Acta Neuropathol (Berl) (1987) 75:147–155

Acta Neuropathologica

Esthesioneuroepithelioma: a tumor of true olfactory epithelium origin

An ultrastructural and immunohistochemical study

H. Takahashi[1], S. Ohara[1], M. Yamada[1], F. Ikuta[1], K. Tanimura[2], and Y. Honda[2]

[1] Department of Pathology, Brain Research Institute, Niigata University, 1 Asahimachi, Niigata, 951 Japan
[2] Department of Neurosurgery, Sannocho Hospital, Sanjo, 955 Japan

Summary. A case of esthesioneuroepithelioma was investigated ultrastructurally and immunohistochemically, using antibodies against neurofilament protein (NFP), glial fibrillary acidic protein (GFAP), keratin, neuron-specific enolase (NSE), S-100 protein (S-100), and tyrosine hydroxylase (TH). The tumor initially manifested as an epidural mass in the anterior cranial fossa in a 64-year-old man, and about 3 1/2 years later, autopsy further revealed extensive metastases to the lymph nodes of the neck and thoracic cavity. In the cranial and nasal cavities, the tumor was composed of fairly uniform, ill-defined cells arranged in nests which were surrounded by a fibrovascular stroma. These histological features were reproduced in the metastatic tumor nodules with frequent occurrence of tubular arrangements of the tumor cells. Ultrastructurally, two different cell types were well recognized by their characteristic morphological features, which were reminiscent of sensory neurons and sustentacular cells of the olfactory epithelium. No dense-cored secretory granules were observed in the tumor cells. Immunohistochemically, the tumor showed a variable number of cells positive for NFP, keratin, NSE and S-100. NFP was present in a relatively small number of cells, which were found diffusely in the nests. Keratin was observed in the cells mainly located at the periphery. NSE-positive cells tended to form irregular clusters in the center. A few S-100-positive cells were found, without any particular arrangement. These findings indicated that the present tumor, which actually arose in the superior nasal cavity, consisted of cells differentiating in at least two distinct directions, neuronal and epithelial, and strongly suggested that the tumor was of true olfactory epithelium origin, or more precisely, derived from the bipotential, undifferentiated basal cells of this epithelium.

Offprint requests to: H. Takahashi (address see above)

Key words: Esthesioneuroepithelioma – Ultrastructure – Immunohistochemistry – Neurofilament protein (NFP) – Keratin

Olfactory neuroblastomas (esthesioneuroblastomas) are rare tumors that arise in the nasal cavity, most often superiorly and laterally near the ethmoidal sinus [29]. Although ultrastructural [5] and histochemical [14, 19] studies strongly suggest that they are apudomas [26] or neurocristomas [3] of neural crest origin, their histogenesis is still a matter of debate.

In 1981, however, Hassoun et al. [10] reported a tumor of the superior nasal cavity infiltrating the frontal lobe. The tumor was composed of compact lobules with tubular arrangements and ultrastructurally showed two different cell types, both devoid of neurosecretory granules and reminiscent of the sensory neurons and sustentacular cells of the olfactory epithelium. They concluded that their case, corresponding to a rare variant, the esthesioneuroepithelioma [2], was a true neurosensorial tumor originating from the olfactory epithelium and differing from olfactory neuroblastomas which are generally considered to be apudomas.

We have recently had the opportunity of ultrastructurally and immunohistochemically examining a similar nasal tumor manifesting initially as an intracranial mass, using antibodies against neurofilament protein (NFP), glial fibrillary acidic protein (GFAP), keratin, neuron-specific protein (NSE), S-100 protein (S-100) and tyrosine hydroxylase (TH). The results obtained appear to contribute to the establishment of the concept of esthesioneuroepithelioma as a clinical entity.

Case report

In March 1983, a 64-year-old man was admitted to the hospital for headache and weakness in the right leg of 2-month duration. Examination revealed bilateral choked disk and slight muscular weakness and hypesthesia in the right leg. Computed tomography (CT) showed a large tumor mainly involving the right anterior cranial fossa. Angiography revealed that the tumor was fed by the right external carotid artery. Surgical removal of the tumor was performed. However, recurrence occurred 4 months later, and a second removal was performed. During the following month, the patient began to complain of bilateral exophthalmos and nasal bleeding. CT showed a continuous tumor mass involving the anterior cranial fossa and nasal cavity, for which a surgical removal procedure and postoperative irradiation (6,000 rad) were performed. After therapy, the patient revealed no obvious neurological abnormalities except for anosmia, and CT suggested only a small residual tumor in the superior nasal cavity. In January 1985, neck tumors appeared, and metastases to the cervical lymph nodes were suspected. Local radiation therapy (5,200 rad) resulted in disappearance of these tumors. Thereafter, however, his general condition gradually deteriorated, and in April 1985, he became bed-ridden with akinetic mutism. CT showed widespread low-density areas in the bilateral frontal lobes. In May 1986, he died of cardiac insufficiency at the age of 67, about 3 1/2 years after disease onset.

Autopsy was performed 3 h after death. Pathological examination revealed extensive metastases to the lymph nodes of the neck, trachea, bronchi, thoracic wall and superior surface of the diaphragm, showing multiple, well-circumscribed whitish tumor nodules of various sizes. In the visceral organs, no tumors were found either grossly or microscopically. Both lungs showed congestion and edema. The brain showed widespread radiation necrosis mainly involving the bilateral frontal white matter with marked hyaline thickening and adventitial fibrosis of the blood vessels. No residual tumors were detected in the cranial or nasal cavity.

Materials and methods

Surgically removed tissues were fixed in 10% formalin, and embedded in paraffin. Metastatic tumor nodules obtained at autopsy were divided into several parts. These were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, Bouin's solution and ethanol, and embedded in paraffin. Paraffin sections, 4 μm in thickness, were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) with or without amylase predigestion, Bodian's method for nerve fibers, and Gomori's method for reticulin.

