Virchows Arch. A Path. Anat. and Histol. 381, 1–11 (1978)

Virchows Archiv A

Original Articles

Cerebral Neuroblastoma

S. Yagishita [1], Y. Itoh [1], Y. Chiba [2], and K. Yuda [2]*

[1] Division of Pathology, Kanagawa Rehabilitation Center Atsugi-Shi, Kanagawa-Ken, Japan
[2] Department of Neurosurgery, Yokohama City University Urafune-Cho Minami-Ku, Yokohama-Shi, Japan

Summary. A cerebral neuroblastoma removed surgically from a female child is presented. Electron microscopy showed numerous neuronal processes with growth cones which are a feature of the developing neurone. In addition there were some rosettes with distinct lumina. The luminal surfaces were covered with a smooth plasma membrane lacking any surface differentiation and the lateral surface of these cells had many cell junctions (terminal bars), reminiscent of a primitive neural tube. These features in a nerve cell tumor help to substantiate it as a neuroblastoma arising from immature rather than differentiated cells.

The nature of this rare tumor is discussed.

Key words: Central neuroblastoma – Electron microscopy – Growth cone – Primitive neural crest – Neuronal cytogenesis.

Human primary cerebral neuroblastomas, unlike those occurring in the peripheral nervous system, are extremely rare and ill-defined both clinically and pathologically. Cerebellar medulloblastomas sometimes show divergent differentiation along either neuronal or glial cell lines or both; by contrast, the differentiating potential of the cerebral neuroblastoma is restricted to ganglion cells only (Horten and Rubinstein, 1976) and this tumor is thought to be derived from the early stage of neuronal cytogenesis (Fujita and Fujita, 1965). On the other hand, Azzarelli et al. (1977) have reported a cerebral neuroblastoma with ultrastructural evidence of divergent differentiation along neuronal, astrocytic and ependymal cell lines.

It is on the basis of these present concepts of tumor cytogenesis that an ultrastructural study of a case of cerebral neuroblastoma was performed. After verifying the neuronal origin, attempts were made to compare some of its pertinent features with the maturation process of a nerve cell in human and murine brains.

* *Present address*: Division of Neurosurgery, Kanagawa Rehabilitation Center

0340-1227/78/0381/0001/$02.20

Case Report

A 3-year-old girl was referred to the neurological clinic of Yokohama City University at the beginning of December 1977 because of frequent headaches, vomiting and lethergy which developed three weeks prior to the admission.

The patient had been diagnosed as having osteogenesis imperfecta on the basis of a history of four fractures of long bones and her blue sclera, but there were no previous neuro-psychiatric symptoms.

The neurological examination showed severe lethergy and bilateral papilledema. Plain skull X-rays revealed diastasis of both coronal and sagittal sutures. Cerebral angiography and CT scan demonstrated a large mass in the trigone of the right lateral ventricle invading the ipsilateral thalamic region, and also a part of the left occipital lobe and corpus callosum (Fig. 1).

An emergency operation was performed with a partial resection of the tumor because of presumed massive hemorrhage into the tumor on December 12, 1977. On January 25, 1978, a second operation was performed and a further partial resection of the tumor was made. Irradiation and chemotherapy were then given. The patient is alive at present in July 1978.

The tumor was relatively well circumscribed. The peripheral parts were homogeneous and grey-white in color and the deeper parts, especially the medial parts, were reddish-purple in color and hemorrhagic.

The specimens were fixed in 2.5% glutaraldehyde solution, post-fixed in 1% osmic acid and embedded in Epon 812. Semi-thin sections were treated with uranyl acetate and lead citrate and were examined with Hitachi HU-12 electron microscope at 75Kv.

