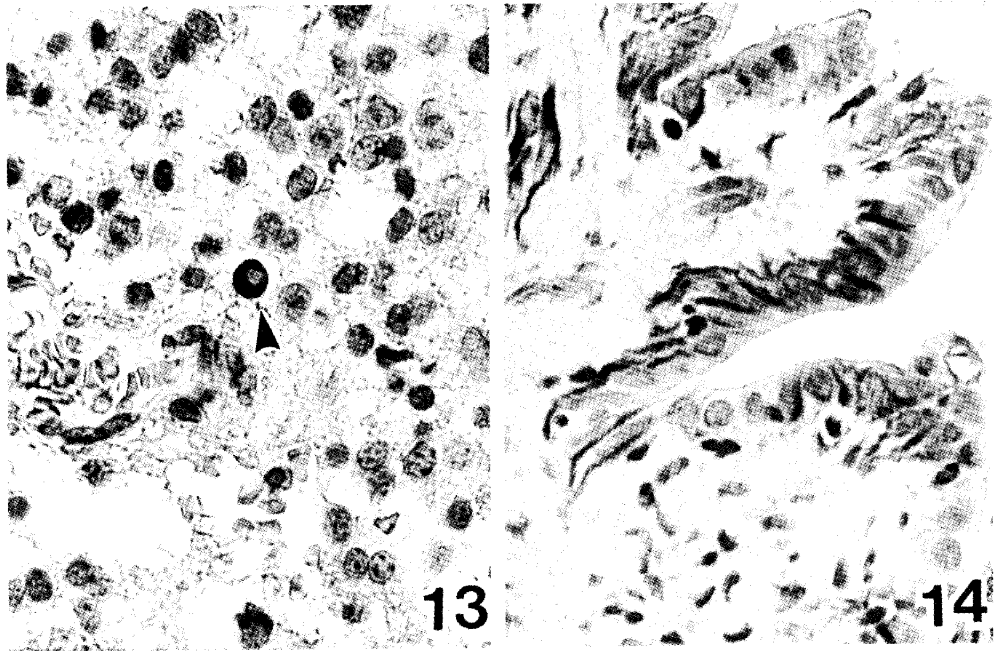


Fig. 1. Electrophoretic pattern of GFAP in a sodium dodecyl sulfate, polyacrylamide gel showing three closed bands (gb from brain and gs from spinal cord). The other bands are (1) chymotrypsinogen A (M.W. 25,000), (2) bovine serum albumin (M.W. 68,000), and (3) rabbit muscle aldorase (M.W. 158,000).

Fig. 2. One common precipitation line is seen between brain GFAP (E), anti-GFAP of spinal cord (A), and anti-GFAP of brain (B), but not with anti-bovine serum albumin (C), and anti S-100 protein (D).

Fig. 3. Fetal ependymal cells of the 21st week of gestation showing positive fibers extending to the parenchyma. ($\times 600$)

Fig. 4. GFAP-positive, long, fine processes in a fibrillary astrocytoma. ($\times 600$)

Fig. 5. Short processes and perikarya of a protoplasmic astrocytoma are GFAP positive. ($\times 600$)

Fig. 6. Abundant cytoplasm of gemistocytic astrocytoma cells is GFAP positive. ($\times 600$)

Fig. 7. Many Rosenthal fibers (arrows) show a thin positive rim. ($\times 600$)

Fig. 8. GFAP in giant cells of a glioblastoma multiforme. (arrow) ($\times 600$)

Fig. 9. In an ependymoma, positive, long, thin processes encase negative cells. ($\times 600$)

Fig. 10. Positive fibrillary fibers beneath a doom-shaped subependymoma. ($\times 60$)

Fig. 11. Intracytoplasmic globular GFAP (arrows) in an astroblastoma. Double arrows indicate lymphocytes. ($\times 600$)

Fig. 12. Positive fibers are close to the proliferate capillaries. Note that stromal cells are negative. ($\times 300$)

Fig. 13. Some oligodendroglioma cells are GFAP positive (arrow). ($\times 600$)

Fig. 14. GFAP-positive, long processes extending toward the stroma in a choroid plexus papilloma. ($\times 600$)