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Case report

Hypothalamic polar spongioblastoma associated with the diencephalic syndrome

Ultrastructural demonstration of a neuro-endocrine organization

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Summary. A tumor was resected from the third ventricle of a four years and eleven month old girl with the diencephalic syndrome. By light microscopy, it was diagnosed as a polar spongioblastoma.

Its ultrastructural study was undertaken and the features were found to be distinctive and previously unreported. The organization of the neoplasm was similar to that of the hypothalamic neuro-endocrine systems: Cellular perivascular arrangement with intra-cytoplasmic microtubules and membrane bound dense-core granules.

Key words: Brain neoplasms, ultrastructure – Diencephalic syndrome, pathology – Hypothalamic neoplasms, ultrastructure – Neurosecretory granules – Polar Spongioblastoma, ultrastructure

Tumors arising from the diencephalon, although rare, often are accompanied by endocrine abnormalities (Burr et al. 1976). The diencepahlic syndrome described by Russell has become the prototype of such association in infants and young children (Russell 1951). In its broadest definition, it comprises a diencephalic tumor with emaciation and minimal neurological signs. The characteristic signs are apparent alertness, hyperkinesis, vomiting, euphoria and nystagmus. Endocrine disorders without emaciation have been described in association with diencephalic tumors; the best known is precocious puberty (Costin 1979; Drop et al. 1980).

The relationship between the neoplasm and the endocrine abnormalities has been difficult to ascertain. This has resulted, in part, from the heterogeneity of the tumors observed. Among these, an often mentioned tumor is the "Polar spongioblastoma" which has been the subject of lengthy debates as to its exact nature. However, according to those who have accepted

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it as a specific entity, it constitutes a rare and relatively benign neoplasm which presents in young subjects and involves midline structures, especially the hypothalamus and lateral walls of the third ventricle, optic chiasma and optic nerves. It is sometimes seen in association with neurofibromatosis (Zulch 1965).

We recently examined a tumor which originated in the third ventricle between the hypophyseal stalk and the optic chiasma (the infundibulum) of a child with the diencephalic syndrome. By light microscopy, it had the features of a "polar spongioblastoma". The ultrastructural study of the tumor showed it to be different from previously described brain neoplasms.

The purpose of this communication is to relate our findings and reconsider the cytogenesis of this neoplasm and its possible relationship with the endocrine abnormalities.

Case history

(K.M.) was seen at four years, eleven months of age. She had had a one and a half month history of progressive nausea and vomiting, wasting and difficulty walking. Investigation revealed markedly increased intra-cranial pressure with hydrocephalus and a suprasellar mass. Following ventriculo-peritoneal shunting, the mass was excised. Later, the patient underwent endocrinological investigation. She had the weight of a three year old, the height of a five year and 6 month old, while her chronological age was four years and eleven months. Growth hormone studies showed a low basal level with no spontaneous peaks and no response to Arginine Tolerance Test-Insulin Tolerance Test (ATT-ITT). The prolactin level was high basal; Thyroid Stimulating Hormone (TSH) was normal with normal response to Thyrotrophin Releasing Hormone (TRH). Adreno-Cortico-Trophic Hormone (ACTH) had elevated basal levels and the Luteinizing Hormone/Follicle Stimulating Hormone (LH/FSH) was interpreted as pre-pubertal with low basal increase with Luteinizing Hormone-Releasing Hormone (LH-RH). The Antidiuretic Hormone (ADH) was normal. In addition she had a normal insulin response to ATT. The patient is still alive four years after surgical excision and radiotherapy.

Material and methods

Several fragments of tumor tissue were obtained. After adequate sampling for electron microscopy, half the tissue was fixed in 10% buffered formalin and processed for light microscopy; the other half was fixed in a mixture of 75% ethyl alcohol, 5% glacial acetic acid and 20% formalin for two hours, then processed as usual.

