Histol Histopath (1991) 6: 503-508

Histology and Histopathology

Immunohistological signposts in central nervous system tumours with neuronal differentiation

F.F. Cruz-Sánchez[1], M.L. Rossi[2], S. Rodríguez-Prados[2], V. Cusi[3] and H.B. Coakham[4]

[1]Neurological Tissue Bank, Department of Medicine, Neurology, Provincial Hospital Clinico, University of Barcelona, Barcelona, Spain, [2]Department of Neuropathology, Midland Centre for Neurology and Neurosurgery (MC NN, Birmingham, UK, [3]Department of Pathology, Hospital Juan de Dios, Barcelona, Spain and [4]Imperial Cancer Research Brain Tumour Group, Frenchay Hospital, Bristol, UK

Summary. 25 neuronal tumours with a panel of antibodies were studied and it was found that vimentin was present in 15 tumours. It was also found in a few cells within rosettes. PGP 9.5 showed a somatic pattern of staining with nuclear and perinuclear positivity in 23. Neurofilament reactivity was found in 14. Retinal S-antigen was detected only in one medulloblastoma, 3/4 pineal tumours and 2/2 retinoblastomas. Reactivity, for synaptophysin was present in 2/5 medulloblastomas, 3/10 neuroblastomas and 2/2 retinoblastomas. GFAP was demonstrated in scattered tumour cells in 4/5 medulloblastomas. Two of these were the only tumours featuring bipolar differentiation whilst it was unipolar in the remainder. The significance of these findings in relation to the ontogeny of these tumours is discussed.

Key words: Neuroblastoma, Pineal tumour, Immunohistology, Embryonal neuroepithelial tumours

Introduction

In the World Health Organization classification (Zulch, 1981), neuronal tumours are divided into 5 distinct groups two of which (neuroblastoma and ganglioneuroblastoma) are classed as embryonal neoplasms. Tumours of the pineal body are another group in which neuronal differentiation has been proposed (Rubinstein and Russell, 1989) and retinoblastoma is considered to be an embryonal tumour of neural derivation (Donoso et al., 1985). Embryonal tumours are composed of cells with similar features and a recent classification (Rorke et al., 1985) has included all of these tumours in the category of primitive neuroectodermal tumours. These tumours may include multipotential cells with the capacity to differentiate along one or more cell lines such as, for example, in medulloblastoma where neuronal differentiation has been demonstrated (Burger et al., 1987; Cruz-Sánchez et al., 1989a). Immunohistological studies may be able to give us, with some provisos (Rubinstein, 1986), the tools to unravel the relationships between the supposed existence of cell lines giving rise to a particular tumour.

25 neuronal tumours were studied with the aim to demonstrate whether histologically similar tumour types had a different immunohistological pattern of staining.

Offprint requests to: Dr. F.F. Cruz-Sánchez, Banco de Tejidos Neurológicos, Servicio de Neurología, Hospital Clínico y Provincial, Villarroel 170, Barcelona 08036, Spain.

