Immunohistochemical Localization of Neurofilaments and Neuron-Specific Enolase in 29 Cases of Neuroblastoma

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Twenty-nine neuroblastomas have been examined with the use of rabbit antibodies specific for each of the three neurofilament polypeptides, with a monoclonal antibody specific for the NF-L polypeptide, and with a rabbit antibody specific for neuron-specific enolase. When frozen material was used, all neuroblastomas were positive with the neurofilaments antibodies. When alcohol-fixed paraffin-embedded material was used, neurofilament staining was weaker and the fixation procedure appeared to destroy the epitopes recognized by the NF-L antibodies preferentially. Although all neuroblastomas were positive for neurone-specific enolase, so were two rhabdomyosarcomas, suggesting that NSE is not an appropriate marker to distinguish the different small blue cell tumors of children. (Am J Pathol 1986, 122:433–442)

NERUOBLASTOMAS are the most common solid tumors of infancy and childhood, if tumors of the central nervous system are excluded. Diagnosis of primitive undifferentiated neuroblastoma is often extremely difficult on the basis of morphologic findings alone,[1-4] and it is often confused clinically and histologically with other small round cell tumors such as Wilms' tumor, rhabdomyosarcoma, lymphoma, and Ewing's sarcoma. To distinguish between the different entities special methods such as electron microscopy, immunochemistry, and catecholamine determination are usually employed. Even then this group of tumors presents a special challenge, because the differential diagnosis is of importance for the subsequent clinical treatment.

Neuroblastomas are characterized at the ultrastructural level in electron microscopy by neurites, core granules, and microtubules.[2,5] Dense core granules are only pathognomonic of neuroblastoma when they are present in neurites, not when they are found as atypical perinuclear granules in the Golgi region of the tumor cells. These perinuclear inclusions are presumably lysosomal in origin, rather than catecholamine granules, and have been observed in a variety of tumors including Ewing's sarcoma, lymphoma, and even rhabdomyosarcoma.[6] It has usually been assumed that neuroblastomas do not contain glycogen and that they are also PAS-negative; however, this has not been supported by a recent extensive study where glycogen was detected in 50% of the neuroblastomas studied.[7]

Although a large number of supposedly neuroblastoma-specific antibodies, and in particular monoclonal antibodies, have been reported, thus far none have proved to be specific only for neuroblastoma; and cross-reactions with Ewing's sarcoma, lymphoid cells, and soft tissue sarcoma have been reported. Currently there are two tissue-specific, but not tumor-specific, proteins which appear helpful for the diagnosis of undifferentiated neuroblastomas, and for the distinction of neuroblastomas from other round cell tumors. These markers are the neurofilament triplet proteins and neuron-specific enolase (NSE).

Neurofilaments are one member of the intermediate filament (IF) family. In addition to neurofilaments, which are typical of most but probably not all neurons of the central and peripheral nervous systems, there are four other IF types, ie, keratins characteristic of both keratinizing and nonkeratinizing epithelia, desmin characteristic of cardiac and skeletal muscle as well as of visceral and most vascular smooth muscle cells, glial fibrillary acidic protein (GFAP) characteristic of astrocytes and certain other nonneural cell types in the cen-

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tral and peripheral nervous systems, and vimentin, the only intermediate filament protein found in most nonmuscle mesenchymal cells. Antibodies specific for a particular IF protein type yield information relevant to the histogenetic origin of normal cells as well as tumor cells. In particular, it has been shown by studying histologic specimens of several hundred human tumors that the IF type characteristic of the cell of origin is preserved in the primary tumor and in its metastases. Thus, carcinomas are keratin-positive, rhabdomyosarcomas express desmin, certain tumors of glial origin express GFA, while nonmuscle sarcomas, malignant lymphomas, and melanomas contain only IFs of the vimentin type (for review and original references see Osborn et al[8] and Moll et al[9]). Neurofilaments (NFs)in vertebrates contain three polypeptides, NF-H, NF-M, and NF-L, which have apparent molecular weights of approximately 200 kd, 160 kd, and 68 kd.[10,11] Extensive studies of the distribution of the individual neurofilament polypeptides have shown that wheras most neuronal cells express all three polypeptides,[12,13] some neuronal cells appear to lack neurofilaments,[14] while in other cell types NF-M and NF-L are present and NF-H is not detected[13,15] (for two reports of nonneuronal cell types reported to express neurofilaments at a particular developmental stage in the chicken, see Granger and Lazarides[16] and Bennett and DiLullo[17]). In a previous study of three neuroblastomas, some were reported to lack all IF types, whereas neurofilaments were detected in one case of ganglioneuroblastoma as well as in pheochromocytoma.[18] A further study[19] also showed that neuroblastomas could be divided into neurofilament-positive and neurofilament-negative tumors.

