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Unusual, Rod-Shaped Cytoplasmic Inclusions (Hirano Bodies) in a Cerebellar Hemangioblastoma *

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Summary. The present study reports unusual, long, tubular, rod-shaped cytoplasmic inclusions found in the stromal cells of a cerebellar hemangioblastoma, associated with von Hippel-Lindau syndrome. These inclusions have two layers of circular, laminated filaments, and longitudinally arranged filaments; and appear similar to the so-called "Hirano bodies", which have been previously found in normal and various pathologic conditions of the brain tissue, skeletal muscle cells in myopathy, and interstitial cells of the testis. A review of previous ultrastructural studies of cerebellar hemangioblastoma shows several different types of cytoplasmic inclusions, but none are found to be identical to the present report.

Key words: Electron Microscopy — Cerebellar Hemangioblastoma — Cytoplasmic Tubular Inclusions.

During the electron microscopic study of a series of cerebellar hemangioblastomas, peculiar, long, rod-shaped, tubular cytoplasmic inclusions of so-called "Hirano bodies" were found in the stromal cells of one of the cases. To the best of our knowledge, these structures have not been previously reported in cerebellar hemangioblastoma. Because of this unusual occurrence, we believe this case worthy of documentation.

Case Report

H. H. is a 39 year old white female who presented to the Medical College of Virginia with a 1—2 months history of headache, ataxia, blurred vision, mental changes, vomiting and weight loss. On physical examination, she had a left homonymous hemianopsia, optic atrophy and gross ataxic cerebellar signs. Angiography showed multiple tumors, at least five. There were three in the posterior fossa, two were in the para third ventricular area. In addition, she was shown to have multiple renal cysts of the left side, as well as retinal angioma in the right eye making a diagnosis of von Hippel-Lindau's syndrome.

She underwent a posterior fossa craniotomy on December 3, 1973 and one of the cystic tumors was removed. Post-operatively she has done well except for a wide base gait.

Materials and Methods

The tissue for light microscopy was processed in the usual manner following fixation in Zenker's solution. For electron microscopy, tissue was obtained immediately following excision and was minced into 1 cu mm blocks. They were fixed in 2% cacodylate buffered glutaraldehyde for 2 hrs, post-fixed in 2% buffered osmic acid for 1 h, dehydrated in alcohol and propylene oxide, and embedded in Epon 812. Thick sections (1—2 μ) were stained with toluidine blue for correlative light microscopy. Ultrathin sections were then cut from

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the selected areas with a diamond knife using an MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi HS 8F2 electron microscope.

Results

Pathologic Findings. The submitted specimen consisted of a membranous, cystic structure lined by soft, brown, hemorrhagic tissue.

Microscopically, there were multiple irregular vascular channels, lined by endothelial cells. Outside the endothelial cells were large, polygonal shaped cells containing abundant acidophilic or clear or vacuolated cytoplasm. The nuclei, not infrequently, demonstrated hyperchromatic, bizarre, and occasionally even a pleomorphic appearance (Fig. 1). Mitosis was not found.

Electron Microscopic Findings. The vascular spaces were lined by endothelial cells with usual appearance and multiple fenestrations. The stromal cells were separated from the endothelial cells and pericytes by a layer of basal lamina and collagen fibrils. The outline of the stromal cells was smooth, except for occasional coarse cytoplasmic processes. The irregular nuclei contained diffusely dispersed chromatin. The cytoplasm was characterized by the presence of abundant lipid droplets, compact microfilaments of 65—85 Å in thickness, and whorls of laminated smooth endoplasmic reticulum (Fig. 2). Additional features included occasional desmosome type junctions (Fig. 2) and focal basal lamina formation.

