Virchows Arch. Abt. A Path. Anat. 356, 111—118 (1972)

The Ultrastructure of Medulloblastoma in Tissue Culture* **

Filippo Gullotta and Günter Kersting
Institut für Neuropathologie der Universität Bonn

Received March 20, 1972

Summary. Electron microscopic investigation of the tissue cultured from medulloblastoma fail to demonstrate a glial or neuronal differentiation of the neoplastic cells in vitro. Particularly impressive are the differences between medulloblastoma cells and the cells of the tissue cultures of sympathicoblastomas, the neuronal nature of which is readily recognized. These results confirm former findings of light microscopy and enzyme histochemistry and support the opinion that medulloblastoma has to be regarded as an embryonic sarcoma rather than a neuroectodermal tumor.

Zusammenfassung. Bericht über die elektronenmikroskopische Untersuchung in vitro kultivierter Medulloblastome, die in Übereinstimmung mit den Ergebnissen lichtmikroskopischer und enzymhistochemischer Methoden Zeichen einer neuronalen oder glialen Differenzierung der Geschwulstzellen nicht nachzuweisen vermag. Dies in deutlichem Gegensatz zu den Gewebekulturen der Neuroblastome des Sympathicus, die eindeutig neuronale Strukturen erkennen lassen. Die Ergebnisse sind geeignet, die Konzeption vom Medulloblastom als einem embryonalen Sarkom nachhaltig zu unterstützen.

In continuation of our investigation on the formal pathogenesis of medulloblastoma (1967, 1970) we report on the ultrastructural findings of two medulloblastomas cultivated in vitro.

Case Material

No. 1 (N 374/70). 26 year old male patient with subarachnoidal metastasis within the cauda equina of a cerebellar medulloblastoma removed two years before (N 112/68).

No. 2 (N 572/70). 8 year old male patient with rather sharply circumscribed tumor of cerebellar hemisphere.

In *light microscopy* the *histological sections* of both tumors show the typical criteria of a medulloblastoma or arachnoidal sarcoma. Abundance of isomorphous cells and nuclei, mitotic figures, a tendency to the formation of whorls. Metallic impregnations for neural and glial fibrils according to Hortega and Polak give negative results. Only the nuclei are distinctly darkened. Comparing the metastasis of case No. 1 with the structure of the primary tumor of 1968 no differences can be perceived.

The *tissue cultures* of both tumors (GK 1412, GK 1444)—after particle explantation in roller tubes according to the technique described by Kersting in 1961—show the growth of longish, sometimes spindle shaped cells with round

* Supported by Deutsche Forschungsgemeinschaft.
** Dedicated to Prof. B. Ostertag.