Tumor tissues for electron microscopy were fixed in 1% glutaraldehyde-3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and post-fixed in 1% osmium tetroxide, then dehydrated through a graded ethanol series, and embedded in Epon 812. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with an electron microscope.

Immunohistochemical studies for the presence of NFP, GFAP, keratin, NSE, S-100 and TH were carried out using the above paraffin-embedded sections. Deparaffinized sections were pretreated with 0.3% H_2O_2 -methanol for 1 h at room temperature and with normal goat serum overnight at 4°C. These sections were examined using the peroxidase-antiperoxidase method [32] employing rabbit anti-rat neurofilament 68-, 150- and 200-kDa sera [13, 21] diluted 1:100 for 1 h, rabbit anti-human GFAP serum (Dako, Santa Barbara, Calif, USA) diluted 1:500 for 2 h, rabbit anti-bovine keratin serum (Dako, USA) diluted 1:200 for 1 h, rabbit anti-bovine NSE serum (Wako, Osaka, Japan) diluted 1:500 for 1 h, rabbit anti-bovine S-100 serum (Dako, USA) diluted 1:2,000 for 2 h, and rabbit anti-bovine TH serum [23, 33] diluted 1:40 for 1 h. The sections were then treated with goat anti-rabbit IgG (Tago, Burlingame, Calif, USA) diluted 1:20 for 30 min and rabbit PAP (DAKO, Glostrup, Denmark) diluted 1:50 for 30 min. In addition, the ethanol-fixed, paraffin-embedded sections were examined using the avidin-biotin-peroxidase complex (ABC) method [12] employing mouse monoclonal antibodies against 68-, 160- and 200-kDa neurofilament (Boehringer Mannheim, West Germany), and cytokeratin (Becton Dickinson, Mountain View, Calif, USA). These antibodies were used at 20 μg/ml, 5 μg/ml, 5 μg/ml and 25 μg/ml, respectively. We used a Vectastain ABC kit (Vector, Burlingame, Calif, USA) in this study. To block nonspecific binding of biotin/avidin system reagents, deparaffinized sections were pretreated with a Blocking Kit (Vector, USA). The sections were then successively incubated in normal horse serum for 30 min, in mouse monoclonal antibodies for 1 h, in biotinylated horse anti-mouse IgG diluted 1:200 for 30 min, and finally in ABC for 30 min. In both methods, the peroxidase reaction was carried out with freshly prepared 0.02%, 3,3′-diaminobenzidine tetrachloride (DAB) and 0.005% H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.6, for 10 min. Each reaction was performed at room temperature. For control sections, the primary antiserum was replaced with normal rabbit serum. When mouse monoclonal antibody was used, it was replaced with normal mouse serum or ascites. Several neural and non-neural tissues from the present patient served as positive or negative controls.

Results

Light microscopy

In the cranial and nasal cavities, the tumor was composed of fairly uniform, ill-defined cells with round to oval, relatively hyperchromatic nuclei and scanty cytoplasm; the tumor cells were compactly arranged in nests and cords surrounded by a fibrovascular stroma (Fig. 1a). Mitotic figures were frequently observed. The tumor cells were occasionally found in rosette-like arrangements. Bodian's staining demonstrated few positive fibers. Some of the tumor cells showed PAS-positive material in their cytoplasm. With amylase predigestion, PAS-positive material almost totally disappeared. These histological features were reproduced in the metastatic tumor nodules (Fig. 1b). In addition, the tumor cells were often found in characteristic tubular arrangements (Fig. 1c) and Bodian's staining revealed many positive fibers (Fig. 1d).

Electron microscopy

Ultrastructural findings were essentially the same in all the materials examined, although postmortem artefacts were conspicuous in the autopsy specimens. The tumor was mainly composed of round to oval cells with relatively sparse cell organelles arranged in