Stains and histochemical techniques employed included hematoxylin and eosin, hematoxylin-phloxine-saffronin (HPS), periodic-acid Schiff (PAS), von Kossa, Grimelius, alizarin red, cresyl violet, Bielschowsky, Bodian, luxol fast blue, Holzer and Mallory's phosphotungstic acid-hematoxylin.

Tissue for electron microscopy was sliced into 0.5 to 1 mm cubes, fixed in 2.5% phosphate buffered glutaraldehyde, and postfixed in osmium tetroxide (2%). The fragments were washed in phosphate buffer, stained "en block" for 30 min with uranyl acetate, rewashed in buffer, dehydrated in graded alcohols, treated with propylene oxide, left in a 50/50 mixture of propylene oxide and Epon (Fisher Scientific Co., Fairlawn, New Jersey) for 90 min, then in 1:2 propylene oxide and Epon overnight. The specimens were then embedded in Epon. Thick sections were stained with toluidine blue. Ultrathin sections were cut and doubly stained with uranyl acetate and lead citrate. The stained sections were examined using a Philips EM-201 electron microscope (Philips Electronic Instruments, Mount Vernon, New York).

For Immunohistochemistry, all the paraffin embedded sections of tumor tissue as well as two sections from a known case of cerebellar fibrillary astrocytoma and a section of normal brain were used. These were deparaffinized, treated with hydrogen peroxide, then incubated

with normal swine serum. Rabbit antiglial fibrillary acidic protein (GFAP) was then allowed to react with the sections. Later, these were rinsed and covered with swine anti-rabbit immunoglobulins. The sections were again washed and the peroxidase-antiperoxidase (PAP) reagent was added. Unbound PAP reagent was then removed by washing. Finally, a solution of hydrogen peroxide and amino-ethylcarbazole (AEC) was added. The sections were counterstained and coverslipped for study. The entire procedure was done according to the instructions received with the kit used and provided by Dako Corporation (Santa Barbara, California).

Results

Light microscopy

The neoplasm was very vascular with loosely arranged thin unipolar and bipolar cells disposed in palisading rows with delicate fibrils extending at right angles to the vascular walls (Fig. 1). The relationship with the vessels was more clearly demonstrable when excessive tissue shrinkage was prevented by the use of a mixture of glacial acetic acid, ethyl-alcohol and concentrated formaldehyde for primary fixation (see methods) (Fig. 2, 3). Embedded within the mass of the tumor, many calcospherites were seen. There were no Rosenthal fibers and no necrotic areas. Mitoses were exceptional. Based on these features, it was diagnosed as a polar spongioblastoma (Russell 1971).

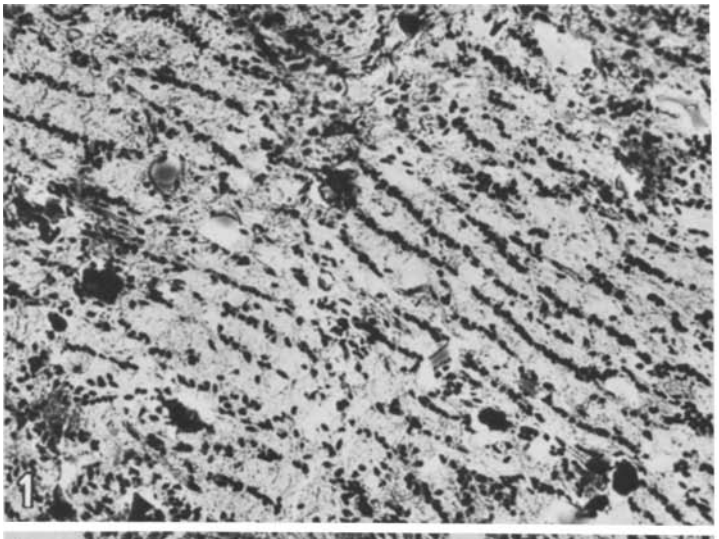
The histochemical stains did not further elucidate the nature of the neoplastic process. In particular, the Grimelius stain was negative.