Materials and methods

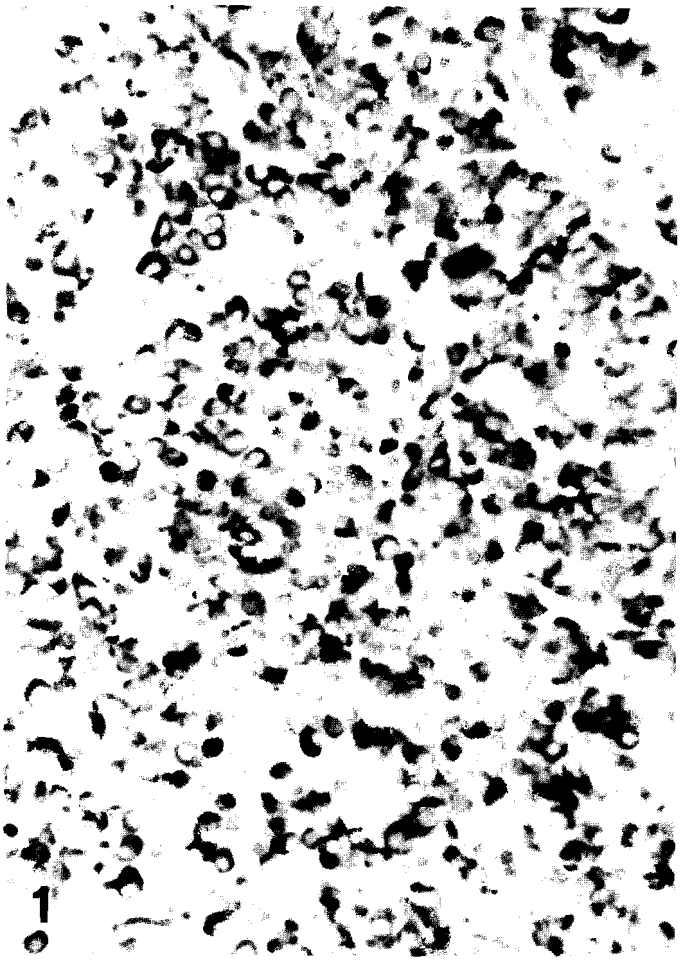
Tissue from 25 primitive CNS tumours including 5 medulloblastomas, 10 neuroblastomas, 2 ganglioneuroblastomas, 2 gangliocytomas, 2 pinealoblastomas, 2 pinealocytomas and 2 retinoblastomas was examined. The mean age of the whole group of patients was 26 years with a a range from 3 months to 50 years; 17 cases were children (< 15 years) and their mean age was 8 years. Eight cases were adults and their mean age was 44 years. There were 15 males and 10 females.

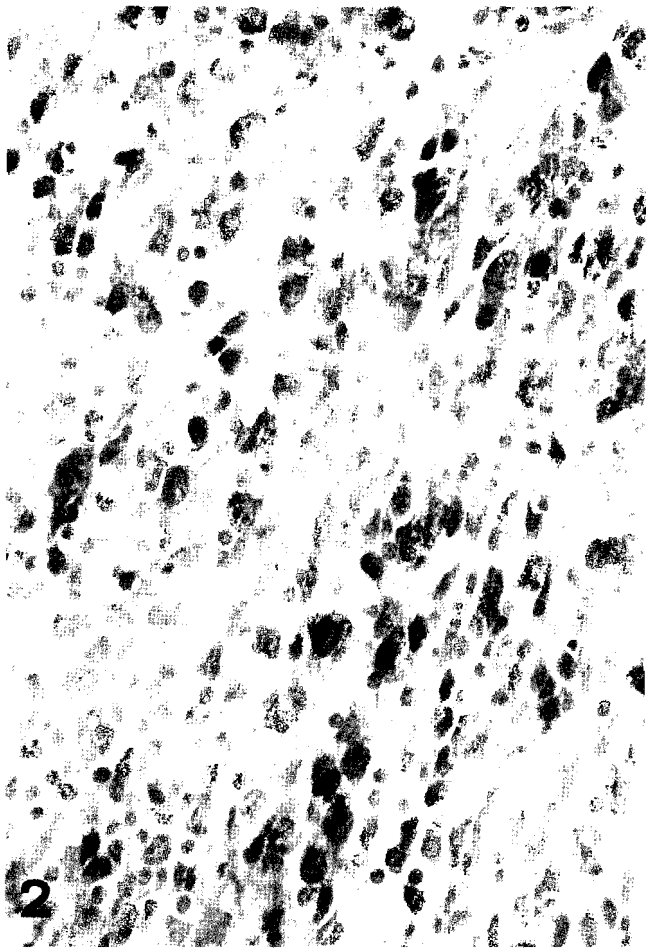
Tumour tissue was fixed in 10% formalin and embedded in paraffin. 7 μm sections were cut and stained with haematoxylin and eosin (H & E) and reacted with a panel of antibodies (Table 1).