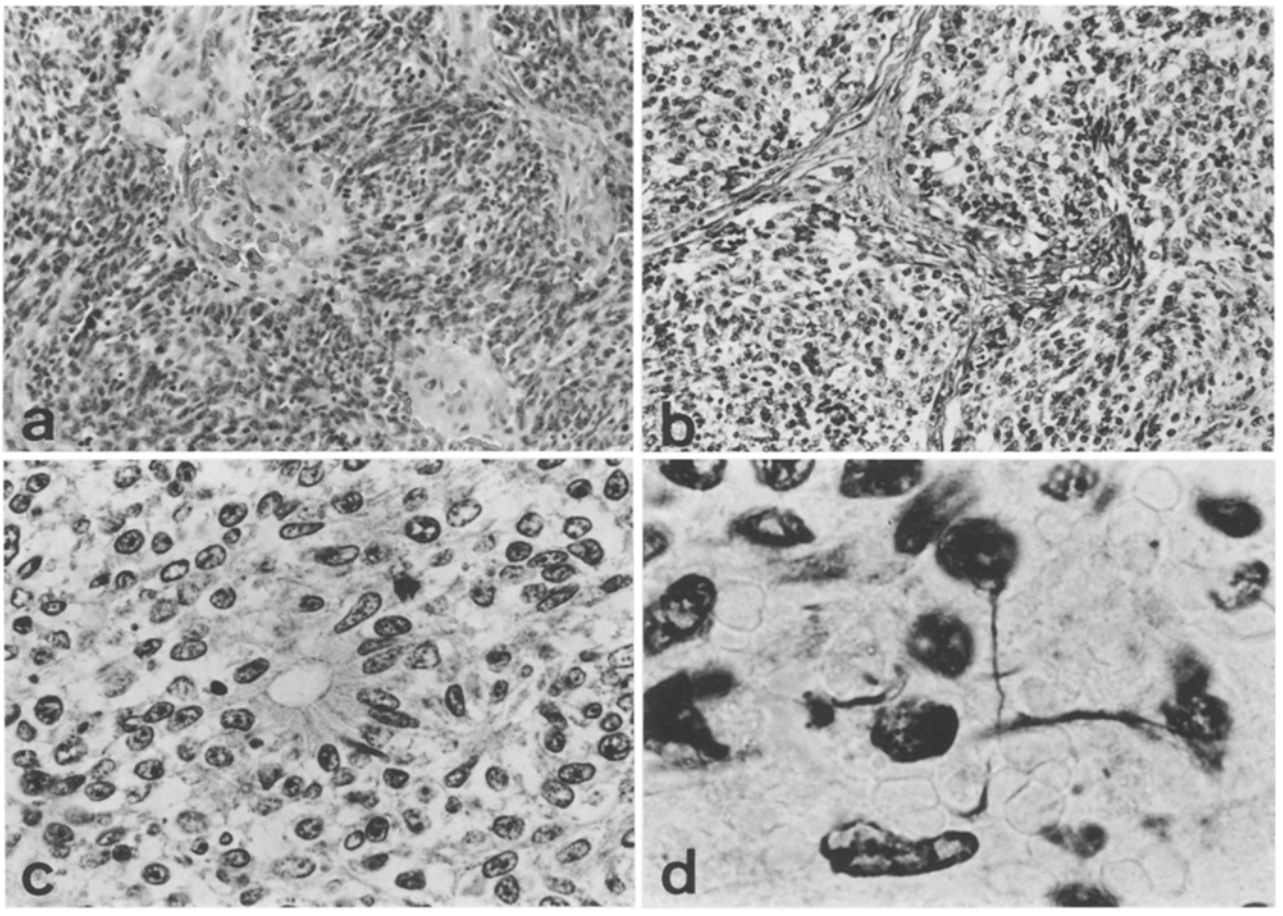

Fig. 1. **a** Histology of the surgically removed intracranial tumor. **b** A cervical metastatic tumor nodule, showing essentially the same histology. **c** Tubular arrangement of the tumor cells. **d** Argyrophilic fibers extending from the tumor cell bodies. **a, b** H&E, ×170; **c** H&E, ×520; **d** Bodian, ×1,300

a cell-to-cell pattern; many cell processes were also present between the cell bodies. These were joined together by occasional punctate adhesions and desmosomes. At the margins of the nests, the tumor cell bodies and processes were seen to rest on the basal lamina.

Among these tumor cells, at least two different cell types could be distinguished on the basis of additional ultrastructural features. The tumor cells of the first type were often seen in rosette-like or incomplete tubular arrangements; they showed relatively well-developed cell organelles such as Golgi's apparatus, rough ER and mitochondria (Fig. 2a). Few intermediate filaments were found. Many centrioles, probably serving as basal bodies, were seen in these cells (Fig. 2a). In addition, cilia were occasionally encountered (Fig. 2b). Some processes showed the features of neurites (Fig. 2c) or of dystrophic axons.

In the tubular arrangements, tumor cells of another distinct type were well demonstrated; they were joined by apical junctional complexes, such as tight junctions and desmosomes, and showed numerous microvilli originating from their free surfaces (Fig. 3a). The microvilli and free cell membranes were coated with granular or spike-like material (Fig. 3b). These cells were further characterized by cytoplasmic bundles of intermediate filaments (tonofilaments) (Fig. 3b, c). No cilia or basal bodies were found in the cells of this type.

No convincing dense-cored secretory granules could be found in the tumor cells.

Immunohistochemistry

Both surgically removed and autopsied tumor tissues showed cells immunoreactive for NFP, keratin, NSE and S-100. With regard to NFP, positive immunostaining was more strongly demonstrated in the ethanol-fixed, paraffin-embedded autopsied tissues.

Some of the tumor cells were shown to be positive for each of the three neurofilament polypeptides when stained with the rabbit polyclonal antibodies. There