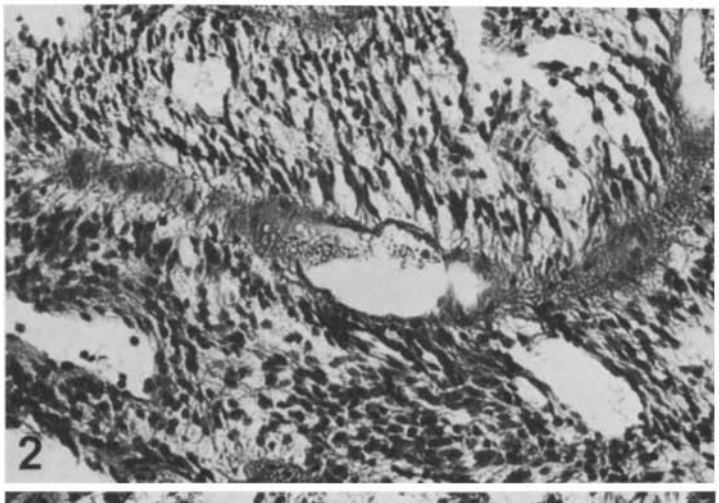
Electron microscopy

The tumor was made of closely-apposed units in each of which, for the sake of clarity, 3 zones will be described (Fig. 4): 1: a densely cellular zone corresponding to the palisades seen by light microscopy. 2: a fibrillary zone intervening between the palisades and the vascular walls. 3: a perivascular zone with corresponding vessels.

The cells making up the palisading layer were homogeneous in appearance. Their bodies were closely approximated and embedded within a feltwork of intermingled slender cytoplasmic processes (Fig. 5). The nuclei were oval with eccentric, usually single, nucleoli. The karyoplasm was fine and only small amounts of heterochromatin were clumped along a double-layered nuclear membrane. A few nuclear pores were seen; nuclear bodies were rare. These cells contained a moderate amount of free ribosomes, a few scattered mitochondria and rare lysosomal bodies. The Golgi apparatus was often prominent and, in parts of the cytoplasm, parallel stacks of rough endoplasmic reticulum could be seen. The cells contained no glycogen and only rare lipid vacuoles.

The fibrillary zone, intervening between the palisade layer and the corresponding vessels, was composed of parallel, slender elongated, cytoplasmic processes emanating from the cell bodies in the palisading zone (Fig. 6). The cell processes extended to reach the neighbouring vessel wall, thus connecting the two structures. Their cytoplasmic profiles were identical to those wedged between the cell bodies within the feltwork of the palisades.





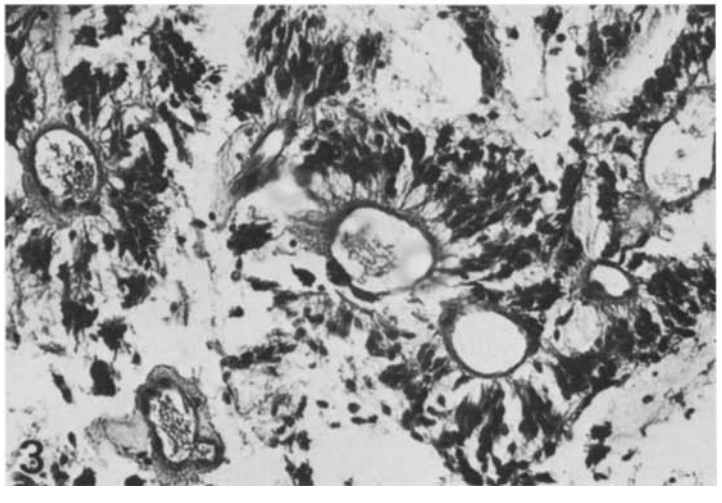


Fig. 1. The classical appearance of a polar spongioblastoma is seen. The calcospherites and the palisading effect are obvious. (HPS, original magnification $\times 125$)