The most striking finding was the presence of 16—38 long, rod-shaped, tubular cytoplasmic inclusions found in about 20 % of the stromal cells. These structures were divided into two groups and in each group, they were aggregated parallel

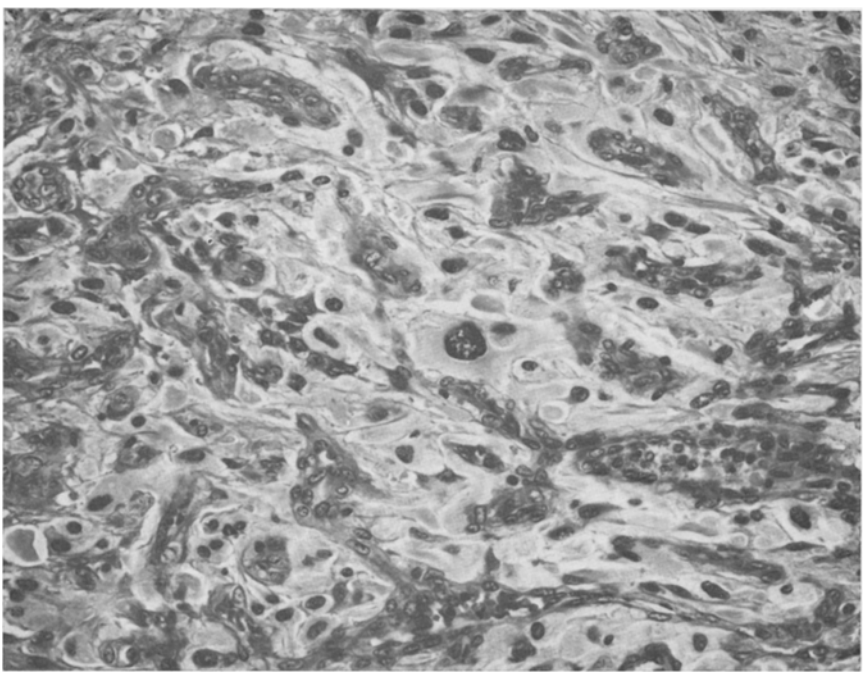


Fig. 1. Photomicrograph of the tumor shows tortuous capillaries which are surrounded by stromal cells. Some of them have atypical, bizarre nuclei. (×250)

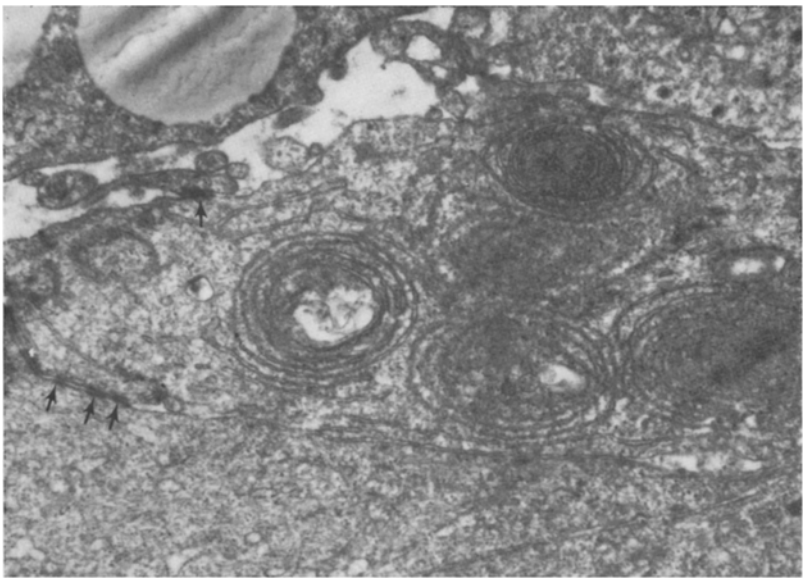


Fig. 2. The presence of multiple whorls of laminated endoplasmic reticulum, usually of the agranular type, is one of the characteristic features of the stromal cells. In addition, several desmosome-like cell junctions are present (arrows). (×12400)

to each other (Fig. 3). The lumen of the inclusions contained cytoplasmic organelles, such as ribosomes and vacuoles (Figs. 3—5). The lumen at both ends sometimes became narrowed or even occluded (Figs. 3, 5).