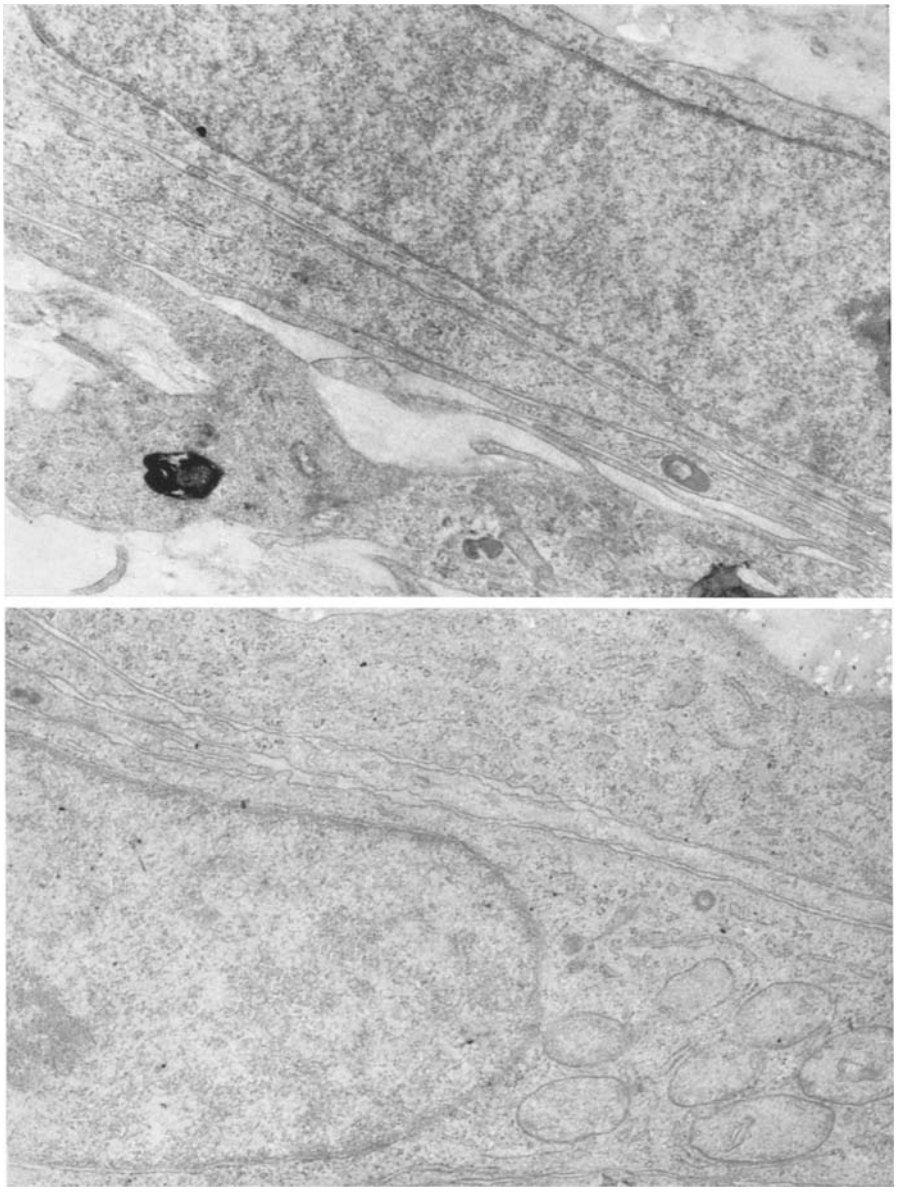

Fig. 1. Medulloblastoma cells in vitro (8th day of cultivation). The tumor elements grow freely floating in the medium. They are bipolar, elongated and have an oval nucleus. The cytoplasm consists of a small perinuclear rim with few organelles and shows many collateral sproutings like microvilli. The cells lie parallel and closely packed to each other, nevertheless neither a thickening of the cell membranes nor so-called cell junctions can be observed. 10000:1

oval nuclei arranged parallel to the cell axis. The cell colonies of case No. 1 present a higher proliferation rate than those of case No. 2. Otherwise there is no difference, not even in the cell colonies of the metastasis and those of the pri-