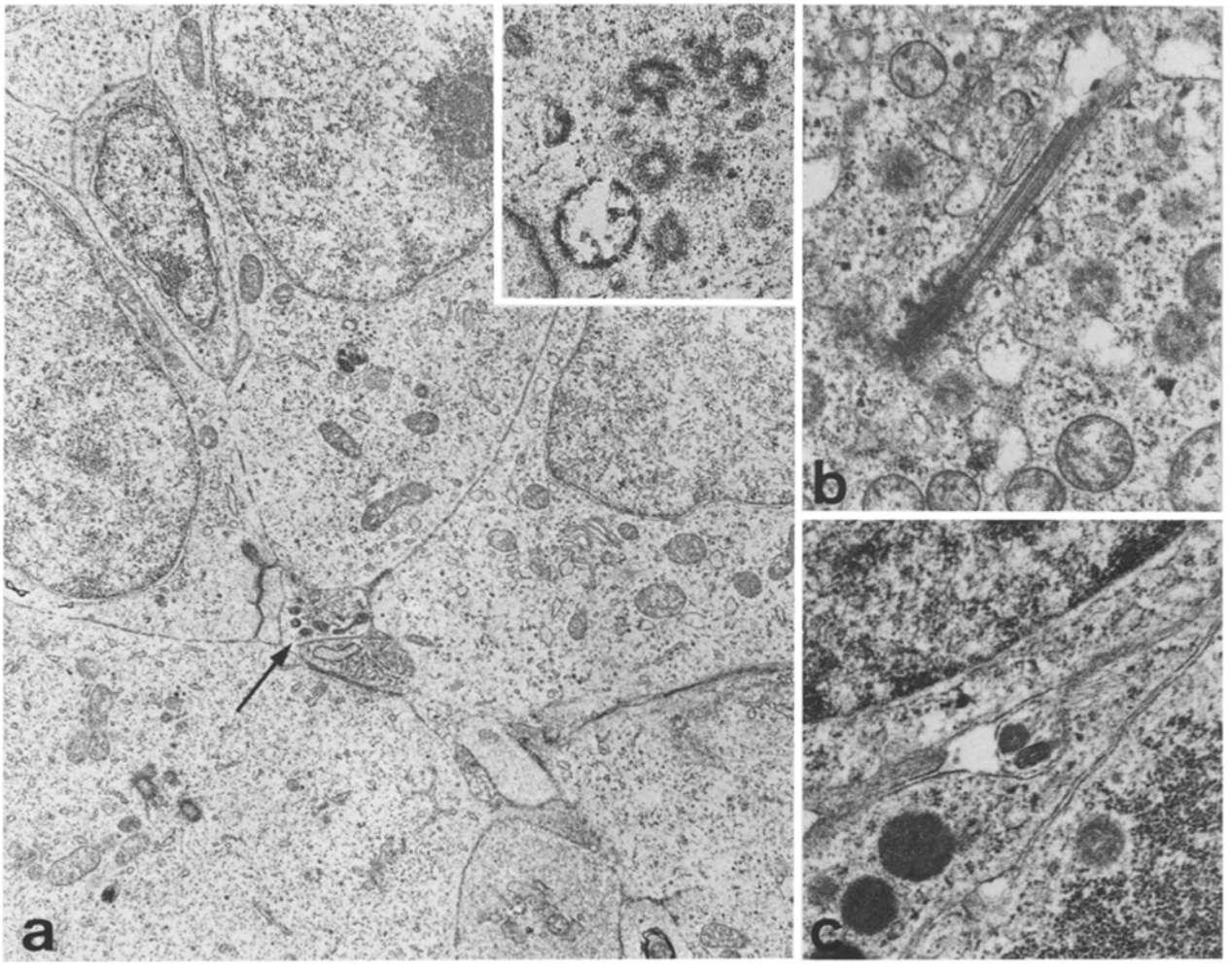

Fig. 2 a–c. Surgically removed intracranial tumor. **a** Electron micrograph illustrating a rosette-like arrangement of tumor cells with apical junctional complexes. Centrioles are seen at the *lower left*. *Arrow* indicates a neurite-like process containing many mitochondria. *Inset:* Tumor cell displaying many centrioles in the apical cytoplasm. **b** Electron micrograph illustrating a cilium extending into a tiny lumen. Many mitochondria are also seen. **c** A neurite-like process (*center*) containing microtubules and mitochondria. **a** ×10,000, inset ×16,000; **b** ×22,000, **c** ×24,000

was no obvious difference in the number of a positive cell between the three polypeptides. The 68-kDa and 200-kDa polypeptides were mainly localized in the cell bodies, and rarely in the processes (Fig. 4a). On the other hand, the 150-kDa polypeptide was well demonstrated in the cell processes. These positive cells were occasionally found in rosette-like or tubular arrangements (Fig. 4b). When ethanol-fixed, paraffin-embedded sections were stained with mouse monoclonal antibodies, the tumor showed positive cells for each of the three neurofilament polypeptides, 68, 160 and 200 kDa. However, these cells were much smaller in number compared with those stained with the rabbit polyclonal antibodies.

Positive immunostaining for keratin was observed in the cells mainly located at the periphery of the nests (Fig. 4c), although the number of positive cells varied considerably from nest to nest. In the tubular arrangements (olfactory rosettes), most cells were found to be immunoreactive for keratin (Fig. 4d). The serial sections failed to demonstrate coexpression of NFP and keratin in the tumor cells. Simultaneously, it was also noted that a large number of tumor cells were negative for both NFP and keratin. When the mouse monoclonal antibody to cytokeratin was employed, the staining results obtained were very similar to those observed with the rabbit polyclonal antibody.

A relatively large number of tumor cells were shown to be positive for NSE. They tended to form irregular clusters in the centers of the nests (Fig. 4e). A few cells showed positive staining for S-100 without any particular arrangement, and rarely showed slender, long processes resembling axons or dendrites (Fig. 4f). However, serial sections failed to demon-