The individual inclusion was composed of longitudinally arranged filaments which were ensheathed by two laminated, concentric, circular layers of filaments. These longitudinal and circular filaments were perpendicular to each other and had a thickness of 100—130 Å with an average of 115 Å.

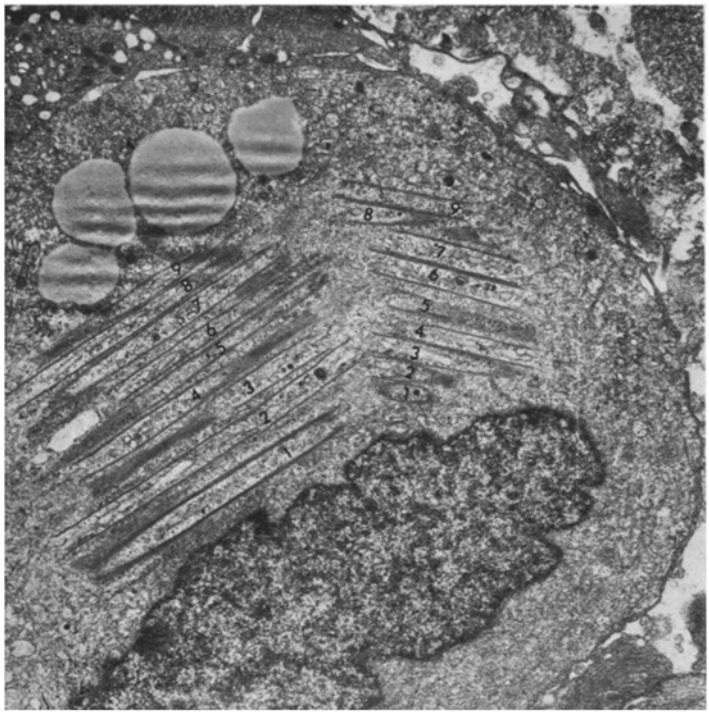
On cross sections, the diameter of the inclusions ranged from 575—660 mμ and showed two concentric, circular layers of filaments. Between these two layers and within the inner layer were closely approximated, longitudinal filaments running parallel to the long axis of the inclusion. These longitudinal filaments appeared as concentric rings of punctate densities on cross sections (Fig. 4).

On the longitudinal sections, the inclusions ranged in length from 1.5 μ to 6.5 μ and consisted of 2—6 layers of longitudinally arranged filaments. This variation in the number of the layers was due to the fact that not all the filaments were always included on the plane of sectioning, but the two circular filaments were always included (Fig. 5).

A criss-cross appearance was particularly common at both ends, where the circular and longitudinal filaments tended to intersect, and this produced a periodicity of 120—150 Å.

Discussion

On the histological examination of this lesion, the possibility of a metastatic renal cell carcinoma is seriously entertained. This is because of the presence of



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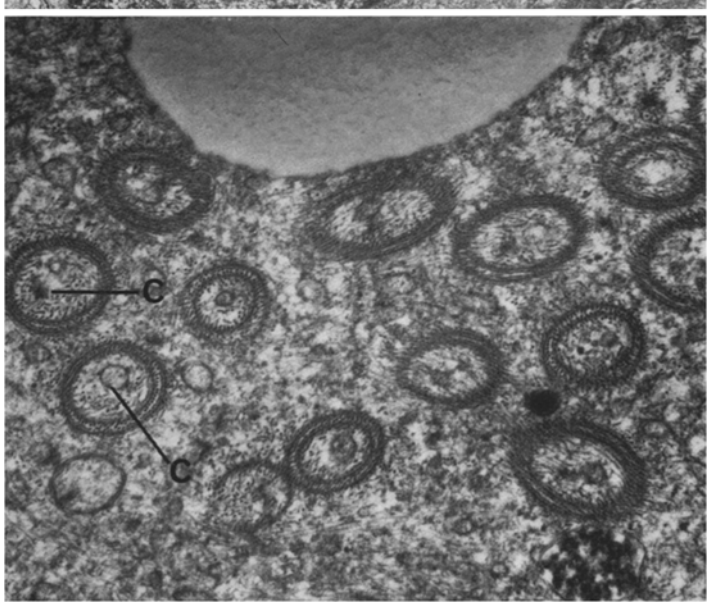