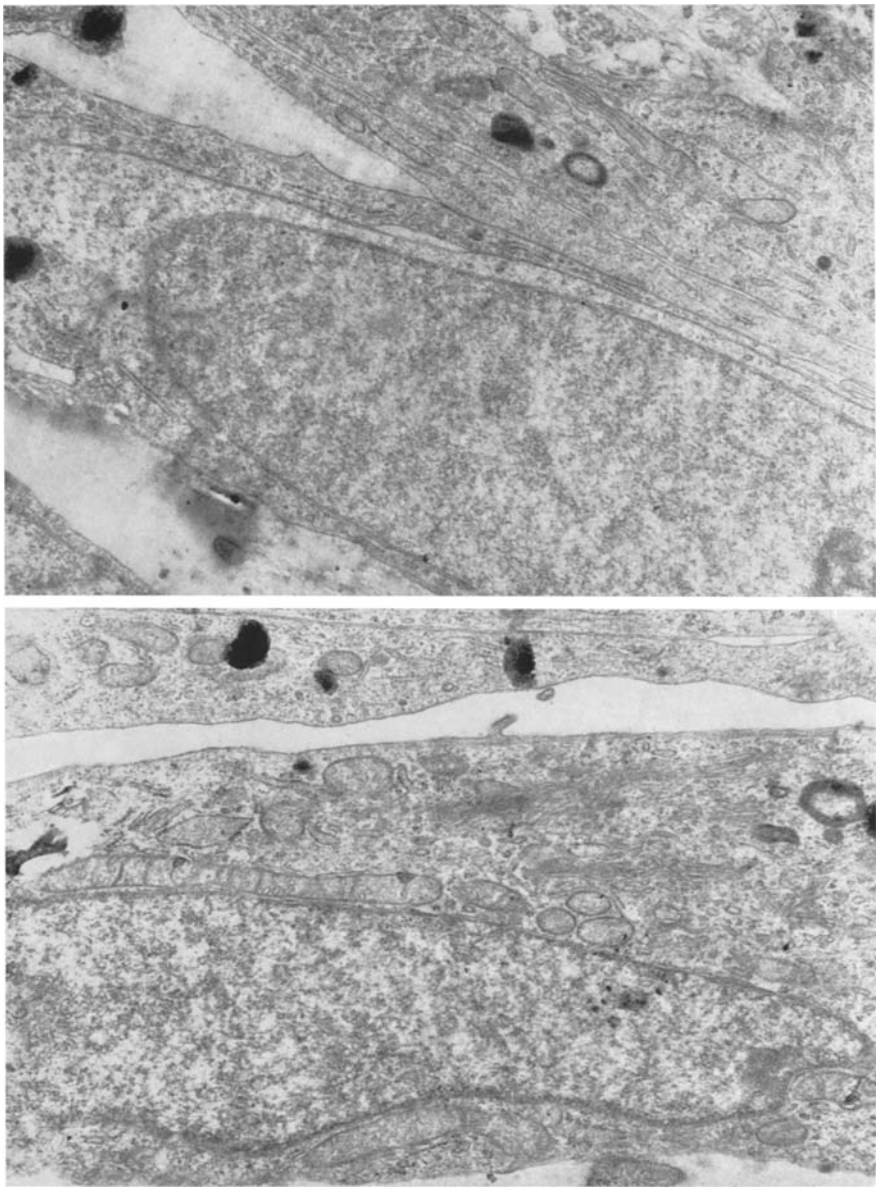

Fig. 2. Medulloblastoma cells in vitro (8th day of cultivation). Same as Fig. 1. The thin lamellar prolongations of cell cytoplasm are well recognized: no cell junctions. Some elements show a well developed Golgi apparatus and elongated mitochondria. 10000:1

mary tumor removed in 1968 (GK 1107). During all the proliferation time the tumor cell colonies preserve their original cytological shape until the latest stages of cultivation just before the final degeneration. No differentiation on a cytological basis takes place.