NSE[20] is an isoenzyme ($\gamma\gamma$) of the glycolytic enzyme enolase, which has been shown to be present at high concentrations in tissues of the nervous system and in the neuroendocrine system.[21,24] Some reports (eg, Tsokos et al[22]) have indicated that the presence of NSE positively identifies neuroblastomas, pheochromocytomas, and so-called "APUDomas," including carcinoids and insulinomas (reviewed by Schmechel[24]), although the specificity of this marker for tumors of neural origin has recently been questioned.[21,24]

The major objectives of the current study were 1) to present our immunocytochemical findings on a large number of neuroblastomas, with the use of rabbit antibodies specific for individual NF polypeptides, as well as a monoclonal antibody to the NF-L polypeptide, and, in addition, a rabbit antibody to neuron-specific enolase; 2) to compare the immunocytochemical results obtained with frozen sections with that seen with ethanol-fixed and paraffin-embedded material; and 3) to compare the diagnostic value of the neurofilament and NSE markers.

Materials and Methods

Tissue Samples

In this study, tumor tissue and cytologic specimens of neuroblastoma were examined. Each specimen was divided into two parts. The first part was immersed in formalin and embedded in paraffin. Hemotoxylin and eosin (H&E) stained slides were prepared, and the neuroblastomas were then graded according to evidence of maturation (Table 1; see Hughes et al[25] and Harms and Wilke[26]). In the Hughes classification, III represents the least differentiated neuroblastomas; II, moderately differentiated neuroblastoma; and I, ganglioneuroblastoma.

Immunohistochemical Methods

The second part of each tumor or smear was treated as follows.

Ethanol-Fixed Paraffin-Embedded Material (15 Specimens)

Sections nominally 1–2 μ in thickness were cut on a microtome and dried overnight. Slides were subsequently deparaffinized with the use of a xylol and alcohol series.

Peroxidase Staining

The deparaffinized sections were incubated with 0.3% hydrogen peroxidase in methanol to block the endogenous peroxidase activity for 30 minutes and then washed in TRIS buffer, pH 7.4. The nonspecific background staining was blocked with normal swine serum diluted 1:10 for 15 minutes at room temperature. The appropriate neurofilament antibody was added, and the sections were incubated for 30 minutes at room temperature. The NSE antibody was incubated at a dilution from 1:500 to 1:2000 overnight at 4 C. Subsequently, sections were washed well, and the peroxidase-labeled second antibody was added for another 30 minutes at room temperature. We treated specimens with diaminobenzidine and H_2O_2 for 5–10 minutes to develop the peroxidase stain and counterstained them with hematoxylin, dehydrated them, and mounted them in Eukitt (Riedel de Haen AG, Seelze, FRG).

Immunofluorescence Procedure

All specimens were fixed in acetone at −10 C for 10 minutes. Ten microliters of the first antibody was then added, and the slides were incubated for 45–60 minutes at 37 C in a humid chamber. After washing well with PBS, 10 μl of the appropriate FITC-labeled second antibody was added, and the samples incubated for a further 30–45 minutes at 37 C. They were then washed in

Table 1—Neurofilament Positivity of Neuroblastomas

Case	Age	Sex	Location	Material*	Hughes grading	NSE	NF antibodies: NF 297 (NF-L)	NF 298 (NF-M)	NF 301 (NF-H)	NR4 (NF-L)
1	4 years	M	Retroperitoneum	E	III	+	0	+	+	0
2		F		E	III	+	0	+	+	0
3	3 years	M	Abdomen	E	III	+	(+)	+	+	(+)
4	2 months	F	Liver metastasis	E	III	+	0	+	+	0
5		M		E	III	+	+	+	+	0
6	3 years	F	Metastasis	E	III	(+)	0	+	(+)	(+)
7	5 years	M	Retroperitoneum	E	III	+	0	(+)	0	(+)
8	0.5 years	M	Abdomen	E	III	+	(+)	+	+	+
9	11 years	M	Retroperitoneum	E	II	+	(+)	+	+	(+)
10	8 years	M	Retroperitoneum	E	III	+	0	+	+	+
11	3.5 years	M	Retroperitoneum	E	II	+	0	+	(+)	0
12	4 years	M		E	II	+	+	+	+	+
13	15 years	F	Mediastinum	E	I	+	(+)	(+)	+	(+)
14	3 years	F	Mediastinum	E	I	+	+	+	+	(+)
15	1 year	M	Retroperitoneum	E	I	+	(+)	+	+	0
16	2 years	F	Retroperitoneum	F	II	(+)	+	+	+	+
17	0.5 year	F	Abdomen	F	I	+	+	+	+	+
18	3 years	F	Abdomen	F	III	+	+	+	+	+
19	3 years	M	Retroperitoneum	F	II	(+)	+	+	+	+
20	3.5 years	M	Retroperitoneum	F	II	+	+	+	+	+
21	1 year	F	Retroperitoneum	F	II	+	(+)	+	+	(+)
22	0.5 year	F	Mediastinum	F	II	(+)	+	+	+	+
23	2.5 years	F	Metastasis lower leg	F	II	+	(+)	+	+	+
24	3 months	M		F	III	(+)	+	(+)	(+)	+
27	1 year	M		F	III	ND	+	ND	ND	ND
25	5 years	F	Retroperitoneum	T	III	ND	+	ND	ND	ND
26	1 year	F	Mediastinum	T	III	ND	+	ND	ND	ND
28		F	Abdomen	T	III	ND	+	ND	ND	ND
29	6 years	M	Abdomen	A	III	ND	+	ND	ND	ND

NSE, neuron-specific enolase; NF 297, rabbit antibody against NF-L; NF 298, rabbit antibody against NF-M; NF 301, rabbit antibody against NF-H; NR4, monoclonal antibody against NF-L.