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Fig. 3. In the stromal cell, many parallel arranged tubular inclusions are separated into two groups. There are 9 inclusions in each group. Within the lumen of the inclusions, cytoplasmic organelles are present. At the end of the inclusions, there is a tendency to become narrowed and in some, the lumen is occluded. (×5920)

Fig. 4. On typical cross sections (C) of the inclusions, two circular, laminated layers are apparent. Between the two layers and within the inner layer, are longitudinal filaments which appear as concentric, punctate densities on the cross sections. Other inclusions are cut at different oblique cross sections which result in various, complicated appearance. (×21900)

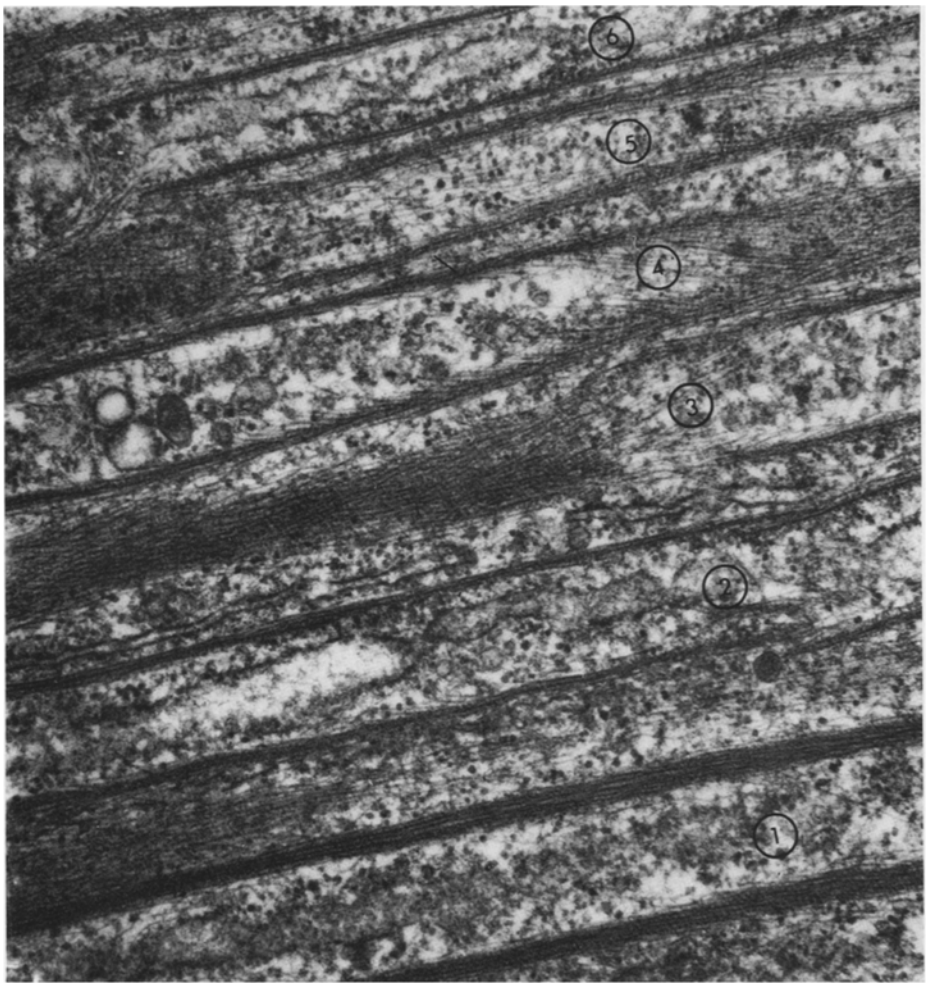

Fig. 5. Higher magnification of longitudinally cut inclusions shown in Fig. 3. They correspond to the inclusions 1–6 in the left side. The number of the layers depends upon whether filaments, which are running longitudinally, are cut on the plane of the sectioning. Inclusions 3 and 4 show narrowed ends with intermingling of the longitudinal and circular filaments, resulting in a lattice pattern. Cytoplasmic organelles are present in the lumens of the inclusions. (× 29180)

a marked nuclear pleomorphism, and because patients with von Hippel-Lindau syndrome may indeed develop renal cell carcinoma (Melmon and Rosen, 1964; Goodbody and Gamlen, 1974). On rare occasions, a differentiation between a cerebellar hemangioblastoma and metastatic renal cell carcinoma, especially those predominantly clear cell lesions, is impossible on the basis of light microscopy (Silver and Hennigar, 1952; Zimmerman, 1963). The electron microscopic findings in our case with abundant lipid droplets, abundant microfilaments, and whorls of laminated smooth endoplasmic reticulum, are essentially in agreement with

previous reports of cerebellar hemangioblastomas (Cancilla and Zimmerman, 1965; Castaigne *et al.*, 1968; Cervos-Navarro, 1971; Kawamura *et al.*, 1973). Our case has again depicted that moderate to severe degree of nuclear atypism may be seen in cerebellar hemangioblastoma (Zimmerman, 1963).