Fig. 2. The same tumor as in Fig. 1 fixed in a special fixative to prevent shrinkage (see methods). It clearly shows a longitudinal cut through a vessel, the polar configuration of the cells and their attachment to the vascular walls. (HPS, original magnification $\times 125$)

Fig. 3. A transverse section through the vascular axes of the tumor. (HPS, original magnification $\times 250$)

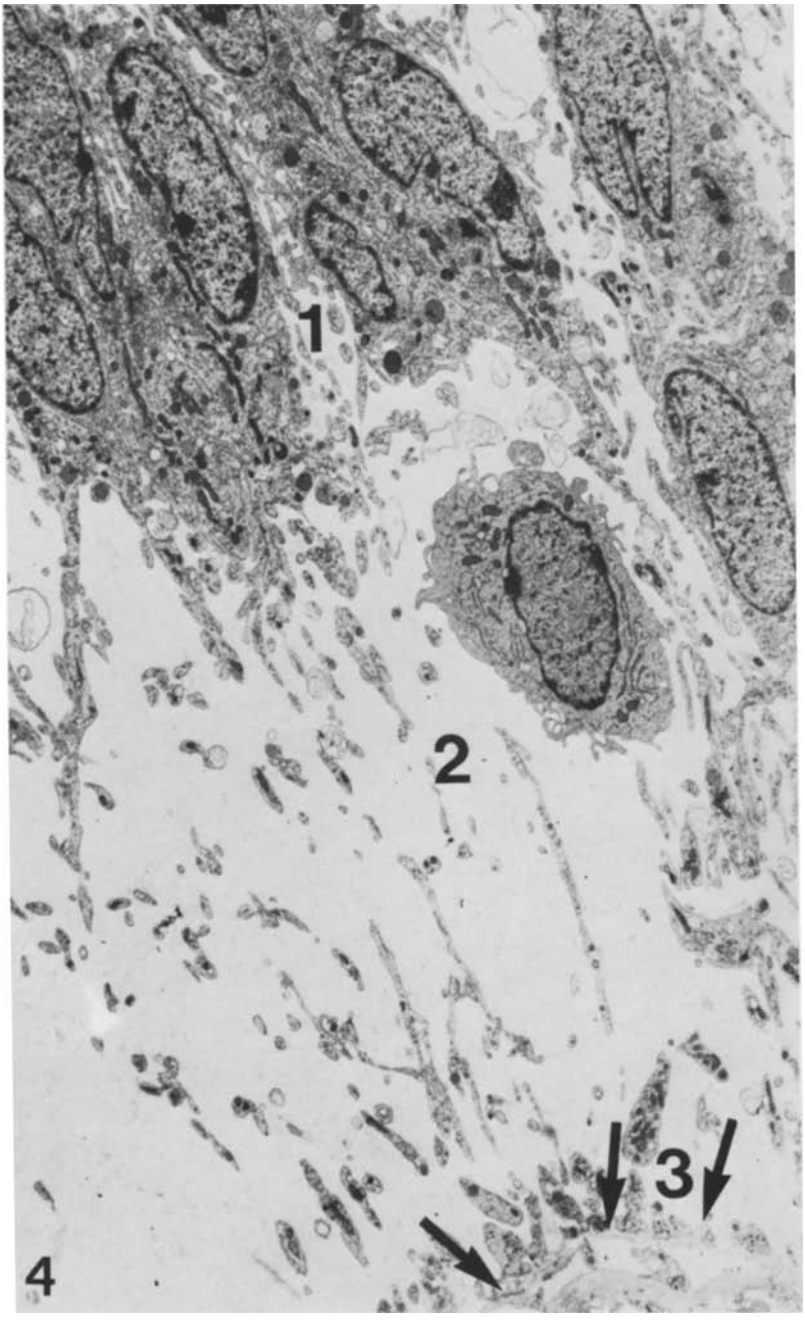


Fig. 4. Overview of the ultrastructural units comprising the tumor: (1) The bodies of the polar cells are seen in the top of the micrograph and represent a palisading row. At the bottom (*arrows*), the vascular zone (3) is indicated. Between the two, one can see the fibrillary zone (2) made of cytoplasmic processes. Compare with Fig. 3. (Original magnification × 2,000)