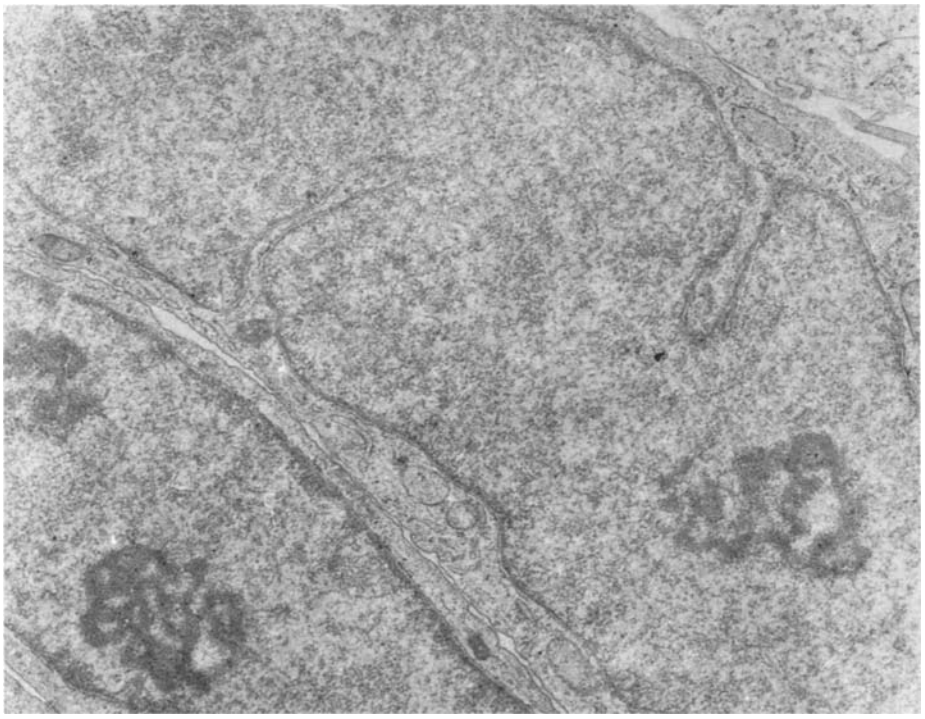

Fig. 3. Medulloblastoma cells in vitro (21st day of cultivation). Irregularly folded nuclei characterize the cells in this culture. The structure of cytoplasm corresponds to that of foregoing pictures. Signs of a neuronal differentiation are missing (compare Fig. 4). 10000:1

In *electron microscopy* the identity of both tumor biopsies (EM 114/70, EM 170/70) is clearly demonstrated. All closely packed tumor cells with narrow edges of cytoplasm, only a few organelles, sometimes rosette-like structures. The nuclei are round oval, often irregularly shaped. Among the viable tumor cells small degenerated elements partly representing necrobiotic granular cells of the cerebellar cortex can be found. (For a more detailed description of the electron microscopy of bioptic material from medulloblastomas see: Galatioto.)

The *ultrastructural investigation* of both tumor cell colonies (EM 119/70, GK 1412, cultures 8 days old, EM 178/70, GK 1444, cultures 21 days old) confirms the results obtained in the bioptic materials. The investigation concerned above all the intermediate proliferation zone which was selected by semi-thin sectioning.

The spindle shaped freely growing tumor cells are packed closely. Their fine processes are long, cytoplasmic and sometimes show lateral sprouts like microvilli extending as thin lamellae to neighbouring cells. The tumor cell nuclei are surrounded only by a thin edge of cytoplasm which contains only a few organelles. Filaments are very rare whereas microtubuli can be seen more often. The mitochondria are round oval, sometimes longish or irregularly shaped. With some exceptions Golgi apparatus and endoplasmic reticulum are not developed exten-

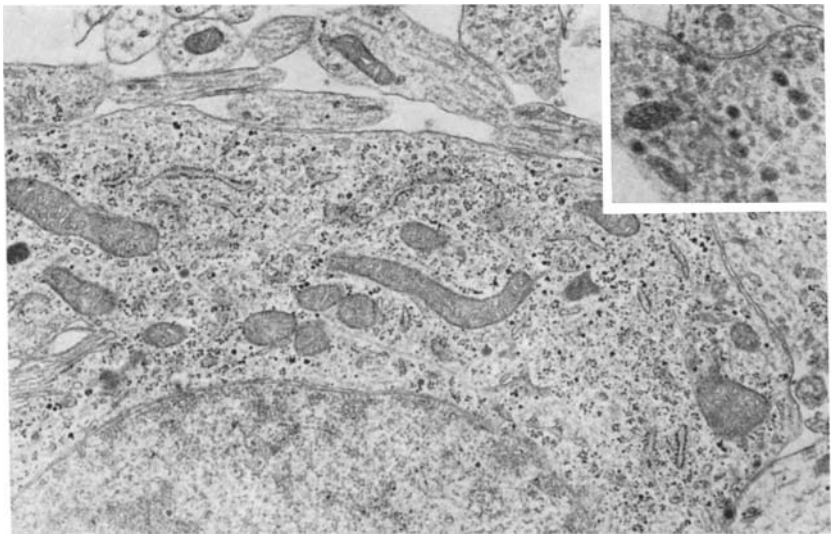

Fig. 4. Sympathicoblastoma cells in vitro (9th day of cultivation). Well developed endoplasmic reticulum, microtubules and filaments characterize these cells which are obviously neuronal in type. The tumor cells have many extremely long processes growing in all directions; they appear here transversely sectioned in the proximity of a neoplastic element. Inset: many osmiophilic, douple outlined round bodies (neurosecretion granula) are frequently found in the cytoplasm and the processes of these elements. 10000:1. Inset: 10000:1