Several types of cytoplasmic inclusions have been described in the cerebellar hemangioblastoma. Cancilla and Zimmerman (1965) reported in the stromal cells aggregates of rods, whorls and spirals with a characteristic radial periodicity of 60 Å. Similar structures have also been observed by Castaigne *et al.* (1971), but who considered them to be granules of the mast cells.

In a case of multiple, supratentorial hemangioblastomas, Ishwar *et al.* (1971) have found the presence of electron-dense, membrane bound granules with a diameter ranging from 1200 Å to 2000 Å. They regarded these granules as evidence of erythropoietin formation, even though their patient was not associated with erythrocythemia.

In the endothelial cells of a cerebellar hemangioblastoma, Kawamura *et al.* (1973) observed tubular intracytoplasmic bodies appearing as electron-dense, membrane-limited cylindrical rods.

The rod-shaped, concentric, laminated and filamentous inclusions described in this study are similar to "Hirano bodies", originally described in amyotrophic lateral sclerosis-Parkinsonism dementia complex (Hirano, 1965, 1966). Subsequently, these structures have been found in normal and various pathologic conditions of the brain, including dementia, degenerative, metabolic, vascular, traumatic, infectious, and neoplastic diseases (Hirano, 1935, 1966; Toga *et al.*, 1971; Ogata *et al.*, 1972; Schochet and McCormick, 1972; Anzil *et al.*, 1974; and Hadfield *et al.*, 1974). More recently, similar structures are found in the myopathic muscle cells (Fisher *et al.*, 1972; Afifi *et al.*, 1974), and testicular interstitial cells of the dog (Setoguti *et al.*, 1974). The significance and function of these inclusions are not fully understood. That these inclusions probably represent a non-specific, cytoplasmic manifestation is supported by the presence of these structures in diverse conditions and in several different tissues (Schochet and McCormick, 1972; Ogata *et al.*, 1972).