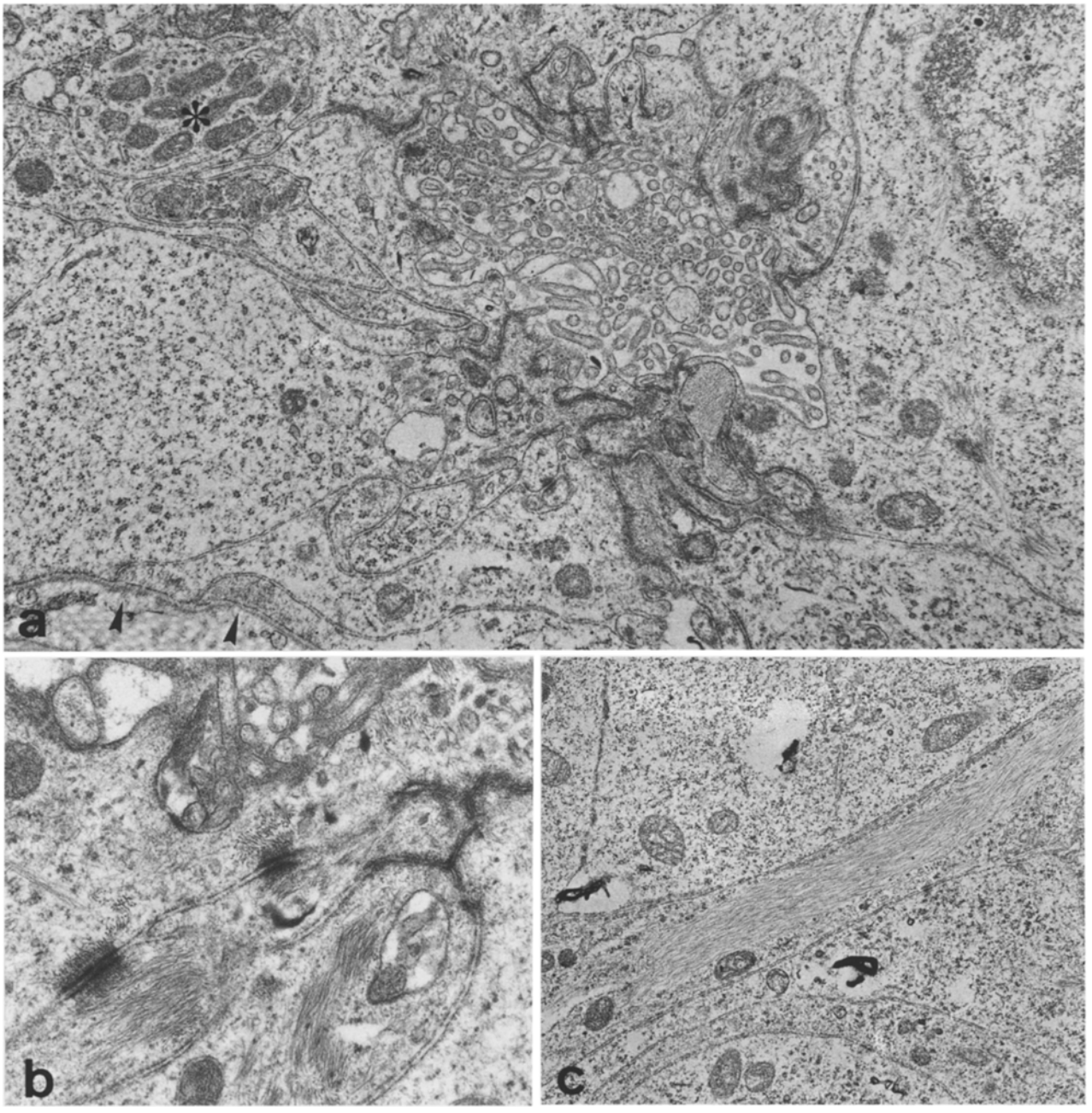

Fig. 3 a–c. Surgically removed intracranial tumor. **a** Electron micrograph illustrating tubular arrangement of the tumor cells. Numerous microvilli and apical junctional complexes are clearly seen in the *center*. A process, probably of the first cell type, is also seen at the *upper left* (*asterisk*). *Arrowheads* indicate basal lamina. **b** Tubular arrangement of tumor cells, containing desmosomes and dense intermediate filaments (tonofilaments). **c** Bundles of tonofilaments frequently present in the cell processes. **a** ×22,000; **b** ×33,000; **c** ×13,000

strate coexpression of NFP and S-100. GFAP and TH were not detected immunohistochemically in any of the materials examined.

Discussion

Previous ultrastructural studies on so-called olfactory neuroblastoma have repeatedly demonstrated the presence of dense-cored or membrane-bound secretory granules in the tumor cells [5, 7, 11, 18, 19, 31, 35]. Histochemical examinations have also shown that such tumor cells possess biogenic amines [14, 19]. These features of olfactory neuroblastoma are common with those of peripheral neuroblastoma, which has been considered to originate from cells of the APUD system [26].