sively. Numerous cells show lipid inclusions, particularly in case No. 2. In this latter case the degenerative changes of cells are very pronounced. Synaptic structures like vesicles, increase of membrane thickness at cell junctions are not to be seen (Figs. 1–4).

The *enzyme histochemical reactions* for acetylcholinesterase (Gomori, Karnowsky) give negative results as well in the biopsies as in the in vitro cultivated material.

Discussion

These new results complete and confirm the former findings of Kersting (1968), Kreutzberg and Gullotta (1967), Matakas *et al.* (1970). The proliferating tumor cells of medulloblastomas are highly undifferentiated, possess only a few organelles and do not show any signs of a neuroectodermal maturation. They look like or are morphologically identical with the cell elements of all embryonic tumors (e.g. Ewing-sarcoma, Friedmann and Gold, 1968; liver tumors, Misugi *et al.*, 1967; immature reticulosarcomas, Matsui, 1964) or all embryonic cells, as long as these do not show any signs of a specific differentiation. Only this further differentiation allows the histological classification of an embryonic cell population. In the same way the cells of a medulloblastoma correspond morphologically to the immature parts of a real neuroblastoma, which separates itself however as soon as the neuronal differentiation starts.

The cells of medulloblastoma do not show the characteristic criteria of maturing ganglional and glial cells. Fujita and Fujita (1963), Glees and Meller (1968),

Meller *et al.* (1969), demonstrated the differentiation of embryonic neuroectodermal elements (neuroblasts) into ganglional and glial cells in vitro within a few days after explantation. Especially Seil and Herndon (1970), Kim (1970), Wolf and Dubois-Dalcq (1970), Lapham and Markesbery (1971) observed by light and electron microscopy the in vitro differentiation of embryonic cerebellar granular cells. Their pictures obviously demonstrate the neuronal nature of these cells: numerous organelles, well developed endoplasmic reticulum, masses of microtubuli. The sympathoblastoma cultures (Goldstein *et al.*, 1964–1968, as well as unpublished personal observations) in their ultrastructural appearance confirm the above findings. Within a few days in vitro cells of neuronal type appear with masses of long processes in which numerous organelles and double outlined neurosecretion granula can be found. The enzyme histochemical reactions for acetylcholin-esterase give repeated positive results in our own observations.

In all medulloblastoma cultures no signs of any differentiation could be found, neither in case No. 1 (8 days old culture) nor in case No. 2 (21 days old culture). Kadin *et al.* (1970) found cell-junctions in old formalin fixed material of medulloblastoma, a finding which we could not verify in bioptic or in cultivated material. From the illustrations of Kadin *et al.*, it seems to be possible that some of the examined tumor areas show preexistent neuroectodermal structures of the cerebellum. Kadin as well as Escourolle and Poirier (1967) interpreted microtubuli as criteria for the neuroectodermal nature of the medulloblastoma cells. But these organelles probably are of no specific importance. Provided the material is adequately fixed microtubuli may be found in cells from all animal and vegetable sources (Behnke, 1964; Sandborn *et al.*, 1964; Anderson *et al.*, 1966; Gibbins *et al.*, 1969; Tilney 1971). The exact function of the microtubuli is not clearly understood yet. They probably form a kind of "cytoskeleton" (Anderson *et al.*) and have something to do with the preservation of the cell shape and its motility (Tilney).

According to our own and other author's experiences only an overwhelming number of microtubuli could indicate the neuronal nature of a cell whereas an occasionally well developed endoplasmic reticulum alone does not give evidence enough of a ganglionic nature or a neuroectodermal differentiation. Maturing neuroblasts are marked by an additional differentiation of the *whole* cell, especially by a proliferation of dendritic and axonal processes (Meller *et al.*, 1969). The same holds true for the sympathoblastomas examined so far.