References

Afifi, A. K., Derkaloustian, V. M., Bahuth, N. B., Mire-Salman, J.: Concentrically laminated membranous inclusions in myofibres of Dyggve-Melchior-Clausen syndrome. J. neurol. Sci. **21**, 335—340 (1974)

Anzil, A. P., Herrlinger, H., Blinzinger, K., Heldrich, A.: Ultrastructure of brain and nerve biopsy tissue in Wilson disease. Arch. Neurol. (Chic.) **31**, 94—100 (1974)

Cancilla, P. A., Zimmerman, H. M.: The fine structure of a cerebellar hemangioblastoma. J. Neuropath. exp. Neurol. **24**, 621—628 (1965)

Castaigne, P., David, M., Pertuiset, B., Escourolle, R., Poirier, J.: L'Ultrastructure des hemangioblastomes du système nerveux central. Rev. neurol. **118**, 5—26 (1968)

Cervos-Navarro, J.: Elektromikroskopie der Hemangioblastome des ZNS und der angioblastischen Meningiome. Acta neuropath. (Berl.) **19**, 184—207 (1971)

Fisher, E. R., Gonzalez, A. R., Khurana, R. C., Danowski, T. S.: Unique, concentrically laminated, membranous inclusions in myofibers. Amer. J. clin. Path. **58**, 239—244 (1972)

Goodbody, R. A., Gamlen, T. R.: Cerebellar hemangioblastoma and genitourinary tumors. J. Neurol. Neurosurg. Psychiat. **37**, 606—609 (1974)

Hadfield, M. G., Martinez, A. J., Gilmartin, R. C.: Progressive multifocal leukoencephalopathy with paramyxovirus-like structures, Hirano bodies and neurofibrillary tangles. Acta neuropath. (Berl.) **27**, 277—288 (1974)

Hirano, A.: Pathology of amyotrophic lateral sclerosis. In: Slow latent and temperate virus infections, NINDB monograph., No. 2, pp. 23—37, D. C. Gajdusek and C. L. Gibbs (eds.). Bethesda: National Institute of Health 1965

Hirano, A.: Neuropathology of amyotrophic lateral sclerosis and Parkinsonism-dementia complex on Guam. In: Proceedings of the fifth International Congress of Neuropathology. International Congress of Neuropathology Series No. 100, pp. 190—194. A. Bischoff and F. Lüthy (eds.). Amsterdam: Excerpta Medica Foundation 1966

Ishwar, S., Taniguchi, R. M., Vogel, F. S.: Multiple supratentorial hemangioblastomas. Case study and ultrastructural characteristics. J. Neurosurg. **35**, 396—405 (1971)

Kawamura, J., Garcia, J. H., Kamijyo, Y.: Cerebellar hemangioblastoma: Histogenesis of stromal cells. Cancer **31**, 1528—1540 (1973)

Melmon, K. L., Rosner, S. W.: Lindau's disease: review of the literature and study of a large kindred. Amer. J. Med. **36**, 595—617 (1964)

Ogata, J., Budziolovich, G. N., Cravioto, H.: A study of rod-like structures (Hirano bodies) in 240 normal and pathological brains. Acta neuropath. (Berl.) **21**, 61—67 (1972)

Schochet, S. S., Jr., McCormick, W. F.: Ultrastructure of Hirano bodies. Acta neuropath. (Berl.) **21**, 50—60 (1972)

Setoguti, T., Esumi, H., Shimizu, T.: Specific organization of intracytoplasmic filaments in the dog testicular interstitial cell. Cell Tiss. Res. **148**, 493—497 (1974)

Silver, M. L., Hennigar, G.: Cerebellar hemangioma (hemangioblastoma). A clinicopathological review of 40 cases. J. Neurosurg. **9**, 484—494 (1952)

Toga, M., Berard-Badier, M., Gambarelli, D., Pinsard, N., Hassoun, J.: Un cas de dystrophie neuroaxonale infantile ou maladie de Seitelberger. III. Etude ultrastructurale du muscle strié. Acta neuropath. (Berl.) **18**, 327—341 (1971)

Zimmerman, H. M.: Vascular tumors of the brain. Clin. Neurosurg. **9**, 245—276 (1963)

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