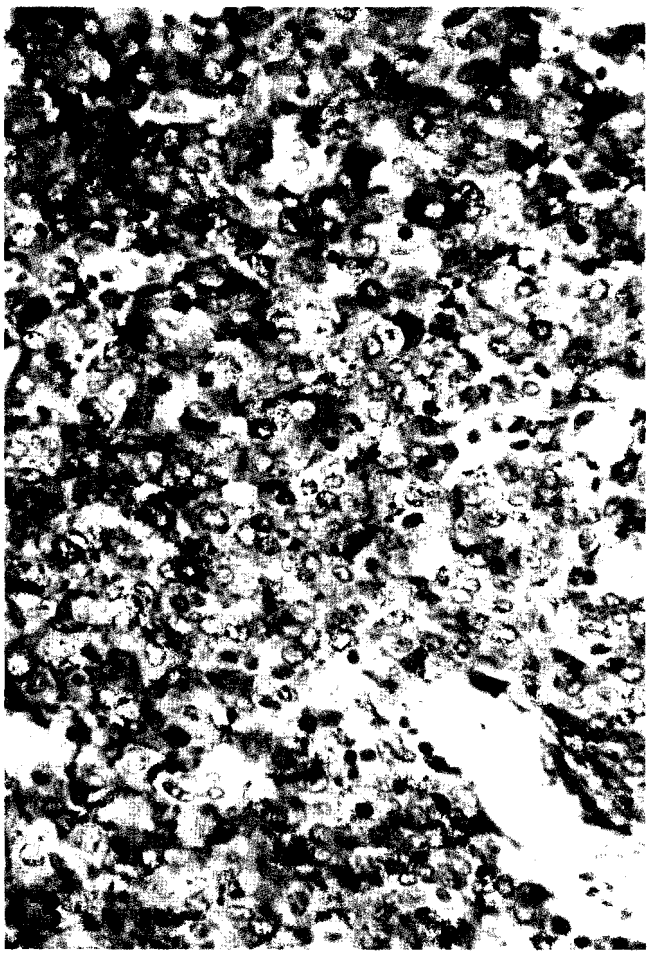
As positive controls we used normal retina (A9-C6) and brain (all other antibodies). All the monoclonal antibodies were used in an indirect two stage immunoperoxidase technique (Warnke et al., 1983) and the polyclonal ones (GFAP & PGP 9.5) were used in peroxidase anti-peroxidase reaction (PAP) (Sternberger, 1979). Sections were not trypsinized.

Results

Vimentin expression by tumour cells was widespread in most tumours (with the exception of gangliocytomas and pineal neoplasms) and the pattern of positivity was somatic (Fig. 1). A few cells within rosettes were positive. Medulloblastomas showed scattered positive