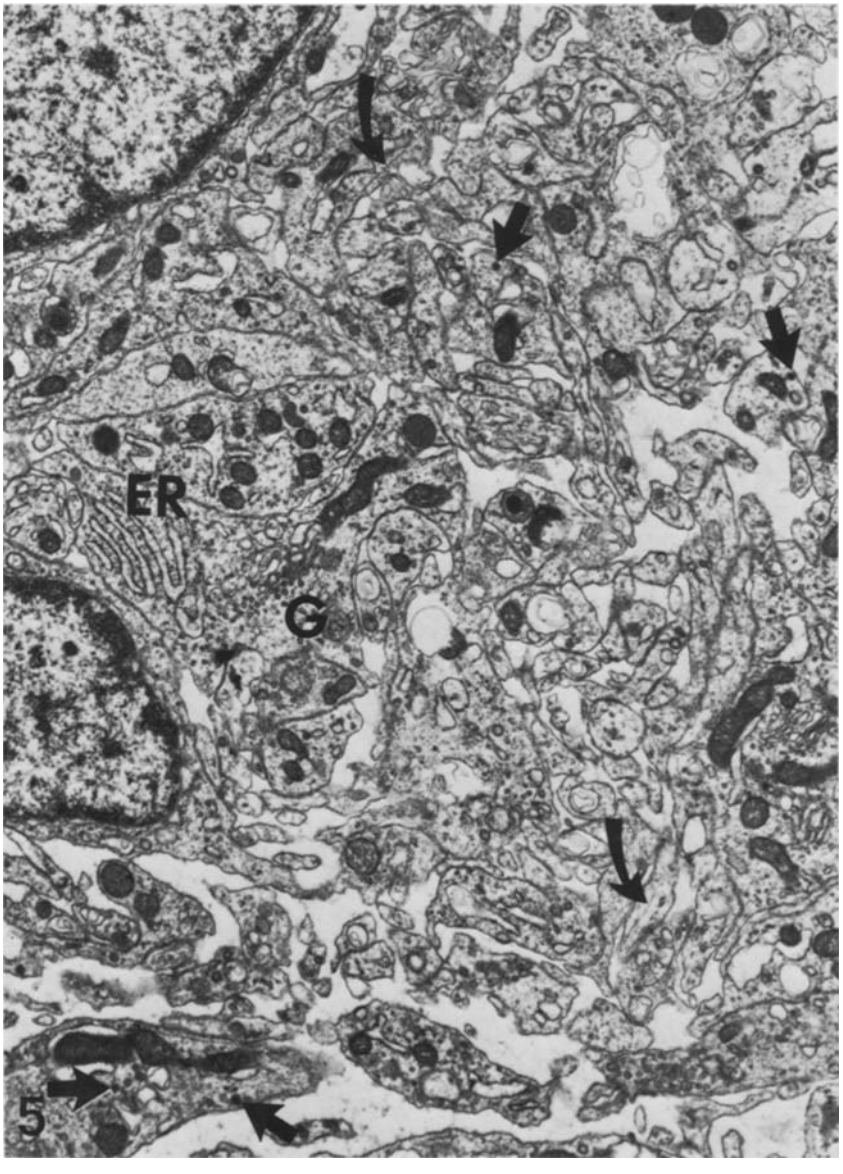


Fig. 5. Detail of the compact zone: cellular bodies within a feltwork of cytoplasmic processes; stacks of rough endoplasmic reticulum (*ER*), mitochondria, microtubules (*long arrow*) and occasional dense-core granules (*short arrows*), prominent Golgi (*G*) and a background of free ribosomes. (Original magnification ×4,500)

They contained a few mitochondria, microtubules oriented parallel to the long axis of the processes and very fine filaments, especially in the more distal portions of the processes which made contact with vascular walls.