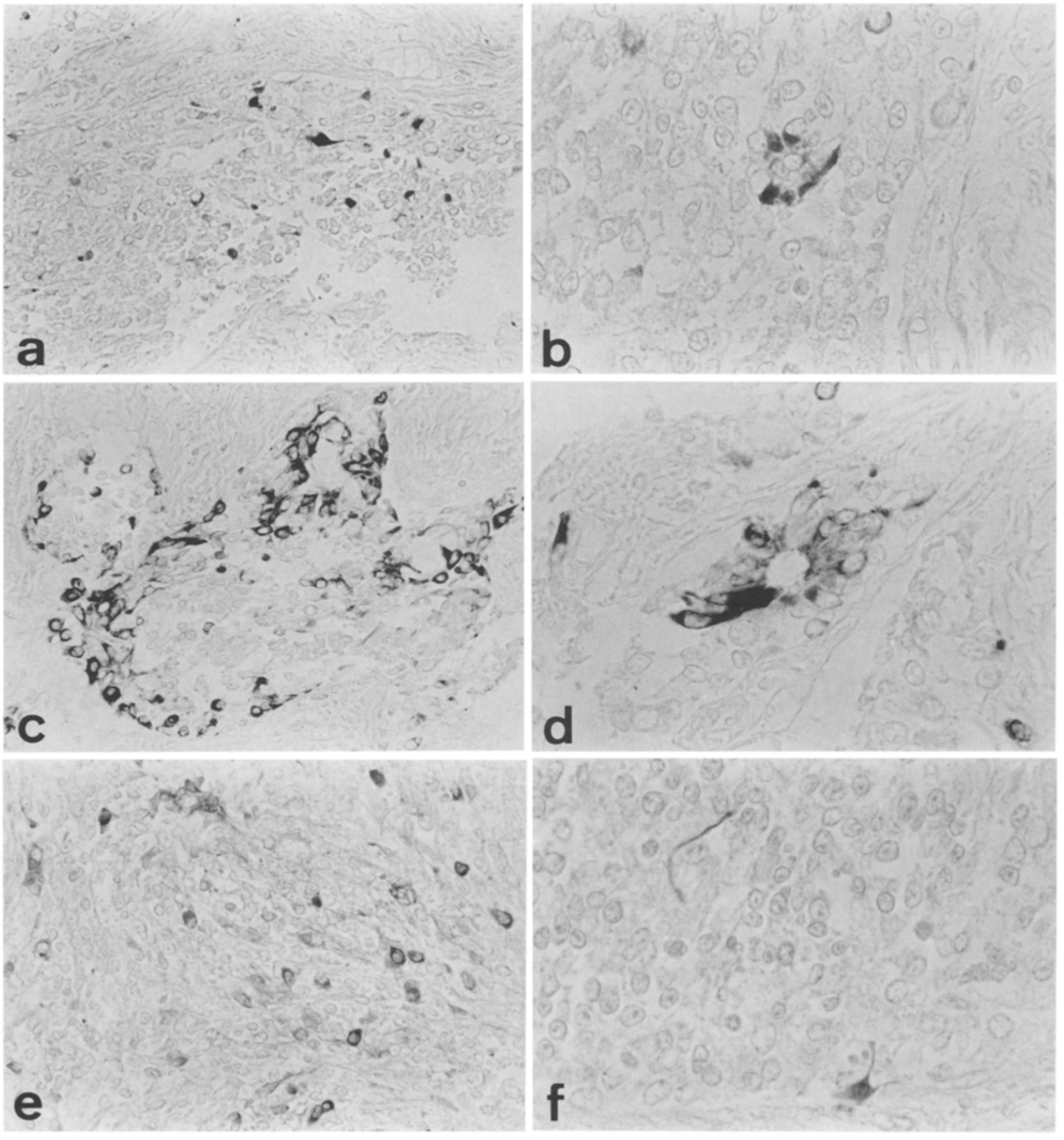

Fig. 4. a, b Tumor stained with rabbit neurofilament 200-kDa antiserum. **a** Positive immunostaining is observed in scattered tumor cells. **b** Positive cells are seen in a rosette-like arrangement. **c, d** Tumor stained with rabbit keratin antiserum. **c** Positive cells are seen at the periphery. **d** Most tumor cells seen in this tubular arrangement are positive for keratin. **e, f** Tumor stained with rabbit NSE antiserum (**e**) or with rabbit S-100 antiserum (**f**). **a–d** Ethanol-fixed, paraffin-embedded; **e, f** Bouin's solution-fixed, paraffin-embedded, **f**, counterstained with hematoxylin; **a, c, e** ×260; **b, d, f** ×520

Histologically, the present tumor showed characteristic tubular arrangements (olfactory rosettes), which were originally described by Berger et al. [2] in a case reported as an olfactory esthesioneuroepithelioma. However, this structure was absent in a case subsequently reported by Berger and Coutard [1] as an olfactory esthesioneurocytoma, which has a marked resemblance to peripheral neuroblastoma. Accordingly, our case was diagnosed as esthesioneuroepithelioma in the strict sense.

At the ultrastructural level, two cell types were clearly demonstrated with characteristic morphological features. The first cell type was characterized by cilia and centrioles, probably serving as basal bodies, in addition to well-developed cytoplasmic organelles. They also bore processes resembling neurites. The second cell type was found in tubular arrangements with numerous microvilli and apical junctional complexes. Bundles of tonofilaments were also a characteristic finding of this cell type. No dense-cored secretory granules could be found in the tumor cells. When the primary site of the present tumor is considered, it is evident that these two cell types were similar to the sensory neurons and sustentacular cells of the olfactory epithelium [8, 27, 28].

A survey of the literature revealed the rare occurrence of nasal tumors which could be considered to be very similar, if not identical, to that in our case [10, 16, 17, 31]. In these previous cases, olfactory rosettes showing tubular arrangements of columnar tumor cell were characteristic histological findings, as in our case. Kudo et al. [16] were the first to demonstrate two different types of tumor cells; the first type showed cilia and basal bodies and the second type was found with microvilli and apical junctional complexes in the tubular arrangements. Dense-cored granules were not observed. They discussed the ultrastructural similarities between the tumor cells and olfactory epithelial cells. Hassoun et al. [10] reported a case of esthesioneuroepithelioma and distinguished this tumor from usual olfactory neuroblastomas. The tumor showed two different cell types reminiscent of sensory neurons and sustentacular cells of the olfactory epithelium. The above authors emphasized the absence of dense-cored secretory granules in these cells in considering the histogenesis of the tumor.

In the present study, NFP, keratin, NSE and S-100 were detected immunohistochemically in a variable number of tumor cells. NFP is composed of three major polypeptides with different molecular weights. This protein, which forms intermediate filaments (neurofilaments), is specific for most, but probably not all, neurons in the central and peripheral nervous systems [24]. In peripheral neuronal tumors such as neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, neoplastic neuroblasts and ganglion cells have been shown to be immunoreactive for NFP [4, 21, 24, 25]. On the other hand, keratin proteins are organized in the cell in the form of intermediate filaments (tonofilaments), which are found in most epithelial cells. The presence of keratin has been shown to be useful in establishing the epithelial nature of a tumor [20, 24]. Therefore, our findings regarding NFP and keratin indicated that the present tumor contained two distinct cell types, neuronal and epithelial, in accord with the ultrastructural findings.