1


2



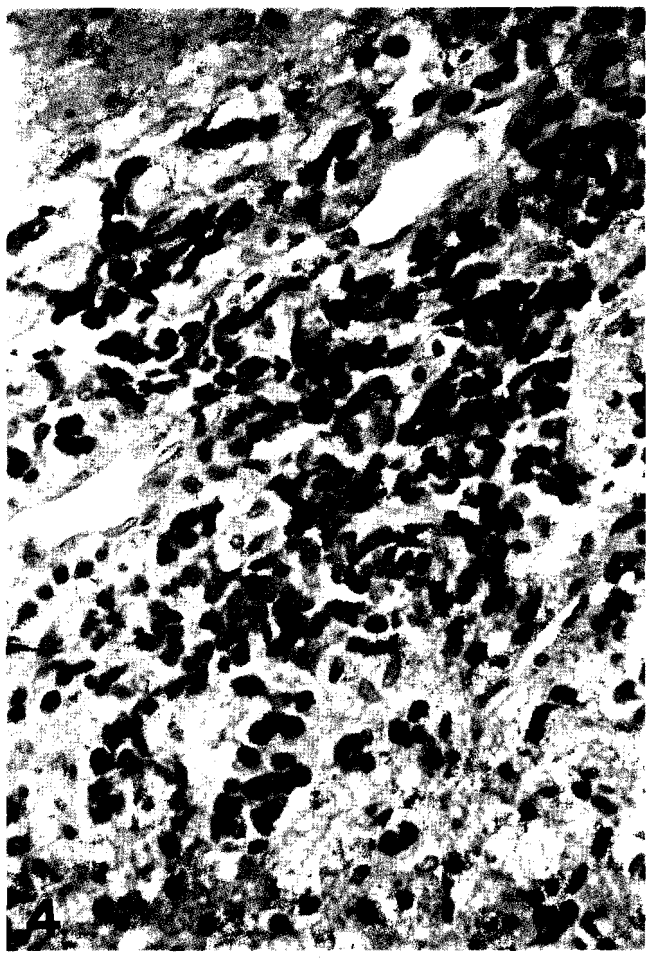

4

Fig. 1. Neuroblastoma: widespread positivity for vimentin. Vimentin. × 200

Fig. 2. Medulloblastoma: somatic pattern of positivity for PGP 9.5. PGP 9.5 × 300

Fig. 3. Pinealoblastoma: Positivity for 155 kd neurofilament (BF10) in most cells. BF10. × 300

Fig. 4. Ganglioneuroblastoma: nests of neurofilament-positive small cells. BF10. × 200

tomour cells. Blood vessel walls and connective tissue were intensely positive and included astrocytes showed co-expression with GFAP.

PGP 9.5 showed a somatic pattern of staining with nuclear and perinuclear positivity in 23 tumours. All medulloblastomas featured positivity within cell cords as well as in a patchy distribution and in clusters or islands of poorly differentiated cells which were strongly positive (Fig. 2). Rosettes were on the whole negative but for slight luminal reactivity. All neuroblastomas were diffusely positive and showed areas with stronger reactivity. Rosettes showed a similar pattern of positivity as in medulloblastoma. The two ganglioneuroblastomas showed strong positivity in nests of small tumour cells and in large tumour cells as did cells in the two gangliocytomas. The two pinealoblastomas and one of the two pinealocytomas were positive and the reactivity was stronger in the former. One of the two retinoblastomas showed a strong positivity but Flexner-type rosettes were negative in both.

BF10-positive cells evinced a somatic pattern of staining as well as few short positive processes. Some neuroblastomas and pinealoblastomas (Fig. 3) showed strong positivity for BF10. The two ganglioneuroblastomas were diffusely positive and showed strong positivity in nests of small cells (Fig. 4). The two gangliocytomas showed moderate positivity in large tumour cells.

RT97 reacted with 8 tumours with a pattern similar to BF10, staining nests of small cells and also large ones (Fig. 5); the two retinoblastomas were focally positive but rosettes were negative.