Throughout, but more so in the palisading portions, occasional small gap junctions were visible between cell bodies, cell bodies and processes and between processes.

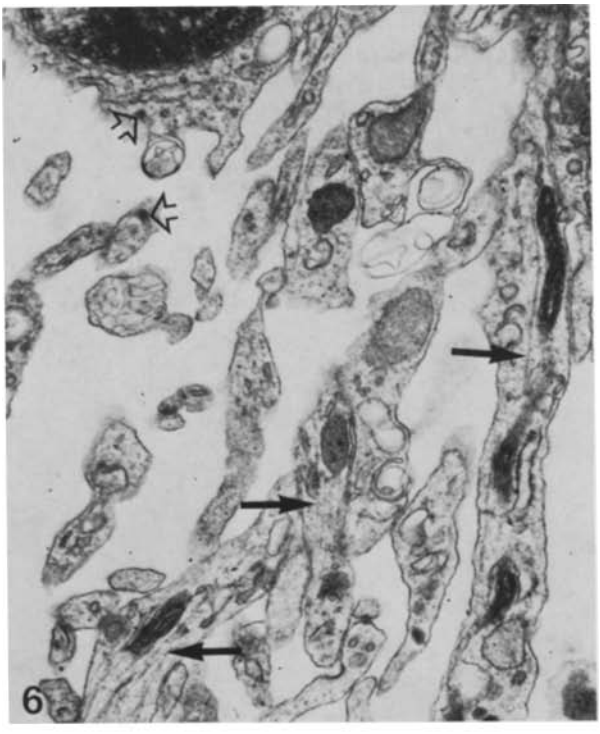


Fig. 6. Detail of the fibrillary zone demonstrating the microtubules running parallel to the long axis of the processes (*arrows*). Some dense-core granules can also be seen (*open arrows*). (Original magnification ×15,000)

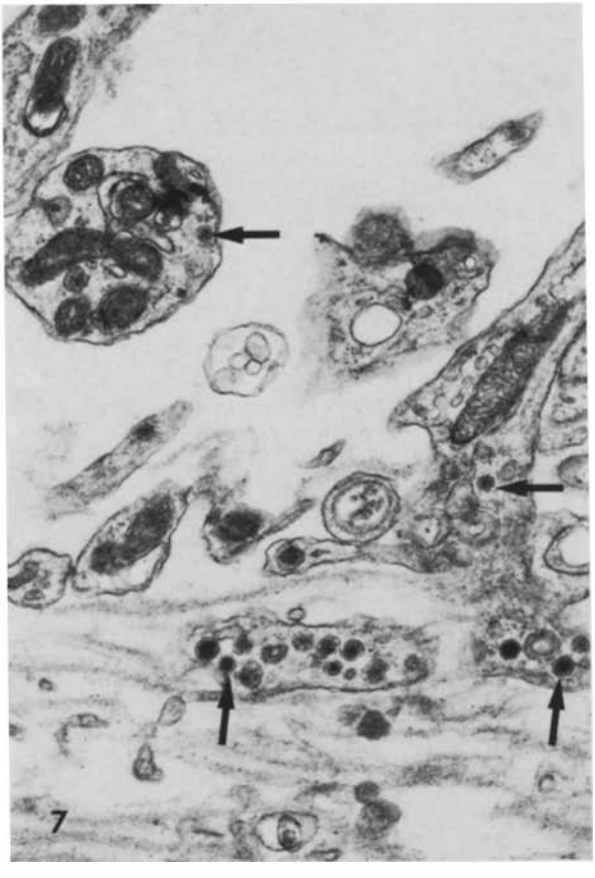


Fig. 7. Detail of the perivascular zone showing the interphase between the cytoplasmic processes and the layered basal lamina. Note the membrane-bound dense-core granules rich cytoplasmic expansions dissecting between the layers of the basal lamina (*arrows*). (Original magnification ×10,000)