* F, frozen; E, ethanol; T, touch imprint; A, aspiration biopsy.

+, All tumor cells stained; (+), rather weak staining or not all tumor cells stained; 0, tested but no staining seen; ND, not determined.

PBS and mounted in Mowiol 4-88 (Hoechst, Frankfurt, West Germany).

Cryostat Sections

Ten specimens approximately 5 μ thick were prepared and allowed to dry for 30–60 minutes at 37 C or were held at −70 C until use. They were then fixed in acetone and processed as above.

Cytologic Specimens

Four specimens were air-dried and immediately processed or were stored at −20 C for some days if immediate processing was impossible. Smears were fixed in acetone and then processed as above.

Antibodies

Primary Antibodies

The preparation and characterization of neurofilament antibodies which specifically recognized the NF-L, the NF-M, or the NF-H polypeptide has been described.[14] Cross-reacting antibodies were removed by passage of each antibody through Sepharose 4B columns to which the other two neurofilament polypeptides were coupled. Immunoblots showed that these antibodies were specific for a single NF polypeptide when tested on NF polypeptides from a variety of different species.[14,27] Recent experiments show that the antibodies display similar specificities on human material (Moll et al, in preparation). The mouse monoclonal antibody NR-4 specific for the NF-L component has been described and characterized elsewhere.[28]

Glial fibrillary acidic protein (GFAP) antibody was prepared in rabbits against GFAP purified from pig spinal cord.[29] The specificity of the GFAP antibody has been demonstrated on human material.[29]

Both an affinity-purified vimentin antibody raised in sheep against porcine lens[8] and a mouse monoclonal vimentin antibody[30] were used.

NSE antibody prepared in rabbits against bovine brain was purchased from Dako Immunochemicals, Copenhagen, Denmark.

Secondary Antibodies

Peroxidase-labeled swine anti-rabbit IgGs, rabbit anti-mouse IgGs (Dako, Denmark), or FITC-labeled second antibodies (Cappell Laboratories, Cochranville, Pa) were used.

Results

Our initial results with neuroblastomas which had been alcohol-fixed and paraffin-embedded showed that when examined by immunofluorescence microscopy three neuroblastomas were neurofilament-negative, and one ganglioneuroblastoma was neurofilament-positive.[18] Additional work reported only in tabular form identified 6 neuroblastomas fixed in a similar manner as neurofilament-negative, while 6 other neuroblastomas examined by immunofluorescence microscopy of frozen sections were positive for neurofilaments.[8] Carlei et al[19] have also shown that neurofilaments could be detected in some but not all the neuroblastomas they studied. Because neuroblastoma is a relatively rare tumor, it was not clear whether these differences could be attributed to the different fixation methods used or whether, as orginally suggested,[18] neuroblastomas may be derived from a subclass of neuronal cells that lack IFs. In the current study we investigated a total number of 29 neuroblastomas. Fifteen specimens were ethanol-fixed and paraffin-embedded, 10 specimens were frozen sections after acetone fixation, and 4 specimens were touch imprints which were acetone-fixed. The specimens showed different grades of differentiation. The Hughes grading and the results of testing the different specimens are shown in Table 1.

Ethanol-Fixed Paraffin-Embedded Material

In preliminary experiments with ethanol-fixed paraffin-embedded material we compared staining with neurofilament antibodies using either immunofluorescence procedures or the peroxidase technique. In the majority of specimens fixed by such procedures the reaction was stronger when the peroxidase detection system was used, and in some instances a positive result was seen only when peroxidase was used. For this reason Table 1 lists only results obtained with peroxidase. Examination of Table 1 shows that the NF-M antibody specific for the 160-kd polypeptide stained all fixed and embedded neuroblastomas, whereas the NF-H antibody was positive in 14 of 15 specimens and the NF-L antibody in only 7 of 15 specimens. The NR-4 monoclonal antibody against the NF-L polypeptide showed a positive reaction on some neuroblastomas and a negative reaction on others.

The results with peroxidase are illustrated in Figures 1 and 2. Figures 1a–c show an undifferentiated Hughes III neuroblastoma stained with antibodies specific for each of the three different neurofilament polypeptides. All antibodies demonstrate staining of the cytoplasm (arrow) as well as of processes (arrowhead); the stroma is negative (asterisk). Figures 1d–f show a second case of undifferentiated Hughes III neuroblastoma labeled by antibodies specific for individual neurofilament polypeptides. The tumor cells and the processes are again positively stained, and pseudorosettes are detectable. Figure 2 illustrates the better differentiated Hughes II and Hughes I lesions. The tumor cells in Hughes II neoplasms (Figure 2a and b) are characterized by larger nuclei which display polymorphism and also by a larger amount of cytoplasm. The different neurofilament antibodies stained both cytoplasm and nerve processes. Thus, for instance, Figure 2b shows bundles of neurofilaments in axonlike processes (arrowhead). Figure 2b illustrates strong cytoplasmic staining in precursor cells of ganglion cells (neuroblasts, arrow). Results with ganglioneuroblastoma are illustrated in Figure 2c and d. In Figure 2c ganglion cells (arrow), neuroblasts (arrowheads), and lymphocytes (asterisk) are present. The cytoplasm of the ganglion cells as well as of neuroblasts is neurofilament-positive. Figure 2d illustrates an area of ganglioneuroblastoma where the tumor cells are well differentiated and where no neuroblasts are present. Also, here a strong cytoplasmic staining of ganglion cells and nerve processes is visible. The vimentin antibody stained stromal cells but not tumor cells of Hughes III neuroblastomas (not shown), whereas stroma cells as well as Schwann cells were strongly positive in the ganglioneuroblastomas (Figure 2e).