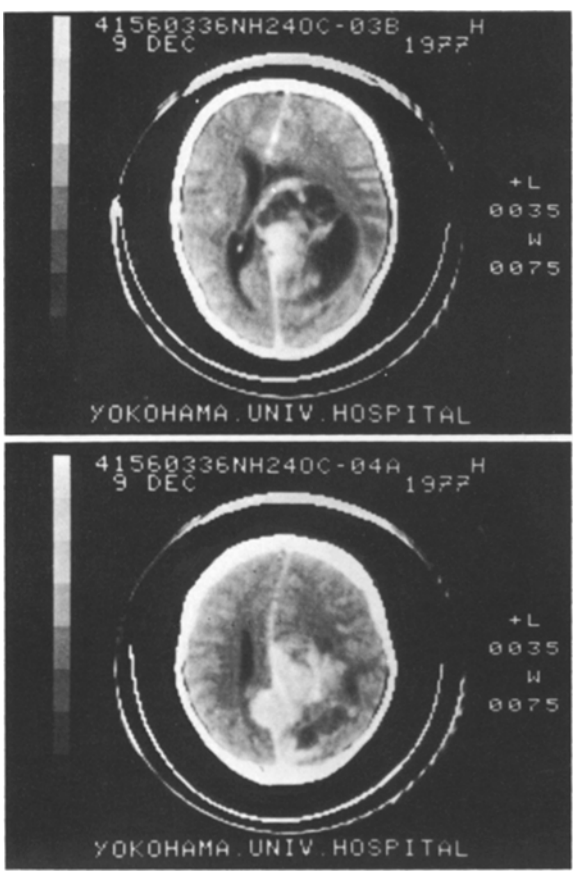


Fig. 1. CT scan. A large tumor in the trigone of the right lateral ventricle

Results

Light Microscopy

The tumor cells were small, uniform and spherical, arranged in broad lobules in abundant delicate fibrillary stroma suggestive of the neuropils. The individual cells had round to oval nuclei surrounded by scanty perinuclear cytoplasm (Figs. 2 a and 2 b). Sometimes the cells were clustered about

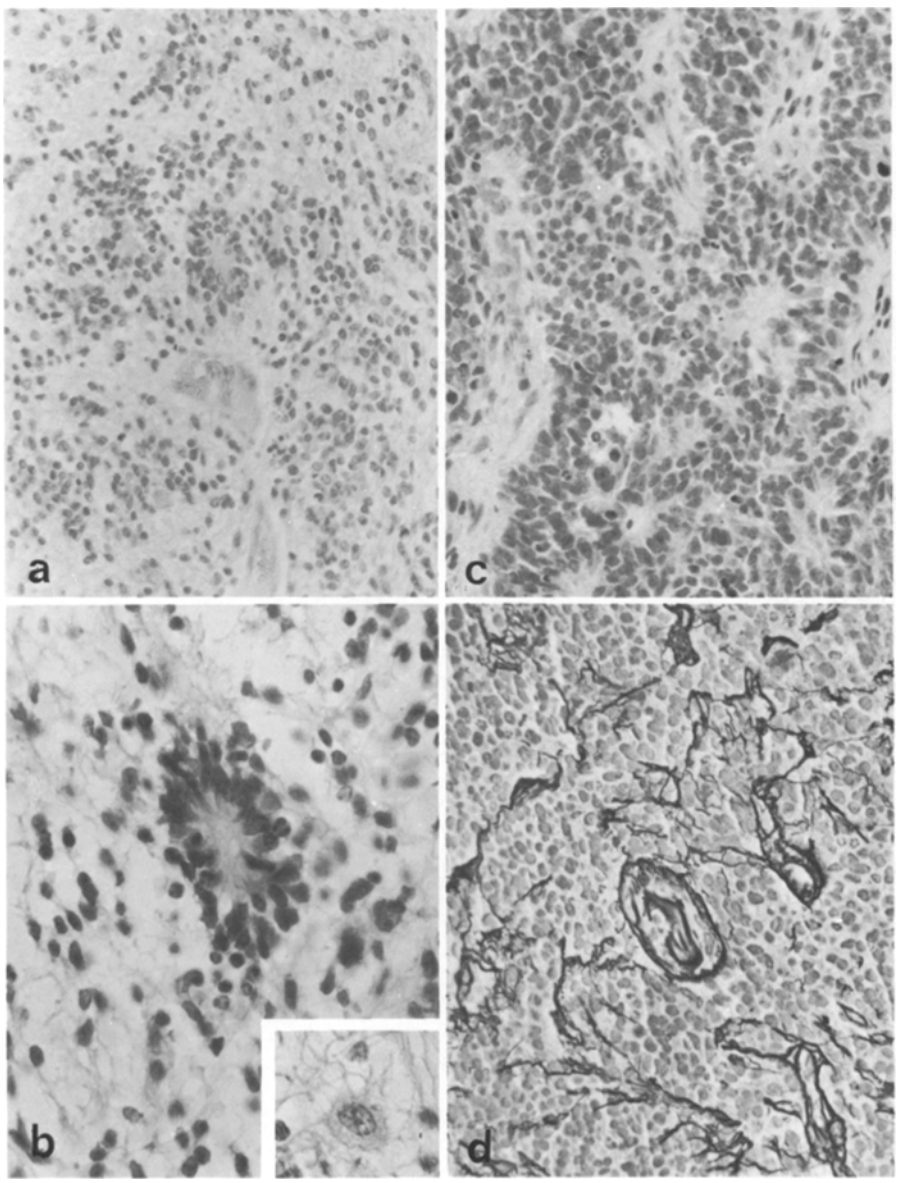


Fig. 2 a small spherical tumor cells in abundant delicate fibrillary stroma. Two rosettes are seen in the upper field. H.E. ×200 **b** Higher magnification of Fig. 1 a. H.E. ×400 **c** highly cellular tumor. Compare with Fig. 1 a. H.E. ×200 **d** The delicate connective tissue stroma separates the tumor cells into groups. Reticulin stain. ×200