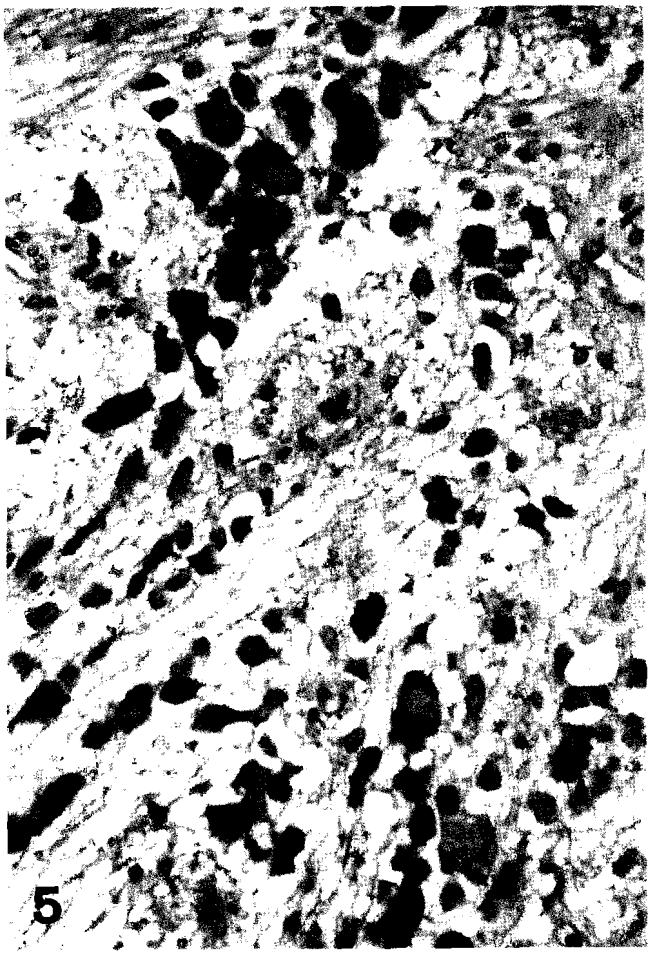


Fig. 5. Ganglioneuroblastoma: somatic pattern of positivity for 210 Kd neurofilament in ganglion cells and more primitive cells. RT97. × 300

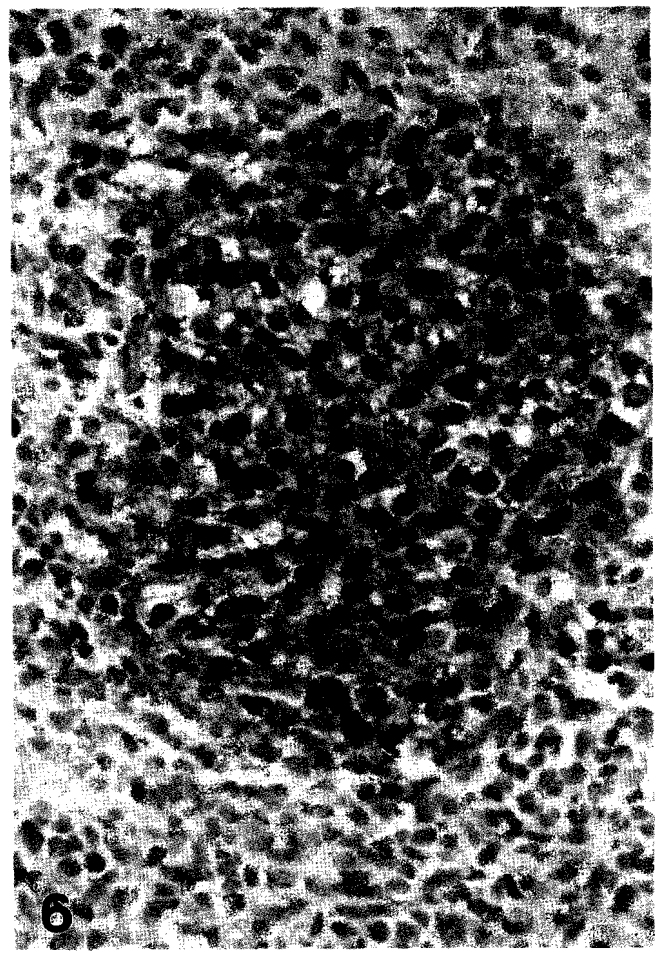


Fig. 6. Medulloblastoma: pale area strongly positive for synaptophysin. Synaptophysin. × 300

Table 1. Antibodies used in the study.