The NSE antibody staining was also performed with the peroxidase technique. All 15 neuroblastomas, irrespective of their degree of differentiation, stained positively. Thus, for instance, Figure 4a shows a Hughes III neuroblastoma which is positive with the NSE antibody, and Figure 4b a Hughes II neuroblastoma which is likewise positive.

Frozen Sections and Smears

Frozen sections from 10 neuroblastomas were examined. The different neurofilament antibodies stained all neuroblastoma specimens regardless of whether an immunofluorescence or an immunoperoxidase detection system was used. The reactions of the different neurofilament antibodies were indistinguishable.

The results are illustrated in Figure 3. Figure 3a shows a moderately differentiated neuroblastoma and Figure 3c and d a ganglioneuroblastoma stained with different neurofilament antibodies. Neuroblasts (Figure 3a)

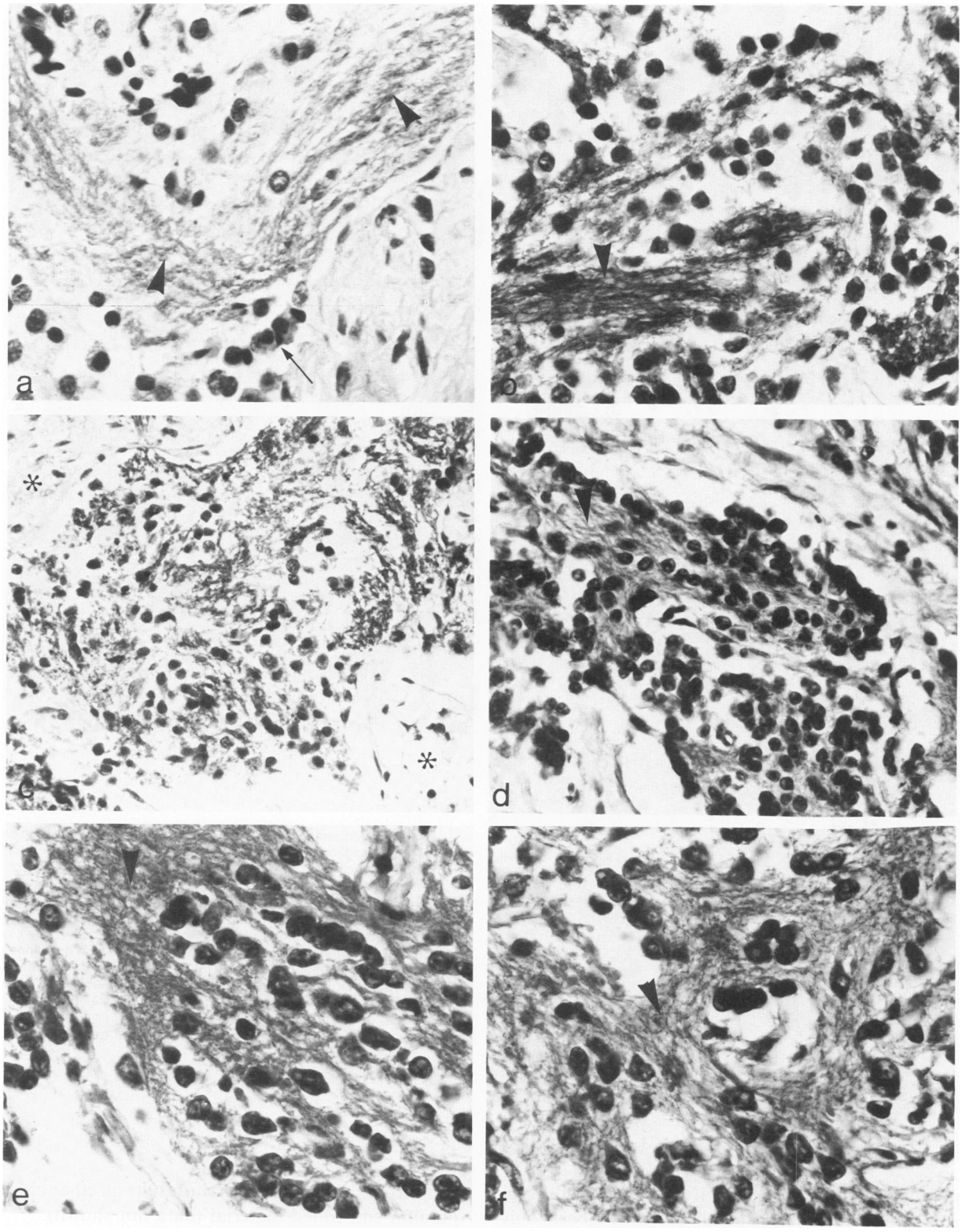

Figure 1—Undifferentiated neuroblastoma (Hughes III) stained by rabbit antibodies specific for NF-L (**a**), for NF-M (**b**), and for NF-H (**c** and **d**) as well as by a mouse monoclonal NF-L antibody (**e** and **f**). Tumor cells show strong cytoplasmic staining (**a**, *arrow*) as well as strong staining of processes (*arrowhead*); the stroma is unstained (**c**, *asterisk*). The different antibodies show equivalent results. **a–c**, Case 3; **d–f**, Case 5. (Ethanol-fixed, paraffin-embedded, stained with peroxidase, **a**, **c**, **d**, ×330; **b**, **e**, **f**, ×530)