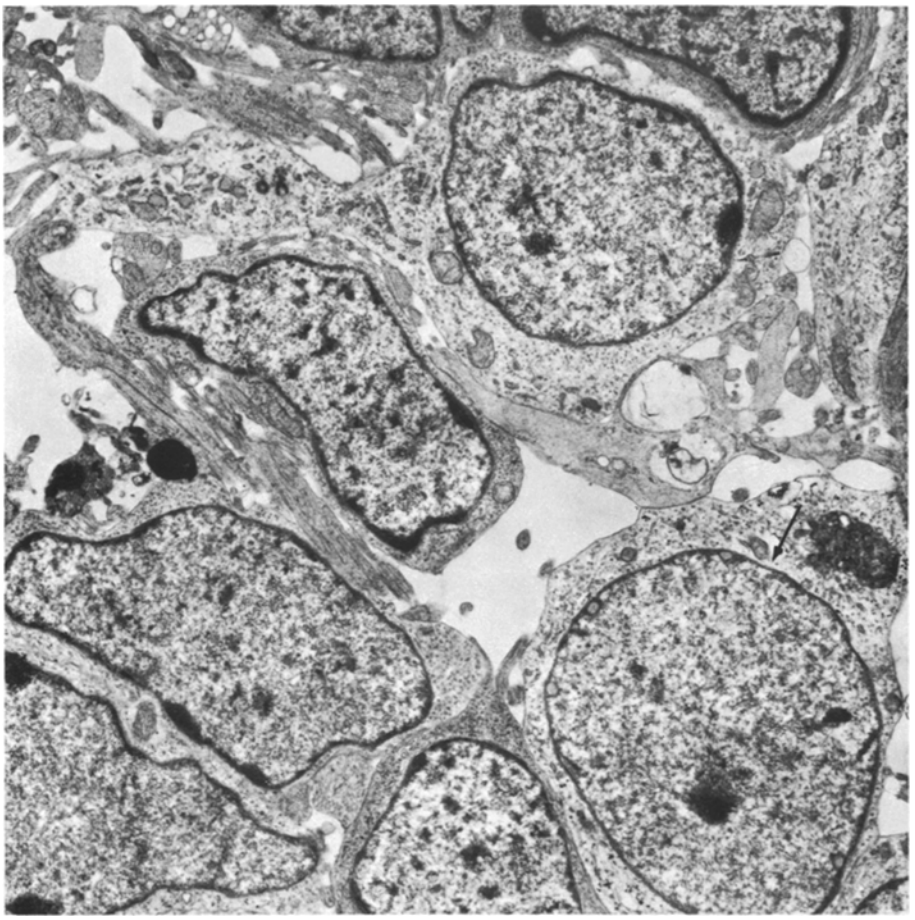


Fig. 3. Tumor cells and interspersed cell processes are seen. Two cells show a round nucleus containing evenly dispersed chromatin. ×6,500

anuclear foci forming abortive rosettes. Some cells were large with a pale vesiculated nucleus and a conspicuous nucleolus (Fig. 2. inset). Blood vessels and fibrous tissue trabeculae were not especially conspicuous. Mitoses were relatively sparse. Silver impregnation revealed many neuronal processes distributed at random throughout the tumor.

In the second surgical specimen, the tumor was much highly cellular and composed of closely packed cells with ill-defined, scanty cytoplasm showing moderate variation in size (Fig. 2c). In some instances a delicate connective stroma separated the cells into groups (Fig. 2d). Rosette formation was not observed. The nuclei were round or oval and the chromatin was finely granular and unevenly dispersed; mitotic figures were moderately abundant. There were multiple foci of necrosis and scattered macrophages. Bodian silver preparations failed to show neurites.

Electron Microscopy

The tumor cells had a large round or oval nucleus with a fine and evenly dispersed chromatin. Occasional indentations rendered the nuclear profiles more