Repeatedly we pointed out not to take the local glial and neuronal elements of the cerebellar tissue for the true tumor cells of medulloblastoma. It necessarily leads to false conclusions if reactive or degenerated neuroectodermal cells are considered as different grades of maturity of tumor elements. This is extremely important in electron microscopic investigations of very small particles in which the histological characteristics of the tissue can hardly be recognized.

The investigation of cells growing in tissue culture is of great importance in so far as the proliferating cell colonies consist of genuine tumor cells—quite apart from fibroblastic elements derived from stromal mesenchyma—. In tissue cultures of cerebellar medulloblastomas sometimes rows and clusters of Purkinje and granular cells appear which have been explanted together with the tumor cells and which can easily be recognized by their degenerative changes as non

neoplastic (Kersting, 1961, 1968). Besides this the mixed structure of some medulloblastomas in the sense of an overgrowth sarcoma has to be considered (Gullotta, 1966). We however do not share the opinion that gangli"oid" elements within a brain tumor tissue prove its neuronal nature (Misugi and Liss).

The results of classic morphology, enzyme histochemistry, electron microscopy and tissue culture have not been able so far to support the opinion of the neuroectodermal origin of medulloblastoma. Therefore and for the reasons cited in earlier publications (Gullotta, 1967) we take the medulloblastoma as an embryonic mesenchymal tumor of the central nervous system, the main representative of a whole tumor group originating from the embryonic mesenchyma of the brain, which according to its localization is able to develop into several kinds of tumors. In this sense Ostertags principles of local dysgenesia find their full application in medulloblastomas too, considering the special conditions in brain development as a whole.

Acknowledgment. The technical assistance of Mrs. F. Lorenz is gratefully acknowledged.

References

Andersson, W. A., Weismann, A., Ellis, R. A.: A comparative study of microtubules in some vertebrate and invertebrate cells. Z. Zellforsch. **71**, 1–13 (1966).

Behnke, O.: A preliminary report on "microtubules" in undifferentiated and differentiated vertebrate cells. J. Ultrastruct. Res. **11**, 139–146 (1964).

Escourolle, R., Poirier, J.: L'ultrastructure du médulloblastome cérébelleux. Ann. Anat. path. **12**, 121–136 (1967).

Friedman, B., Gold, H.: Ultrastructure of Ewing's sarcoma of bone. Cancer (Philad.) **22**, 307–322 (1968).

Fujita, H., Fujita, S.: Electron microscopic studies on neuroblast differentiation in the CNS of domestic fowl. Z. Zellforsch. **60**, 463–478 (1963).

Galatioto, S.: Contributo ultrastrutturale allo studio del medulloblastoma. Acta neurol. (Napoli) in press.

Gibbins, J. R., Tilney, L. G., Porter, K. R.: Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. I. The distribution of microtubules. J. Cell Biol. **41**, 201–226 (1969).

Glees, P., Meller, K.: Morphology of neuroglia. In: The structure and function of nervous tissue, ed. by G. H. Bourne, vol. I, p. 301–323. New York-London: Academic Press 1968.

Goldstein, M. N.: Neuroblastoma cells in tissue culture. J. pediat. Surg. **3**, 166–167 (1968).

Goldstein, M. N., Burdman, J. A., Journey, L. J.: Long-term tissue culture of neuroblastomas. II. Morphologic evidence for differentiation and maturation. J. nat. Cancer Inst. **32**, 165–200 (1964).

Gullotta, F.: Über angeborene Mischgeschwülste des Kleinhirns. Dtsch. Z. Nervenheilk. **189**, 354–374 (1966).

Gullotta, F.: Das sogenannte Medulloblastom. Berlin-Heidelberg-New York: Springer 1967.

Gullotta, F.: The specific localisation of mesenchymal tumors of central nervous system. Proc. VIth Intern. Congr. of Neuropathology (Paris). Paris: Masson 1970.