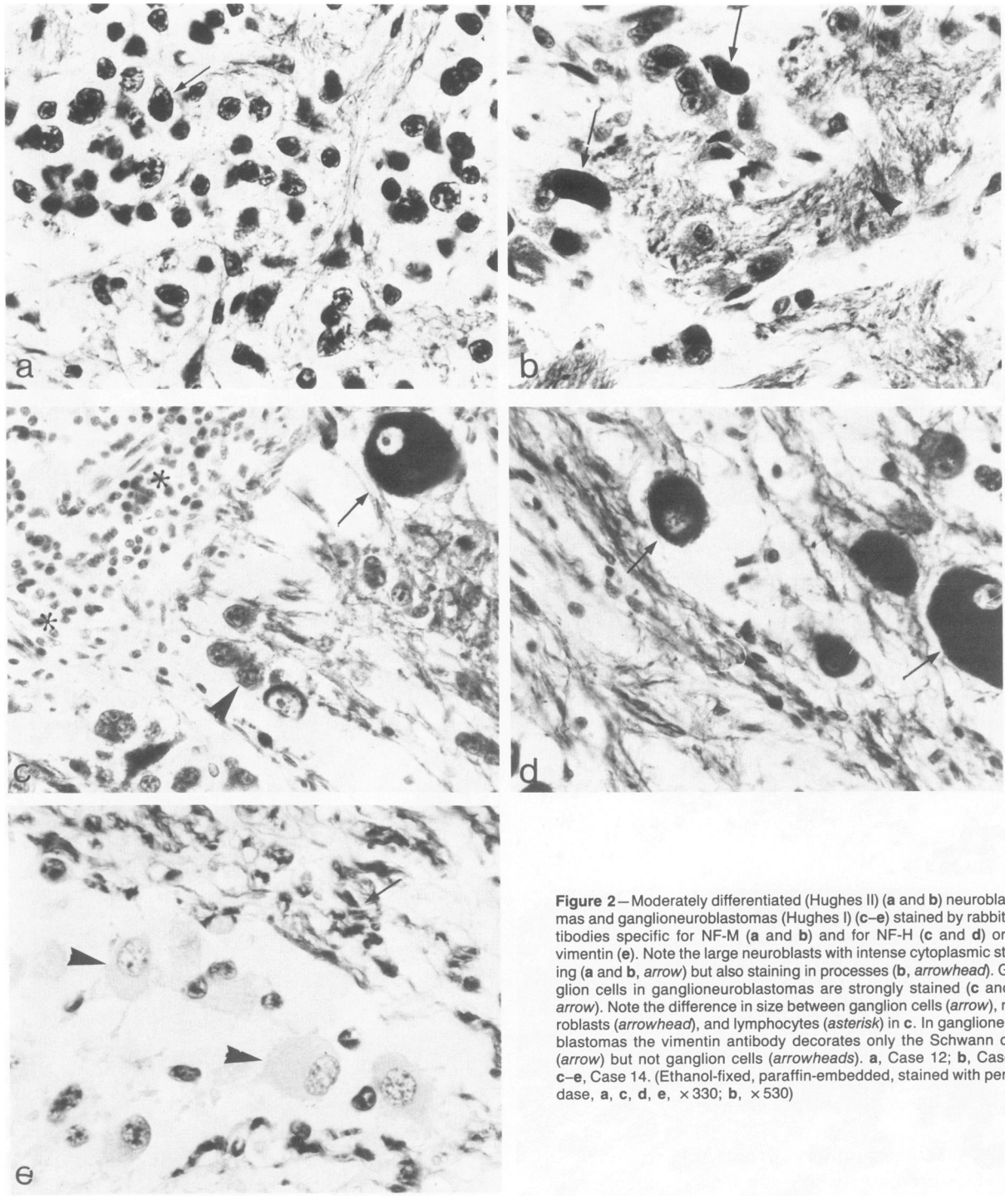

Figure 2—Moderately differentiated (Hughes II) (**a** and **b**) neuroblastomas and ganglioneuroblastomas (Hughes I) (**c–e**) stained by rabbit antibodies specific for NF-M (**a** and **b**) and for NF-H (**c** and **d**) or for vimentin (**e**). Note the large neuroblasts with intense cytoplasmic staining (**a** and **b**, *arrow*) but also staining in processes (**b**, *arrowhead*). Ganglion cells in ganglioneuroblastomas are strongly stained (**c** and **d**, *arrow*). Note the difference in size between ganglion cells (*arrow*), neuroblasts (*arrowhead*), and lymphocytes (*asterisk*) in **c**. In ganglioneuroblastomas the vimentin antibody decorates only the Schwann cells (*arrow*) but not ganglion cells (*arrowheads*). **a**, Case 12; **b**, Case 9; **c–e**, Case 14. (Ethanol-fixed, paraffin-embedded, stained with peroxidase, **a**, **c**, **d**, **e**, ×330; **b**, ×530)

and processes (Figure 3c) as well as ganglion cells (Figure 3d) are positive. The vimentin antibody labeled only cells of stroma and vessels in both neuroblastoma (Figure 3b) and ganglioneuroblastoma (not shown). GFA could not be detected in any of the 10 specimens.