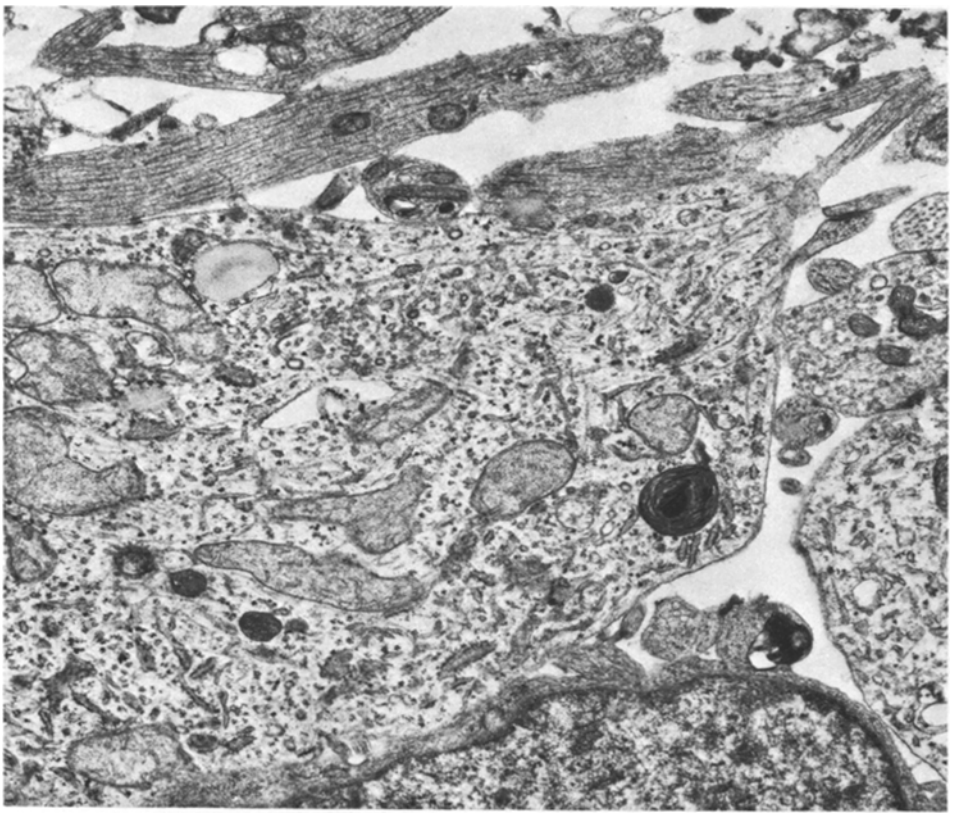
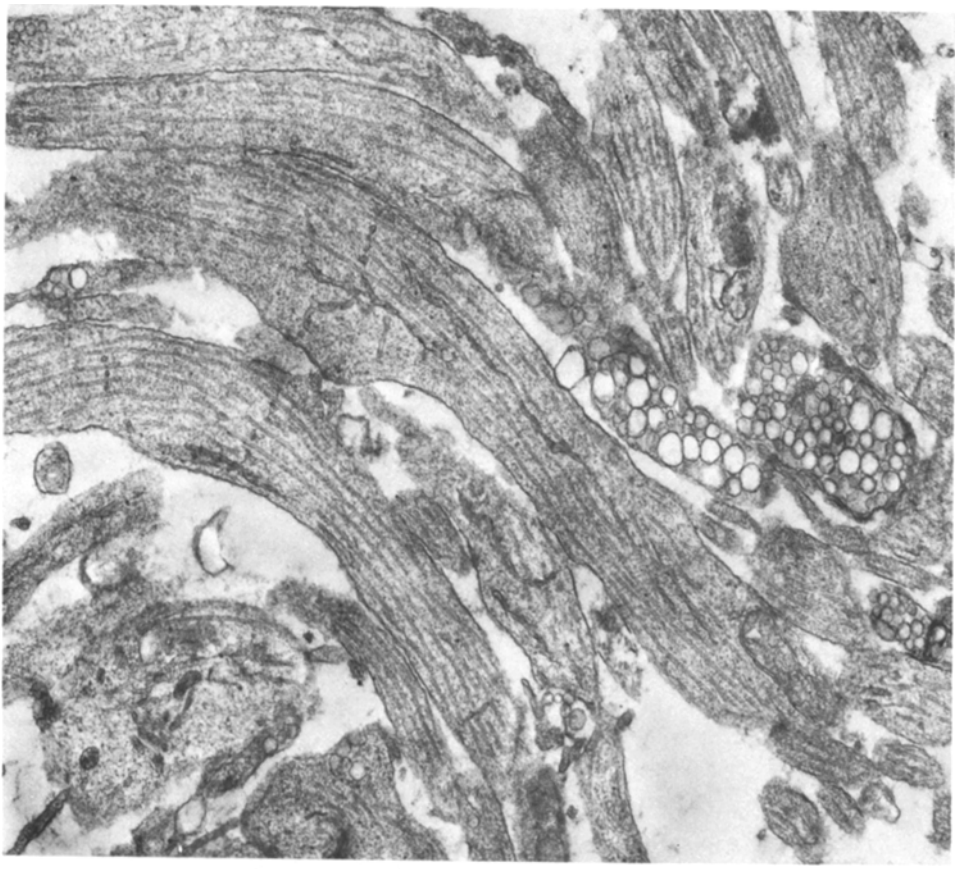