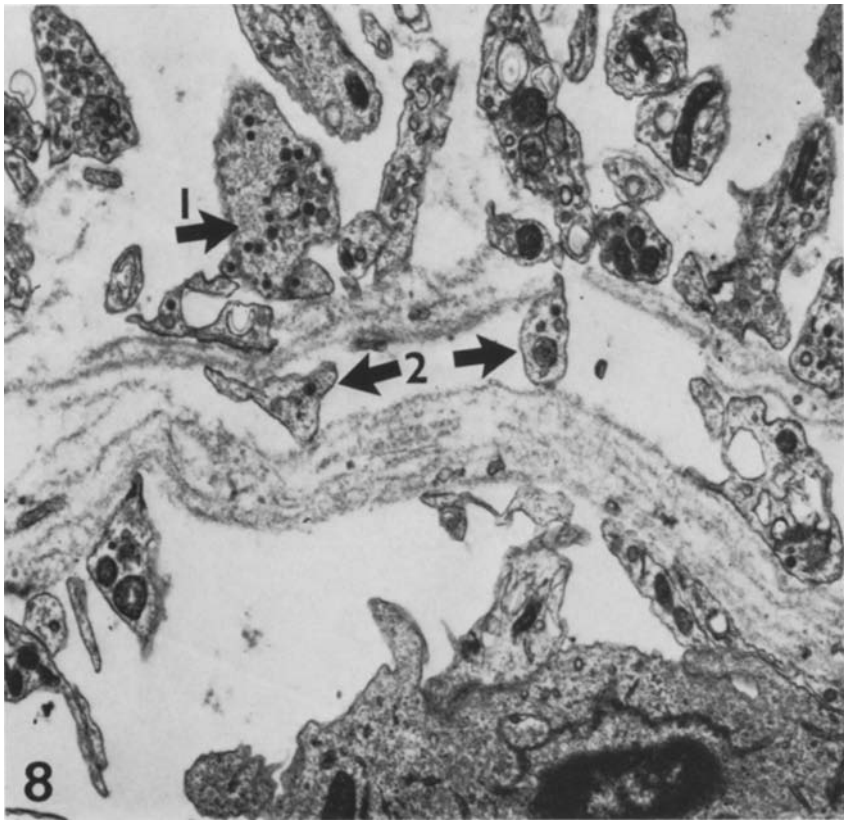


Fig. 8. Another perivascular zone (the same as in Fig. 4) showing cytoplasmic expansions (*arrows*) resting on the outer layer (*arrow 1*) and within the layers of basal lamina (*arrow 2*) (Original magnification × 10,000

At the level of the vessel (Fig. 7) in which a multi-layered basal lamina was seen, the cytoplasmic processes not only abutted on its outer layer, but also seemed to insinuate between the various layers. (Fig. 8)

Membrane-bound granules with an electron-dense core were present throughout the cytoplasmic processes and within the cell bodies. They averaged 100 nm in diameter and were more abundant in the distal segments of the processes near the vessels. (Figs. 5, 6, 7, 8)

Immunocytochemistry

As expected from the ultrastructural study, when the peroxidase-antiperoxidase reagent was added to slides of the tumor previously exposed to rabbit anti-GFAP then to swine anti-rabbit immunoglobulins, no chromogenic reaction was observed. The controls showed definite positivity in the cytoplasmic bodies and processes of a large number of astrocytes both benign reactive and neoplastic.