The NSE antibody reacted more weakly on frozen sections in comparison with the results on ethanol-fixed paraffin-embedded material (data not shown).

We also investigated touch imprints from four neuroblastomas. In all four specimens tumor cells are neurofilament-positive. Some of the tumor cells form clusters (Figure 3f), and some form rosettes (Figure 3e).

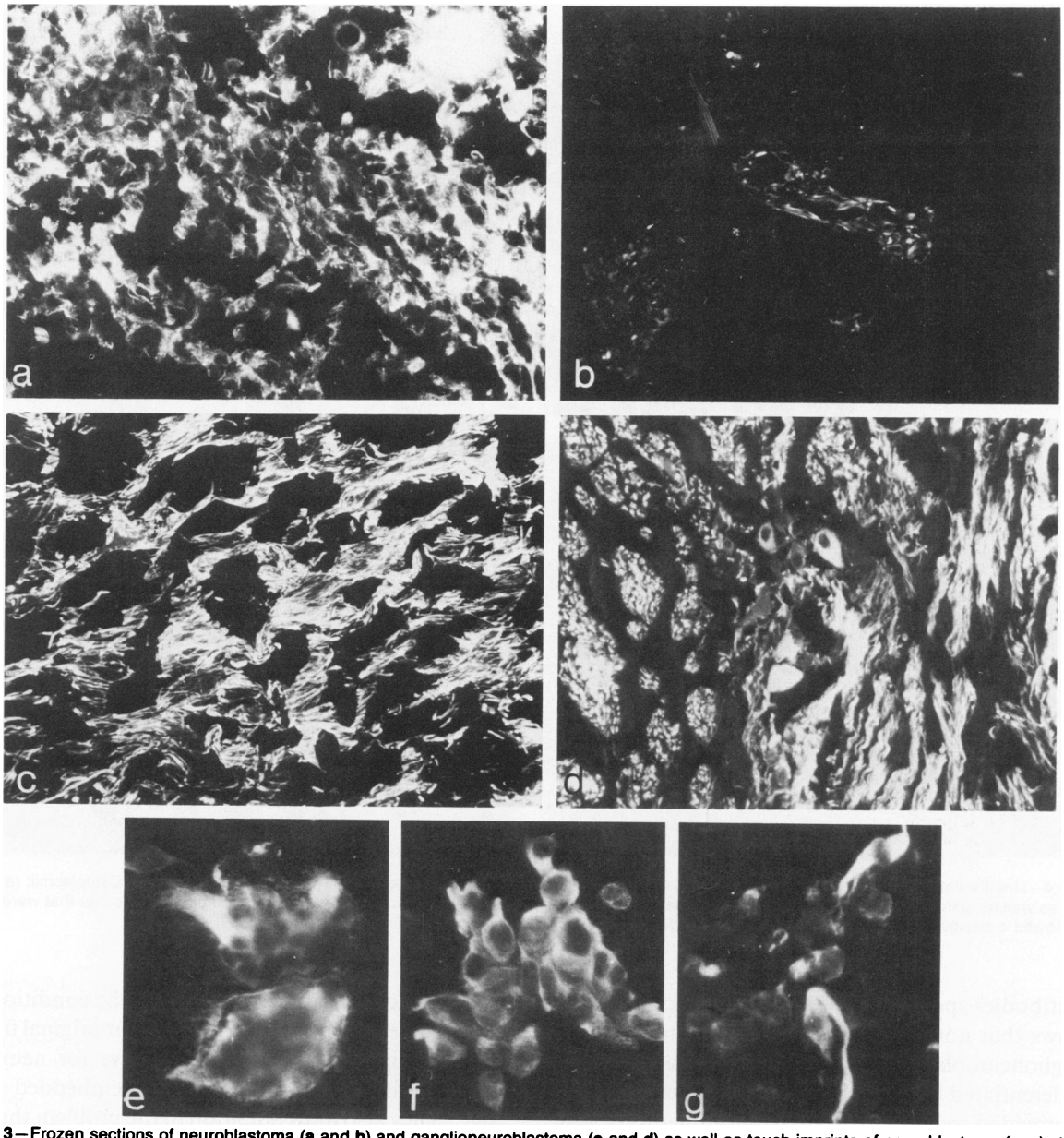

Figure 3—Frozen sections of neuroblastoma (**a** and **b**) and ganglioneuroblastoma (**c** and **d**) as well as touch imprints of neuroblastoma (**e–g**) stained by rabbit NF-L (**a** and **d**), rabbit NF-M (**c**), and mouse monoclonal NF-L (**e–g**) neurofilament antibodies or by vimentin antibody (**b**) and viewed by fluorescence microscopy. Neuroblasts (**a**), ganglion cells (**d**), and processes (**e**) are strongly labeled by the different neurofilament antibodies. In contrast, vimentin antibody stains blood vessels but not neuroblasts (**b**). In touch imprints rosettelike formations (**e**) and long processes are strongly stained. **a**, Case 21; **b**, Case 7; **c** and **d**, Case 17; **e–g**, Case 29. (FITC, **a** and **c**, ×330; **b** and **d**, ×200; **e–g**, ×530)

In some instances tumor cells are characterized by long axonlike processes which are neurofilament-positive (Figure 3g).