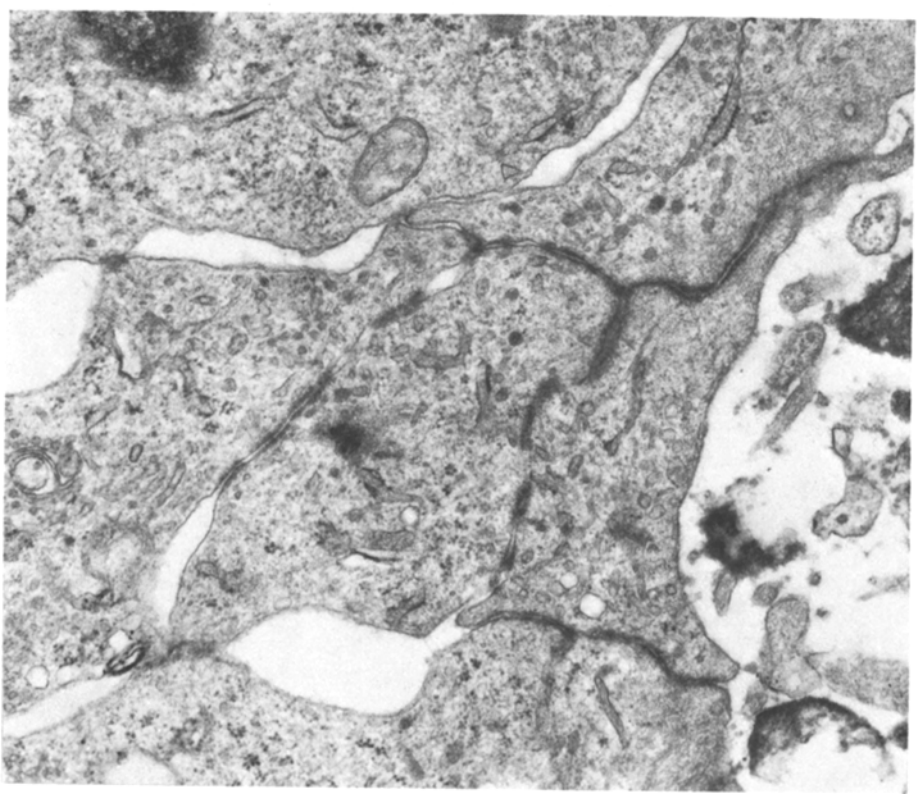
Fig. 4. A part of the cytoplasm of a tumor cell. Many polysomes, cisternae of rough-surfaced endoplasmic reticulum and microtubules are seen. × 14,000

irregular in many places. The cytoplasm was usually inconspicuous (Fig. 3) but abundant in some instances and contained many cell organelles. The cisterns of the rough endoplasmic reticulum were disorderly scattered in the cytoplasm. Mitochondria, Golgi complexes, lysosomes and lipid bodies were also present (Fig. 4). In addition, microtubules were dispersed in the cell bodies but they were more numerous in the cell processes.

The cell surfaces either juxtaposed to one another or exposed to the extracellular space were smooth. At first glance, the perikaryal outlines seemed complicated by numerous cellular expansions, however, this was actually due to the presence of abutting swollen bulbs and tiny cell processes distinct from the plasma membrane and originating from the nearby cells; the extracellular spaces surrounding each cell were occupied by intricate networks of slender cytoplasmic expansions presumably leaving the cell bodies (Fig. 3). Several of these interstitial cell processes exhibited terminal or subterminal dilatations studded with apparently empty, smoothwalled pleomorphic vesicles. Most processes contained many microtubules running parallel to their long axis, together with a few ribosomes mitochondria and dense bodies. On the other hand, some distended cell processes