Antibody	Clone	Antigen	Source/Reference	Dilution
GFAP	Polyclonal	52 Kd polypeptide	DAKO	1:200
PGP9.5	Polyclonal	Neuronal marker	Ultraclone(+)	1:400(·)
BF10	IgG2a	155 Kd NF	(●)	1:1
RT97	IgGl	210 Kd NF	(●)	1:1
A9-C6	Monoclonal	Retinal S-antingen	(*)	1:1
Vimentin	Monoclonal	57 kd Vimentin	(X)	1:1
Synaptophysin	IgGl	Presynaptic Vescicles	Boehringer Mann	10μg/ml

(X) from KC Gatter, Oxford
(+) Thompson et al. (1983)
(·) Incubation time of 4 hours for the polyclonal antibodies.
(●) Anderton et al. (1982).
(*) Donoso et al. (1985); also present in photoreceptor cells and pinealocytes as well as in retinoblastoma, pinealocytoma and pinealoblastoma (Donoso et al., 1985; Parentes et al., 1986).
NF = neurofilament subunit

Table 2. Summary of results obtained with 8 antibodies in 25 primitive neural tumours.

	No cases	VI	PG	A9	BF	RT	Sy	GF
Medulloblastomas	5	4	5	1	3	2	2	4
Neuroblastomas	10	7	10	–	5	2	3	–
Ganglioneuroblastomas	2	2	2	–	2	–	–	–
Gangliocytomas	2	–	2	–	2	1	–	–
Pinealoblastomas	2	–	2	2	2	1	–	–
Pinealocytoma	2	–	1	1	–	–	–	–
Retinoblastomas	2	2	1	2	–	2	2	–
Total	25	15	23	6	14	8	7	4

Legend: VI = Vimentin; PG = PGP 9.5; A9 = A9-C6; BF = BF10;
RT = RT97; Sy = Synaptophysin; GF = GFAP

Retinal S-antigen was only detected in scattered and clustered cells of one medulloblastoma, 3/4 pineal tumours and the two retinoblastomas. The pattern was somatic with juxtanuclear accentuation.

Reactivity for synaptophysin was present in 2/5 medulloblastomas, 3/10 neuroblastomas and the two retinoblastomas. Pale areas of medulloblastomas were positive (Fig. 6).

GFAP was demonstrated in scattered tumour cells of 4/5 medulloblastomas with a predominant somatic pattern but few cells showing short prolongations were seen in all tumours. Several cases showed only included or reactive astrocytes.

Discussion

Our results demonstrate that most tumours expressed various proteins related mainly to neuronal differentiation. However, expression, distribution and pattern of positivity were different. We therefore postulate that these tumours show distinct stages of maturation. Only medulloblastomas expressed glial and neuronal proteins simultaneously.

Histologically more undifferentiated cells in

medulloblastomas, neuroblastomas, ganglioneuroblastomas and retinoblastomas contained vimentin; ganglion cells in gangliocytomas and ganglioneuroblastomas nevertheless did not. We agree with Houle and Federoff (1983), who proposed that the expression of vimentin may reflect degrees of undifferentiation; however, poorly differentiated cells of pinealoblastomas showed no vimentin positivity.

PGP 9.5 is a neuronal protein isolated from brain but its function is unknown at present (Thompson et al., 1983; Doran et al., 1983; Rode et al., 1985). We found that it was more frequently expressed by the more undifferentiated cells in medulloblastomas and by the large cells in ganglion cell neoplasms, which would imply that PGP 9.5 is a constant component of neuronal cells.

Synaptophysin is a glycoprotein associated with synpatic vesicles (Miettinen, 1987). We found positivity in 7/25 tumours (2 medulloblastomas, 3 neuroblastomas and 2 retinoblastomas) which may indicate that most of the tumours had still not reached a sufficient degree of maturation.

BF10 is an intermediate neurofilament of 155 kd and RT97 of 210 kd (Anderton et al., 1982). The expression of the two neurofilament subunits recognized by our antibodies varied in the various tumours.