Cross-Reactivity of the NSE Antibody

Two embryonal rhabdomyosarcomas which had been ethanol-fixed and paraffin-embedded were stained with antibodies to desmin, neurofilaments, and NSE. The desmin antibodies stained tumor cells in rhabdomyosarcoma positively as previously reported,[31] and as expected, no staining of rhabdomyosarcomas could be detected with the neurofilament antibody (data not shown). In contrast, the NSE antibody showed a positive reaction with the tumor cells in rhabdomyosarcoma (Figure 4c and d).

Discussion

In this study we have shown that in a large number of neuroblastomas the tumor cells are positively stained by several different neurofilament antibodies as well as

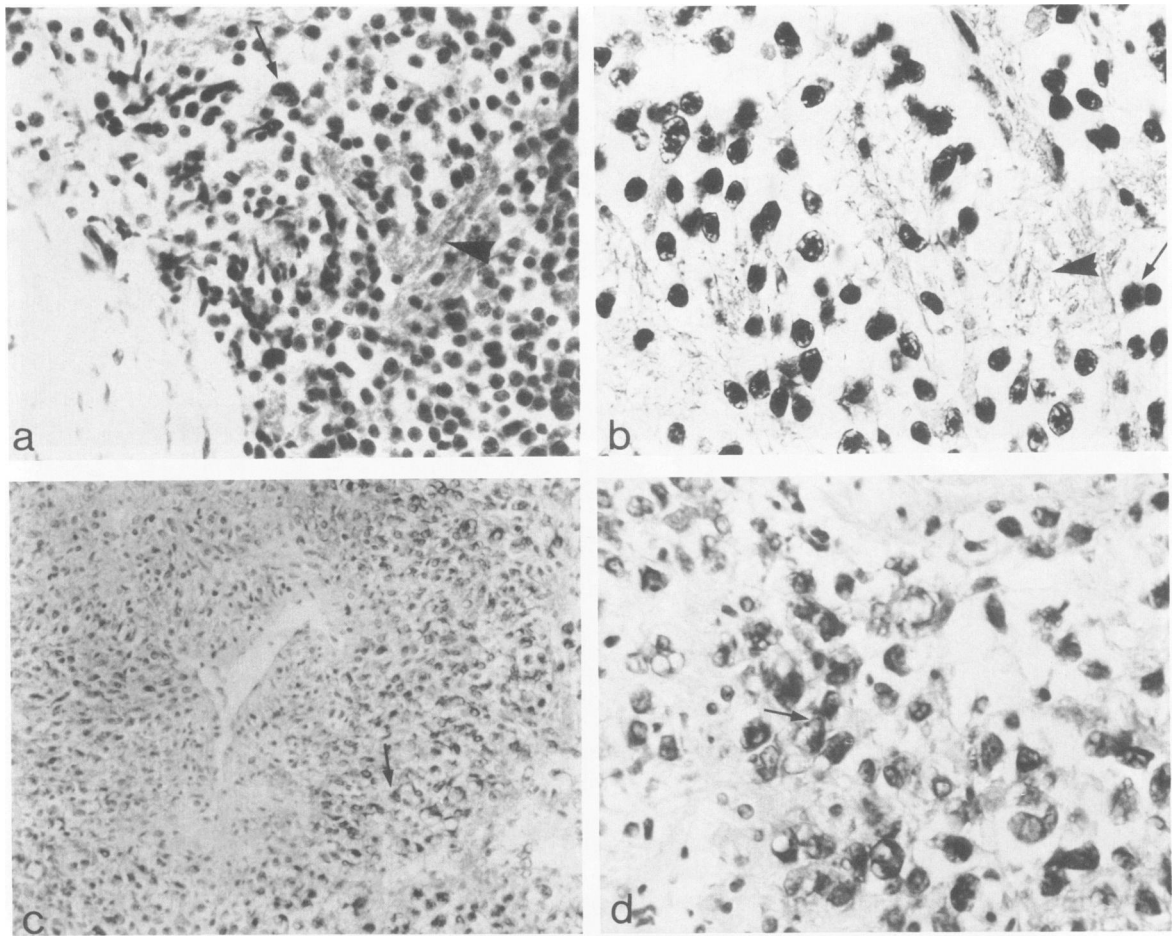

Figure 4—Undifferentiated (**a**) and moderately differentiated (**b**) neuroblastomas were labeled by the NSE antibody (**a** and **b**). Cytoplasmic (**a** and **b**, *arrow*) as well as staining of processes (**a** and **b**, *arrowheads*) could be visualized. Tumor cells in the 2 cases of rhabdomyosarcoma that were tested also showed a positive reaction (**c** and **d**, *arrows*). **a**, Case 3; **b**, Case 11. (Peroxidase, **a** and **c**, ×330; **b**, ×530)

by antibodies specific for NSE. Examination of Table 1 shows that not only the well-differentiated Hughes I ganglioneuroblastomas were positive, but also that the undifferentiated Hughes III neuroblastomas were positive provided that appropriate fixation methods were used. The presence of neurofilaments in the tumor cells, as well as in those instances where it was tested the lack of staining with antibodies specific for other IF types, is consistent with the presumptive origin of neuroblastomas from undifferentiated neuroblasts.