The presence of NSE has been observed in neurons, neuroendocrine cells and neoplastic neuronal cells [36, 38]. However, the specificity of this enzyme for cells of neuronal lineage has recently been questioned by several investigators who have immunohistochemically demonstrated the presence of NSE in non-neuronal cells and their tumors including carcinoma [9, 25, 39]. In the present tumor, the distribution of NSE-positive cells was quite different from that of keratin-positive cells when examined in serial sections. It is our impression that in the present case, NSE could have been expressed by immature neuronal cells. S-100, first detected in astrocytes and Schwann cells, has now been demonstrated in a wide variety of neural and non-neural tissues and their tumors [15, 22]. S-100 was detected in a few tumor cells in the present case, and the cytology shown by immunohistochemistry was suggestive of their neuronal differentiation. Similar observations have been reported in some peripheral neuroblastomas [4, 15]. GFAP forms intermediate filaments (glial filaments) characteristic of astrocytes and other glial cells in the nervous system [24]. However, the present findings suggested that the tumor contained no glial components. TH, a rate-limiting enzyme in the catecholamine-synthesizing pathway, has been considered to be a marker for catecholamine-producing neuronal cells [23]. The presence of this enzyme has often been shown immunohistochemically in peripheral neuronal tumors [4, 33]. However, TH was completely negative in the present case.

Only a few reports are currently available on the immunohistochemical features of olfactory neuroblastoma (esthesioneuroblastoma). Trojanowski et al. [37] demonstrated the presence of NFP in scattered tumor cells in an esthesioneuroblastoma. However, they did not mention any possible keratin content. Vollrath et al. [40] examined an esthesioneuroblastoma for the presence of the five known types of intermediate filament (keratin, vimentin, desmin, GFAP and neurofilament). However, no tumor cells showed positive staining for any of these filaments. More recently, Choi and Anderson [6] examined ten cases of olfactory neuroblastoma using NSE, GFAP and S-100 antibodies. Although NFP and keratin were not investigated, all the cases showed many NSE- and S-100-positive cells with particular arrangements. It is important to note that none of the tumors described in these reports displayed olfactory rosettes. At the ultrastructural level, they showed no epithelial cells characterized by bundles of tonofilaments, but neuronal cells containing typical dense-cored granules were present [6, 37, 40].

A recent immunohistochemical study on the human fetal olfactory epithelium has clearly demonstrated that sensory neurons are positive for NSE and NFP. It is interesting to note that NSE is detected earlier than NFP in the process of development [34]. Immunohistochemical examination of the same fetal materials [34] showed that sustentacular cells were positive for keratin (data not shown). In the adult rat olfactory epithelium, sustentacular cells are strongly positive with antibodies to keratin [41], while sensory neurons, although small in number, are stained with antibodies to NFP [30]. A certain subpopulation of basal cells are negative for these intermediate filament proteins [41]. These unique immunohistochemical properties of the normal olfactory epithelium appear to well explain the histogenesis of the present tumor arising from the superior nasal cavity. S-100 is not present in the normal olfactory epithelium [34]. However, it can be considered that the cells could have obtained this protein in the process of neoplastic transformation.

Our ultrastructural and immunohistochemical findings strongly suggest that the present tumor, an esthesioneuroepithelioma, is actually the neoplastic counterpart of the olfactory epithelium, or more precisely, is derived from the bipotential, undifferentiated basal cells of this epithelium. The olfactory epithelium develops from primordial placodes cytoarchitecturally similar to brain vesicles or neural tubes. On the other hand, olfactory neuroblastomas are considered to be derived from the APUD cell nests of the ethmoidal and maxillary sinuses [5, 14, 19]. APUD cells are of neural crest origin and are distributed widely throughout the body. It should also be emphasized that a diagnosis of esthesioneuroepithelioma may be difficult in certain cases showing predominant epithelial differentiation. Caution must therefore be exercised, since this tumor is not a genuine carcinoma, which is common among tumors arising in the nasal cavity.

Acknowledgements. We wish to thank Dr. Y. Ihara, Division of Clinical Physiology II, Tokyo Metropolitan Institute of Gerontology, Tokyo, who kindly provided us with the rabbit anti-neurofilament polypeptides sera used in this study, and Dr. S. Takahashi, Department of Otorhinolaryngology, Niigata University School of Medicine, who kindly gave us an opportunity to examine the human fetal olfactory epithelia. We also wish to express our appreciation to Mr. T. Ichikawa, Mr. K. Kobayashi and Ms S. Sekimoto for their technical assistance, and to Ms K. Murayama and Mrs. Y. Tanahashi for their help in preparing the manuscript.