Co-expression between PGP 9.5, synaptophysin and both neurofilament subunits was found but the pattern was different. PGP 9.5 reactivity was predominantly somatic in clusters of cells such as for example in pale areas of medulloblastomas and on the lumina of rosettes.

Synaptophysin reactivity also had a somatic pattern with diffuse reactivity and focal accentuation. On the contrary, reactivity for both neurofilaments was somatic and in short prolongations in scattered cells which were diffusely distributed and at the edge of pale areas. This may indicate that the degree of differentiation or stage of maturation play an important role in neurofilament expression. PGP 9.5 is the first protein which appears in neuronal differentiation. Pale areas of medulloblastomas included cells featuring only neuroblastic differentiation and this was reflected by the fact that they reacted only with PGP 9.5. More mature cells also containing neurofilaments were situated at the edge of pale areas. Cells within rosettes were negative for neurofilament and were reactive with PGP 9.5 only on the luminal border, which may imply that they are more primitive than cells within pale areas.

A9-C6 is a monoclonal antibody which reacts with retinal S-antigen (Donoso et al., 1985) which is also present in pinealocytes, retinoblastomas, pinealocytomas and pinealoblastomas (Bonnin and Parentes, 1988).

Pineal tumours showed neuronal differentiation; however, differences between pinealocytomas and pinealoblastomas were found in that the former were neurofilament-negative.

A9-C6 reactivity was found in 1 medulloblastoma and in the two retinoblastomas, thus demonstrating that medulloblastoma may show neuronal photoreceptor differentiation and pointing to the closeness in the stage of maturation of medulloblastomas, pinealo-
blastomas and retinoblastomas.

Our results demonstrate the usefulness of an antibody panel in ascertaining the cellular lineage in embryonal central nervous system tumours and in classifying them correctly according to the degree of differentiation.

Medulloblastomas featured bipolar, glial and neuronal differentiation (including photoreceptor). Other tumours showed only neuronal differentiation but all showed distinct stages of maturation which was evident at the histological and immunohistological level. Whilst several neuronal proteins were expressed in some cells within an individual tumour, other cells did not express any. A possible explanation may be that aberrant ontogenetic information of differentiation is directly related to the expression of these proteins. This has also been proposed for other tumours (Rubinstein, 1986; Cruz-Sánchez et al., 1989b) and relates to the timing in which the ontogenetic information is translated or the oncogenetic factors operate.

The combination of our results allow us to group tumours with the same immunohistological characteristics with or without correlation with the histological appearance.

Rorke (1983) sustained that embryonal neural tumours were composed of small primitive cells with multipotential capacity to differentiate. We have demonstrated only the bipolar (in medulloblastomas) or unipolar (the rest of the tumours) capacity of these tumours which could be grouped not only by histological features but also by their immunohistological behaviour. We note however that Sobel et al. (1981) and Bonnin et al. (1984) have demonstrated glial differentiation in pinealoblastoma.

Further studies will be necessary to ascertain whether the morphological (histological and immunohistological) appearances may be related to outcome.

Acknowledgements. We wish to acknowledge the generous help of Dr. K.C. Gatter, Dr. D.Y. Mason, Dr. L.A. Donoso, Profs. J. O'D. McGee, Dr. B. Anderton, Ms. M. Jones, H. Turley and J. Cordell who provided some of the antibodies. We thank the trustees of the MCNN for their support. Ms A. Pichardo (on a cirit fellowship) is thanked for her assistance. S. Rodríguez-Prados wa supported by a national Univ. of Tucuman (Argentina) fellowiship.

References

Anderton B.H., Breinburg D., Downes M.J., Green P.J., Tomlison B.E., Ulrich J., Wood J.N. and Kahn J. (1982).

Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share antigenic determinants. Nature 298, 84-86.

Bonnin J.M., Rubinstein L.J., Palmer N.F. and Beckwith J.B. (1984). The association of embryonal tumours originating in the kidney and in the brain. Cancer 54, 2137-2141.