When the neurofilament data is considered, it is clear that the staining is stronger and that results were easier to interpret with frozen sections than after alcohol fixation and paraffin embedding. Table 1 shows that in frozen sections neurofilaments could be demonstrated with all four antibodies used in this study. In the paraffin-embedded material, although the NF-M antibody stained all the specimens, not all specimens were positive with either the rabbit NF-L antibody or the monoclonal NF-L antibody, even with the peroxidase technique, which is more sensitive under the conditions we used. These results probably explain our original finding of neuroblastomas that were negative for neurofilaments in alcohol-fixed and paraffin-embedded material.[18] They also draw attention to the problem that certain antigens or epitopes may be particularly sensitive to the fixation and embedding procedure used in a given study. Thus, a comparison of the results shown in Table 1 for paraffin-embedded and for frozen material suggests that the epitopes recognized by the rabbit and mouse NF-L, and to a lesser extent by the rabbit NF-H antibodies, may be partially destroyed by alcohol fixation and by paraffin embedding. That other epitopes on IFs do survive such fixation procedures is shown, for example, by our results showing desmin positivity for rhabdomyosarcomas, vimentin positivity for non-muscle sarcomas, and keratin positivity for carcinomas after such procedures.[8,31] Our results can also be compared to those of Carlei et al,[19] who reported that 9 of 30 neuroblastomas were neurofilament-negative when

assayed on formaldehyde-fixed paraffin-embedded material. One explanation of the difference may be that the formaldehyde treatment, like the alcohol fixation, causes some reduction in antigenicity, and thus tumors with relatively few neurofilaments may seem negative in their assay. Other investigators[32,33] have also pointed out that the phosphorylation state of the neurofilament epitopes recognized by particular neurofilament antibodies can affect reactivity, and this might provide an alternate explanation for some of the differences.

Our results thus far do not allow a subdivision of neuroblastomas by neurofilament content. We have no evidence for a subgroup which lacks NFs, although at the moment we cannot exclude that if more Hughes III cases were examined some might fall into such a category, in view of the finding in the rat of certain neurones which appear to lack neurofilaments.[14] Likewise we have no evidence for a subgroup in which NF-L is present and NF-H is absent, as has again been reported for the rat during normal development.[15] Again, further studies may reveal such a subgroup.

We have not found any other type of intermediate filaments in tumor cells of neuroblastomas. In particular, and in contrast to results in a previous study,[18] we have not detected GFAP in neuroblastomas when assayed with a well-characterized GFAP antibody. Thus, perhaps the GFAP-positive material seen by Carlei et al reflects a cross-reaction of the GFAP antibody with neurofilaments, rather than the existence of pluripotential precursor cells. In our specimens vimentin was only detectable in vessels and fibroblasts of stroma and Schwann cells of ganglioneuroblastomas, but was not found in neuroblasts.

Neuroblastomas of different degrees of differentiation seem therefore to share with pheochromocytoma[18] the property of being neurofilament-positive. Both tumors are thought to be derived from the neural crest. Although neuroblastomas are thought to be derived from neuroblasts, pheochromocytomas are assumed to arise from chromaffin cells or their precursors. Chromaffin cells have been shown to be neurofilament-positive.[34]

Although NSE could be demonstrated in all our neuroblastoma cases, and was particularly strong in the alcohol-fixed paraffin-embedded specimens, our results, like those of others,[21,24] raise the question of how specific a marker NSE is for neuronal and neuroendocrine tissues, and particularly for tumors derived from those tissues. Our finding that the two cases of rhabdomyosarcoma that we examined were NSE-positive as well as results of other studies in which a wide variety of normal tissues[21] and a variety of different non-neuronal tumor types[23] are stained by antibodies specific for γ-enolase suggest that results obtained with the currently available NSE antibodies should be interpreted with caution. Perhaps the recently reported monoclonal antibody to human γγ-enolase[35] may show greater specificity.

Finally, our results suggest again that the differential diagnosis of small, blue, round tumors in childhood can be aided by the use of appropriate antibodies against intermediate filaments,[31,36,37] perhaps used in conjunction with other markers.[38,39] Neuroblastomas are neurofilament-positive, rhabdomyosarcomas are desmin-positive, and Ewing's sarcoma as well as malignant lymphomas express vimentin. The anti-T200 antibody,[39] which detects a surface marker on lymphoid cells and malignant lymphomas, allows a distinction between malignant lymphomas and Ewing's sarcomas. To detect the T200 antigen and neurofilaments in the same specimen, one should use frozen sections.

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