References

1. Berger L, Coutard H (1926) L'esthesioneurocytome olfactif. Bull Assoc Fr Etude Cancer 15:404–414
2. Berger L, Luc G, Richard D (1924) L'esthesiononeuro-epitheliome olfactif. Bull Assoc Fr Etude Cancer 13:410–421
3. Bolande RP (1974) The neurocristopathies. A unifying concept of disease arising in neural crest maldevelopment. Hum Pathol 5:409–429
4. Carlei F, Polak JM, Ceccamea A, Marangos PJ, Dahl D, Cocchia D, Michetti F, Lezoche E, Speranza V (1984) Neuronal and glial markers in tumors of neuroblastic origin. Virchows Arch [A] 404:313–324
5. Chaudhry AP, Haar JG, Koul A, Nickerson PA (1979) Olfactory neuroblastoma (Esthesioneuroblastoma). A light- and ultrastructural study of two cases. Cancer 44:564–579
6. Choi H-SH, Anderson PJ (1985) Immunohistochemical diagnosis of olfactory neuroblastoma. J Neuropathol Exp Neurol 44:18–31
7. Friedmann I, Osborn DA (1974) The ultrastructure of the olfactory neuroblastoma. Minerva Otorhinolaryngol 24: 66–74
8. Frisch D (1967) Ultrastructure of mouse olfactory mucosa. Am J Anat 121:87–120
9. Haimoto H, Takahashi Y, Koshikawa T, Nagura H, Kato K (1985) Immunohistochemical localization of γ-enolase in normal human tissues other than nervous and neuroendocrine tissues. Lab Invest 52:257–263
10. Hassoun J, Gambarelli D, Grisoli F, Henric A, Toga M (1981) Esthesioneuroepithelioma, a true neurosensorial tumor. Light and electron microscopic study of a case with endocranial extension. Acta Neuropathol (Berl) 55:77–80
11. Hirano T, Aida T, Moriyama M, Asano G, Suzuki I, Yuge K (1985) Primary neuroblastoma of the nasal cavity and review of literature. Acta Pathol Jpn 35:183–191
12. Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580
13. Ihara Y, Nukina N, Sugita H, Toyokura Y (1981) Staining of Alzheimer's neurofibrillary tangles with antiserum against 200k component of neurofilament. Proc Jpn Acad 57:152–156
14. Judge DM, McGavran MH, Trapukdi S (1976) Fume-induced fluorescence in diagnosis of nasal neuroblastoma. Arch Otolaryngol 102:97–98
15. Kahn HJ, Marks A, Thom H, Baumal R (1983) Role of antibody to S100 protein in diagnostic pathology. Am J Clin Pathol 79:341–347
16. Kudo M, Aoyama A, Tanaka F, Nakajima T (1972) An electron microscopic investigation of olfactory esthesioneuroepithelioma. Jpn J Clin Pathol 20:754–763
17. Mackay B, Luna MA, Butler JJ (1976) Adult neuroblastoma. Electron microscopic observations in nine cases. Cancer 37:1334–1351
18. McGavran MH (1970) Neurogenous nasal neoplasm. Ann Otol Rhinol Laryngol 79:547–550
19. Micheau C (1977) A new histochemical and biochemical approach to olfactory esthesioneuroma. A nasal tumor of neural crest origin. Cancer 40:314–318
20. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24
21. Mukai M, Torikata C, Iri H, Morikawa Y, Shimizu K, Shimoda T, Nukina N, Ihara Y, Kageyama K (1986) Expression of neurofilament triplet proteins in human neural tumors. An immunohistochemical study of paraganglioma, ganglioneuroma, ganglioneuroblastoma, and neuroblastoma. Am J Pathol 122:28–35
22. Nakajima T, Kamega T, Watanabe S, Hirota T, Sato Y, Shimosato Y (1982) An immunoperoxidase study of S-100

protein distribution in normal and neoplastic tissues. Am J Surg Pathol 6:715–727
23. Nakashima S, Ikuta F (1984) Tyrosine hydroxylase protein in Lewy bodies of parkinsonian and senile brains. J Neurol Sci 66:91–96
24. Osborn M, Weber K (1983) Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. Lab Invest 48:372–394
25. Osborn M, Dirk T, Käser H, Weber K, Altmannsberger M (1986) Immunohistochemical localization of neurofilaments and neuron-specific enolase in 29 cases of neuroblastoma. Am J Pathol 122:433–442
26. Pearse AGE (1974) The APUD cell concept and its implications in pathology. Pathol Annu 9:27–41
27. Polyzonis BM, Kafandaris PM, Gigis PI, Demetriou T (1979) An electron microscopic study of human olfactory mucosa. J Anat 128:77–83
28. Pyatkina GA (1982) Development of the olfactory epithelium in man. Z Mikrosk Anat Forsch 96:361–372
29. Russell DS, Rubinstein LJ (1977) Pathology of tumors of the nervous system, 4th edn. Edward Arnold, London, pp 412–414
30. Schwob JE, Farber NB, Gottlieb DI (1986) Neurons of the olfactory epithelium in adult rats contain vimentin. J Neuroscience 6:208–217
31. Silva EG, Butler JJ, Mackay B, Goepfert H (1982) Neuroblastomas and neuroendocrine carcinomas of the nasal cavity. A proposed new classification. Cancer 50:2388–2405
32. Sternberger LA (1979) Immunocytochemistry, 2nd edn. Wiley and Sons, New York, pp 104–169
33. Takahashi H, Nakashima S, Kumanishi T, Ikuta F (1987) Paragangliomas of the craniocervical region. An immunohistochemical study on tyrosine hydroxylase. Acta Neuropathol (Berl) 73:227–232
34. Takahashi S, Iwanaga T, Takahashi Y, Nakano Y, Fujita T (1984) Neuron-specific enolase, neurofilament protein and S-100 protein in the olfactory mucosa of human fetuses. An immunohistochemical study. Cell Tissue Res 238:231–234
35. Taxy JB, Hidvegi DF (1977) Olfactory neuroblastoma. An ultrastructural study. Cancer 39:131–138
36. Triche TJ, Askin FB (1983) Neuroblastoma and the differential diagnosis of small-, round-, blue-cell tumors. Hum Pathol 14:569–595
37. Trojanowski JQ, Lee V, Pillsbury N, Lee S (1982) Neuronal origin of human esthesioneuroblastoma demonstrated with anti-neurofilament monoclonal antibodies. N Engl J Med 307:159–161
38. Tsokos M, Linnoila RI, Chandra RS, Triche TJ (1984) Neuron-specific enolase in the diagnosis of neuroblastoma and other small round-cell tumors in children. Hum Pathol 15:575–584
39. Vinores SA, Bonnin JM, Rubinstein LJ, Marangos PJ (1984) Immunohistochemical demonstration of neuron-specific enolase in neoplasmas of the CNS and other tissues. Arch pathol Lab Med 108:536–540
40. Vollrath M, Altmannsberger M, Hunneman DH, Goebel HH, Osborn M (1984) Esthesioneuroblastoma: ultrastructural, immunohistological and biochemical investigation of one case. Arch Otorhinolaryngol 239:133–144
41. Vollrath M, Altmannsberger M, Weber K, Osborn M (1985) An ultrastructural and immunohistological study of the rat olfactory epithelium: unique properties of olfactory sensory cells. Differentiation 29:243–253

Received November 19, 1986/Accepted August 4, 1987