Bonnin J.M. and Parentes E. (1988). Retinal S-antigen immunoreactivity in medulloblastomas. Acta Neuropathol. (Berl) 76, 204-207.

Burger P.C., Graham F.C., Bliestle A. and Kleihues P. (1987). Differentiation in the medulloblastoma. A histological and immunohistochemical study. Acta Neuropathol. (Berl.) 73, 115-123.

Cruz-Sánchez F.F., Rossi M.L., Hughes J.T., Esiri M.M. and Coakham H.B. (1989a). Medulloblastoma: an immunohistological study of 50 cases. Acta Neuropathol. (Berl.) 79, 205-210.

Cruz-Sánchez F.F., Rossi M.L., Hughes J.T., Coakham H.B., Figols J. and Eynaud P.M. (1989b). Choroid Plexus Papillomas: An immunohistological study of 15 cases. Histopathology 15, 61-69.

Donoso L.A., Feldberg N.T., Ausburger J.J. and Shield J.A. (1985). Retinal S-antigen and retinoblastoma: a monoclonal antibody and flow cytometric study. Invest. Ophtalmol. Visual Sci. 26, 568-571.

Doran J.F., Jackson P.J., Kynoch P.A.M. and Thompson R.J. (1983). Isolation of PGP 9.5, a new human neurone specific protein detected by high resolution two dimensional electrophoresis. J. Neurochem. 40, 1542-1547.

Houle J. and Fedoroff S. (1983). Temporal relationship between the appearance of vimentin and neural tube development. Dev. Brain Res. 9, 189-195.

Miettinen M. (1987). Synaptophysin and neurofilament proteins as markers for neuroendocrine tumors. Arch. Pathol. Lab. Med. 111, 813-818.

Parentes E., Rubinstein L.J., Herman M.M. and Donoso L.A. (1986). S-antigen immunoreactivity in human pineal glands and pineal parenchymal tumours. A monoclonal antibody study. Acta Neuropathol. (Berl.) 71, 224-227.

Rode J., Dhillon A.P., Doran J.F., Jackson P.J. and Thompson R.J. (1985). PGP 9.5, a new marker for human neuroendocrine tumours. Histopathology 11, 147-158.

Rorke L.B. (1983). The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumours. J. Neuropathol. Exp. Neurol. 42, 1-15.

Rorke L.B., Gilles F.H., Davis R.L. and Becker L.E. (1985). Revision of the World Health Organisation classification of brain tumours for childhood brain tumours. Cancer 56, 1869-1886.

Rubinstein L.J. (1986). Immunohistochemical singpost-not markers-in neural tumour differentiation. J. Neuropathol. Appl. Neurobiol. 12, 523-537.

Rubinstein L.J. and Russell D.S. (1989). Pathology of tumours of the nervous system. 5th Ed. Edward Arnold (ed). London.

Sobel R.A., Trice J.E., Nielsen S.L. and Ellis W.G. (1981). Pinealoblastoma with ganglionic and glial differentiation. Report of two cases. Acta Neuropathol. (Berl.) 55, 243-248.

Sternberger L.A. (1979). The unlabelled antibody peroxidase-antiperoxidase (PAP) method. In: Immunohistochemistry. Chichester, John Willey. pp 104-169.

Thompson R.J., Doran J.F., Jackson P., Dhillon A.P. and Rode J. (1983). PGP 9.5-A new marker for vertebrate neurones and neuroendocrine cells. Brain Res. 278, 224-228.

Warnke R.A., Gatter K.C., Falini B., Hildreth P., Woolston R.-E., Pulford K., Cordell J., Cohen B., Wolf-Peters C. and Mason D.Y. (1983). Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. N. Engl. J. Med. 309, 1275-1281.

Zülch K.J. (1981). Historical development of the classification of brain tumours and the new proposal of the World Health Organisation (WHO). Neurosurg. Rev. 4, 123-127.

Accepted April 10, 1991