5



6

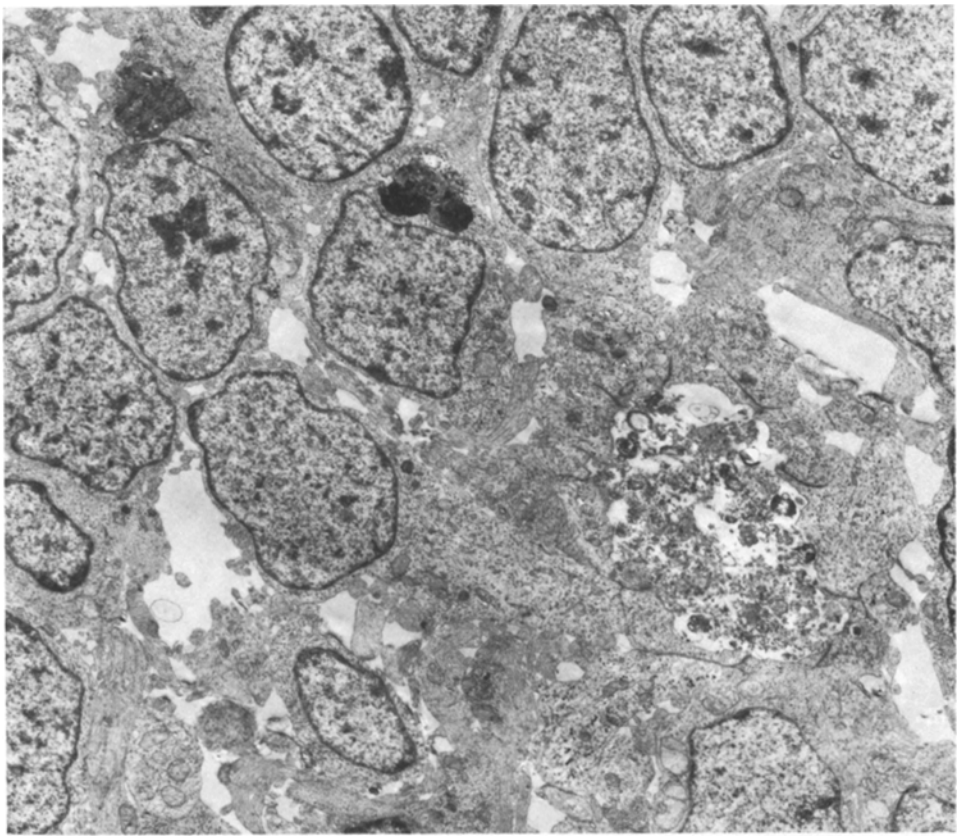


Fig. 7. Rosette-like structure consisting of multilayered cells closely applied to one another. The other cells were separated by many delicate fibrils. × 7,500

exhibited scanty organelles simulating immature dendrites. Very tiny cell processes also existed among the branches of the cell processes and they contained no organelles except for amorphous or fibrillary material (Figs. 4 and 5). There were no secretory granules in the cell processes or in the cell perikarya.

Some processes were closely apposed to one another, showing local thickening and increased density of the adjoining membranes (junctional device of puncta adhaerens type).

Rosettes were formed by multilayered cells surrounding a central acellular space. At first glance, most spaces seemed to be filled with tiny cell processes cut in various planes, but there was a distinct lumen in the central portion.

Fig. 5. Many cytoplasmic processes suggestive of developing axons with abortive growth cones *(arrow)*. × 25,000

Fig. 6. Luminal surface of a rosette. Many intercellular junctional complexes are seen. The luminal surface is covered with smooth plasma membrane without any surface differentiation. × 19,000

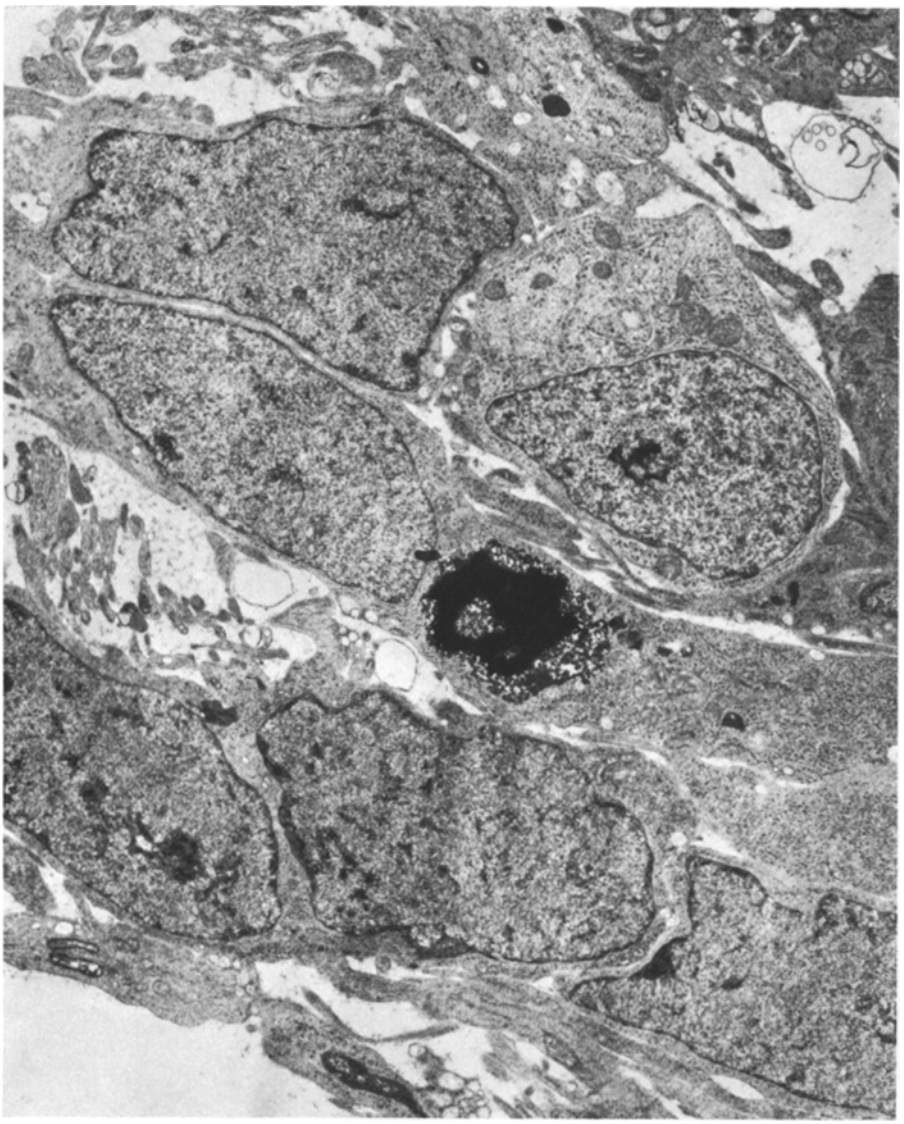


Fig. 8. Neoplastic cells, in the second surgical material, appear to be less differentiated. ×7,000