Discussion

It is tempting to assume that every cell line should have a corresponding neoplasm. However, a true neuro-endocrine hypothalamic benign or malignant neoplasm with features reminiscent of the normal hypothalamic neuroendocrine systems has not been described (Tischler et al. 1977; Peters et al. 1976). The tumors with the closest structure are the rare so-called "hypothalamic hamartomas" (Judge et al. 1977; Hochman et al. 1981). Indeed, some have been demonstrated to be a source of Luteinizing-Hormone-Releasing Factor (LRF) and ultrastructurally, have been shown to be made of unipolar and bipolar cells interspersed among glial cells and to contain membrane-bound electron-dense granules giving a positive fluorescence with LRF antiserum (Judge et al. 1977; Hochman et al. 1981).

The apparent non-existence of specifically hypothalamic neuro-endocrine neoplasms could be explained in two opposing ways. These neoplasms do not develop because the hypothalamic neuro-endocrine cells are, after all, neurones and neurones of neural tube origin are generally not prone to neoplastic transformation. Alternatively, these tumors exist but are extremely rare and/or have not been recognized.

This study has concentrated on a possible candidate which also is the most controversial diencephalic tumor, the "polar spongioblastoma", currently regarded as an astrocytoma (Russell et al. 1971). In the present case, the astrocytic component was minimal. The majority of the cells were spindle-shaped "polar" with long and slender cytoplasmic expansions. The latter were rich in dense core vesicles, contained microtubules, and were negative for GFAP by the PAP technique. In addition, the dense-core vesicle-rich expansions terminated by footplates abutting on the basal laminae of the vessels. There, the granule-bearing footplates, although resting on the basal laminae, also appeared to be insinuating their terminals between its multiple layers. In view of these findings and although the biochemical nature of the granules remains to be determined, one is compelled to interpret the ultrastructural appearance as indicative of a neuroendocrine component, hence a neural, rather than glial, cytogenesis. As far as the relationship that might exist between this tumor and the endocrine abnormalities, only a speculative suggestion can be offered. The usual explanation for the abnormal hormonal levels in patients with the diencepahlic syndrome has not been unanimously accepted. The main reason has been that although the hormonal changes are often stereotyped, the associated tumors circumscribe a spectrum of neoplasms regarded as indistinguishable from their glial counterparts observed elsewhere in the brain. This lead to the conclusion that they were not likely to be actively responsible for the abnormalities. The cause of the disturbances was attributed to the proximity of the tumors to neuro-secretory centers. The present findings suggest that irritation of the neuro-secretory centers may not be the only explanation and that these tumors may have an active secretory role in the pathogenesis of the endocrine disorders.

Since our study is limited to one case, it is impossible to know whether

the present observations constitute the rule or the exception. However, based on data provided in certain case reports and reviews of diencephalic syndrome associated tumors, there are sufficient grounds to suspect the features we have seen may not be exceptional to find. In a review of 67 cases of diencephalic syndrome (Burr et al. 1976), 15% of the tumors were reported as "spongioblastoma, gangioglioma and dysgerminoma with astrocytes". Another 16% were reported simply as gliomas. Knowing how disputed some of these entities are and how mixed the patterns may be, we believe such cases deserve a second look to see whether at least some may not contain a neuro-endocrine component. Regarding the present tumor, we believe it should be referred to with a designator which takes in account its ultrastructural features. In order to preserve the morphological and historical link with the "polar spongioblastoma", we propose to refer to it with the generic name of polar neuroendocrine tumor of the ventral diencephalon.

Prospectively, a biochemical functional study and classification of similar cases should be attempted and the natural history and possible relationship with the "hypothalamic hamartomas" as a precursor lesion should be reviewed. Finally, if the "polar spongioblastoma" associated with neurofibromatosis is found to be a similar neuro-endocrine tumor, it could shed new light on our understanding of neurofibromatosis which might not be, as it is widely perceived today, a disease limited to the neural crest derivatives.

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