Kadin, M. E., Rubinstein, L. J., Nelson, J. S.: Neonatal cerebellar medulloblastoma originating from the fetal external granular layer. J. Neuropath. exp. Neurol. **29**, 583–600 (1970).

Kersting, G.: Die Gewebszüchtung menschlicher Hirngeschwülste. Berlin-Göttingen-Heidelberg: Springer 1961.

Kersting, G.: Tissue culture of human gliomas. In: Prog. neurol. Surg., vol. 2., p. 165–202, ed. by H. Krayenbühl, P. E. Maspes and W. H. Sweet. Basel: Karger 1968.

Kim, S. U.: Observations on cerebellar granule cells in tissue culture. A silver and electron microscope study. Z. Zellforsch. **107**, 454–465 (1970).

Kreutzberg, G. W., Gullotta, F.: Enzymhistochemischer Beitrag zur Histogenese des Medulloblastoms. Arch. Psychiat. Nervenkr. **209**, 378–386 (1967).

Lapham, L. W., Markesbery, W. R.: Human fetal cerebellar cortex: organization and maturation of cells in vitro. Science **173**, 829–832 (1971).

Matakas, F., Cervos Navarro, J., Gullotta, F.: The ultrastructure of medulloblastomas. Acta neuropath. (Berl.) **16**, 271–284 (1970).

Matsui, K.: Ultrastructure of reticulosarcoma. Proc. IVth Intern. Symp. on RES (Otsu and Kyoto, 1964). Kyoto: Nissha Print. Co. 1965.

Meller, K., Breipohl, W., Wagner, H. H., Knuth, A.: Die Differenzierung isolierter Nerven- und Gliazellen aus trypsiniertem Rückenmark von Hühnerembryonen in Gewebekulturen. Eine licht- und elektronenmikroskopische Studie. Z. Zellforsch. **101**, 135–151 (1969).

Meller, K., Eschner, J., Glees, P.: The differentiation of endoplasmic reticulum in developing neurons of the chick spinal cord. Z. Zellforsch. **69**, 189—187 (1966).

Meller, K.,Haupt, R.: Die Feinstruktur der Neuro-. Glio- und Ependymoblasten von Hühnerembryonen in der Gewebekultur. Z. Zellforsch. **76**, 260—277 (1967).

Misugi, K., Okajima, H., Misugi, N., Newton, W.A.: Classification of primary malignant tumors of liver in infancy and childhood. Cancer (Philad.) **20**, 1760–1771 (1967).

Misugi, K., Liss, L.: Medulloblastoma with cross-striated muscle. A fine structural study. Cancer (Philad.) **25**, 1279–1285 (1970).

Ostertag, B.: Einteilung und Charakteristik der Hirngewächse. Jena: Fischer 1936.

Ostertag, B.: Anatomie und Pathologie der raumfordernden Prozesse des Schädelbinnenraumes In: Spezielle Chirurgie der Gehirnkrankheiten, Neue Deutsche Chirurgie, Bd. 50/III. Stuttgart: Enke 1941.

Sandborn, E., Koen, P. F., McNabb, J. D., Moore, G.: Cytoplasmic microtubules in mammalian cells. J. Ultrastruct. Res. **11**, 123–138 (1964).

Seil, F. J., Herndon, R. M.: Cerebellar granule cells in vitro. A light and electron microscope study. J. Cell Biol. **45**, 212–220 (1970).

Tilney, L. G.: Origin and continuity of microtubules. In: Origin and continuity of cell organelles, ed. by J. Reinert and H. Ursprung, p. 222–260. Berlin-Heidelberg-New York: Springer 1971.

Wolf, M. K., Dubois-Dalcq, M.: Anatomy of cultured mouse cerebellum. I. Golgi and electron microscopic demonstrations of granule cells, their afferent and efferent synapses. J. comp. Neurol. **140**, 261–280 (1970).

Prof. Dr. F. Gullotta
Prof. Dr. G. Kersting
Institut für Neuropathologie
der Universität
D-5300 Bonn-Venusberg
Annaberger Weg
Germany