The luminal surface of the cell was smooth and lacked any surface differentiation such as microvilli, cilia or centrisomes, except for several irregular protrusions of cytoplasm (Fig. 6). Many of the multilayered cells were closely applied to one another and the remainder was separated by irregular extracellular spaces containing many delicate fibrils, presumably developing axons (Fig. 7). Many cell junctions were found at the lateral surface of the cells adjacent to the lumina, which resembled terminal bar attachments (Fig. 6). No basement membranes were identified. The tumor cells exhibited no neuroglial differentiation.

The ultrastructure of the second surgical specimen appeared to be essentially similar to that of the first sample. However, the tumor cells were more immature, having a less abundant cytoplasm and fewer organelles, but there were numerous cell processes interspersed in the extracellular spaces (Fig. 8).

Discussion

Human cerebral neuroblastomas are poorly differentiated tumors, and the precise recognition of the tumor cells by means of standard histological techniques is often difficult. The presence of distinct Homer-Wright rosettes, maturation to ganglion cells and demonstrable argyrophilic cell process are said to be characteristic features of this tumor (Horten and Rubinstein, 1976). The histology of the present tumor might satisfy these criteria and the electron microscopic pictures extended the evidence for the diagnosis of neuroblastoma. Our electron microscopic findings, particularly the well dispersed chromatin, the smooth cell contours with empty extracellular spaces, and numerous interstitial processes with microtubules running parallel to the axis constitute substantial features to support the diagnosis of neuroblastoma. The presence of growth cones confirm a neuroblastic lineage.

Growing axons are known to present a growth cone provided with filopodia which form specialized cell junctions, some of which resemble synapses (Mugnaini, 1971; Yamada, 1971). In the present tumor the tiny interstitial cell processes, presumably developing axons, exhibited terminal or subterminal dilatations (Fig. 5) studded with apparently empty, pleomorphic vesicles, simulating abortive growth cones. Some tiny processes closely apposed to one another showed asymmetrical thickening and increased membrane density exclusive for interneuron junctions, but definite synaptic devices could not be found. These growing apparatuses have never been described in any human neuronal tumors apart from experimental or cultured neuroblastomas (Bucher and Ermel, 1974; Lantos and Pilkington, 1977; Hsu and Trupin, 1978). Synaptic structures are usually absent or ill-defined (Staley et al., 1967; Kadin et al., 1971; Nakayama et al., 1975). Only Luse (1967) described synaptic endings on neoplastic cells and their processes.

The neuroblast differentiates first into a neuron through the critical period of Flexner at its peak at eight days in newborn rats; hallmarks of its maturation are the numerical density of cytoplasmic ribosomes and a swelling of the endoplasmic cisterns. Prior to ten days of age, there are numerous cell contacts suggesting presumptive synapses but it is not until ten days that special synaptic devices develop in this portion (Caley, 1971). Although Bucher and Ermel (1974) propose that the rarity of establishment of normal synaptic connections could be responsible for the eccentrically located nuclei which is seen in cultured neurons and neuroblastomas, the poor degree of maturation of the neoplastic cells might explain the rare development of synaptic complexes, at least in the present tumor in which the majority of cells had not differentiated into ganglion cells. Conversely many synaptic contacts are reported in cerebral ganglioglioma (Rubinstein and Herman, 1972).

The largest part of the perikarya and processes of the neoplastic cells contained microtubules 200 Å in diameter. These have been recognized as cytoplasmic components in large numbers of animal and plant cells. They have also been identified in immature neuronal cells including medulloblastoma (Escourelli et al., 1967; Matakas, 1970) and neuroblastoma, and have been considered to be a distinct feature of differentiating neuroblastoma (Azzarelli et al., 1977).

Another important feature was the presence of rosettes with distinct lumina in the centers. The luminal surface was covered with a smooth plasma membrane without any surface differentiation and the lateral surface shows many cell junctions. Thus these appearances mimic those of the primitive neural tube (Pollak and Friede, 1977, Tennyson and Pappas, 1962). Whereas there is no distinct basal lamina in the present tumor, the medulloepithelioma always exhibits a basal surface to the epithelial layer which rests on a distinct basal lamina and usually differentiates into an astrocyte, ependymal cell or primitive neuroblast (Jellinger, 1972, Karch and Urich, 1974; Pollak and Friede, 1974). These findings favour the concept that the neuroblastoma may arise from a kind of cell corresponding to the medullary epithelial cell in a very early stage of cytogenesis.

The minority of central neuroblastomas exhibit secretory granules in the cell body and its processes (Azzarelli et al., 1977; Vuia and Hager, 1975) but these granules are usually seen in peripheral neuroblastomas (Macky et al., 1976; Macky et al., 1975). Vuia and Hager (1975) report a spinal neuroblastoma with smooth muscle fibers and consider that dysgenesis of the ectomesenchyma is the basis of the development of this type of tumor. The question why secretory granules are present in some central neuroblastomas and absent in the others requires further investigation.

References

Azzarelli, B., Richards, D.E., Anton, A.H., Roessmann, U.: Central neuroblastoma. Electron microscopic observations and chatecholamine determinations. J. Neuropath. Exp. Neurol. **36**, 384–397 (1977)

Bucher, J.M., Ermel, A.E.: Central neuroblastoma induced by transplacental administration of methylnitrosourea in Wistar rats. An electron microscopic study. J. Neurol. **208**, 1–16 (1974)

Caley, D.W.: Cellular aspects of neuronal growth and differentiation. UCLA Forum Med. Sci. No. 14. Univ. of Calfornia Press 73–101 (1971)

Escourolli, R., Poirier, J.: Ultrastructure du medulloblastome cerebelleux. Ann. Anat. Pathol. (Paris) **12**, 121–136 (1967)

Fujita, H., Fujita, S.: Electron microscopic studies on neuroblast differentiation in the central nervous system of domestic fowl. Zschr. Zellforsch. **60**, 463–478 (1965)

Goebel, H.H., Cravioto, H.: Ultrastructure of human and experimental ependymomas. A comparative study. J. Neuropath. Exp. Neurol. **33**, 54–71 (1974)

Hortem, B.C., Rubinstein, L.J.: Primary cerebral neuroblastoma. Clinicopathological study of 35 cases. Brain **99**, 745–765 (1976)

Hsu, L., Trupin, G.L.: The in vivo differentiation of murine neuroblastoma. Virchows Arch. B Cell Path. **27**, 49–61 (1978)

Jellinger, K.: Cerebral medulloepithelioma. Acta Neuropath. (Berl.) **22**, 95–101 (1973)

Kadin, M.E., Bensch, K.: Comparison of pheochromocytes with ganglion cells and neuroblasts grown in vitro. An electron microscopic and histochemical study. Cancer **27**, 1138–1160 (1971)

Karch, S.B., Urich, H.: Medulloepithelioma: Definition of an entity. J. Neuropath. Exp. Neurol. **33**, 27–53 (1974)

Lantos, P.L., Pilkington, J.G.: Neuroblasts in cerebral tumors induced by ethylnitrosourea in rats. A fine structural study. Virchows Arch. B Cell. Path. **25**, 243–259 (1977)

Luse, S.A.: Synaptic structures occurring in a neuroblastoma. Arch. Neurol. (Chic) **11**, 185–190 (1964)

Macky, B., Luna, M.A., Bulter, J.J.: Adult neuroblastoma. Electron microscopic observations in nine cases. Cancer **37**, 1334–1351 (1976)

Macky, B., Masses, S.R., King, O.Y., Butler, J.J.: Diagnosis of neuroblastoma by electron microscopy of bone marrow aspirates. Pediatrics **56**, 1035–1039 (1975)

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

Mugnaini, E: Cellualr aspects of neuronal growth and differentiation. UCLA Forum Med. Sci. No. 14, Univ. of Calfornia Press 103–159 (1971)

Nakayama, I., Tsuda, N., Muta, J., Fujii, H., Tsuki, K., Matsuo, T., Takahara, O.: Fine structural comparison of Ewing's sarcoma with neuroblastoma. Acta Path. Jap. **25**, 251–268 (1975)

Pollak, A., Friede, R.L.: Fine structure of Medulloepithelioma. J. Neuropath. Exp. Neurol. **36**, 712–725 (1974)

Rubinstein, L.J., Herman, M.M.: A light- and electron microscopic study of a temporal lobe ganglioglioma. J. Neurol. Sci. **16**, 27–48 (1972)

Staley, N.K., Plesky, H.F., Bensch, K.G.: Fine structural and biochemical studies of the malignant ganglioneuroma. J. Neuropath. Exp. Neurol. **26**, 634–653 (1967)

Tennyson, V.M., Pappas, G.D.: An electron microscope study of ependymal cells of the fetal, early-postnatal and adult rabbit. Zschr. Zellforsch. **56**, 595–618 (1962)

Yamada, K.M., Spooner, B.S., Wessells, N.K.: Ultrastructure and junction of cultured nerve cells. J. Cell Biol. **49**, 614–635 (1971)

Vaghn, J.E., Peters, S.: Electron microscopy of early postnatal development of fibrous astrocytes. Am. J. Anat. **121**, 131–151 (1967)

Vuia, O., Hager, H.: Central neuroblastic tumor associated with smooth muscle fiber. A study in the optic and electron microscopes. Europ. Neurol. **13**, 248–272 (1975)

Received June 20, 1978