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Some Contributions of Electron Microscopy to the Diagnosis of Brain Tumors

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Summary. Selected examples of neoplasms of the central nervous system are reviewed in which the electron microscope has been instrumental in establishing the diagnosis. These include ependymoma, ependymoblastoma, epithelial cyst, paraganglioma in the cauda equina and cerebellar neuroblastoma.

Key words: Ependymoma – Ependymoblastoma – Epithelial cysts – Paraganglioma – Cerebellar neuroblastoma.

The diagnosis of a brain tumor is generally reached in stages. It requires the cooperation of the clinician, the radiologist and the neurosurgeon. However, it is up to the neuropathologist to supply the final diagnosis based on all the information received and his own microscopic analysis. Even with this substantial armamentarium at his disposal certain cases can resist analysis leaving a measure of doubt. Among these cases the electron microscope can sometimes be useful in establishing a final diagnosis (Poon et al., 1971, 1978; Toga et al., 1976; Russell and Rubinstein, 1977).

It is the purpose of this communication to present selected examples of diagnostic problems which arose in our laboratory and in which we found the electron microscope useful.

Ependymomas and Related Neoplasms

Among the various cells of the central nervous system the ependymal cell is probably the most easily recognizable on the basis of both its location and its cytological characteristics (Hirano et al., 1975; Peters et al., 1976). The ependyma is arranged in a single layer lining the ventricles. The cells are cuboidal in shape and display three distinct kinds of surfaces. There is the luminal surface characterized by cilia and microvilli, the lateral surface which shows well developed junctional devices and the basal surface which is free of any surface specialization and abuts the neuropil with no intervening basal lamina.

In most ependymomas the ependymal cells lose their precise alignment and form cellular masses although the presence of ependymal rosettes in addition to vascular rosettes provide diagnostic markers. Electron microscopic study of the solid masses reveals characteristic details of the cells although they may be severely distorted in the neoplastic process (Hirano et al., 1975). These consist of junctional complexes as well as cilia and microvilli; the latter usually aggregated in a confined space (Fig. 1).

On the other hand, some ependymoma-like tumors are not as easily classifiable. In a case of a large mid-line tumor in a three-year-old girl, the light microscope showed many compactly arranged cells strongly reminiscent of a medulloblastoma (Hirano et al., 1973). At the same time occasional ependymal rosettes were also seen. The electron microscopic findings of the solid portions of the tumor were not as clear-cut as might be wished for. Neither cilia nor microvilli could be found. On the other hand, the cells were sometimes arranged in "mini"-rosettes around a minute lumen devoid of cilia or microvilli. The telltale feature, however, consisted of well developed junctional specializations between the cells near the tiny lumen. In addition, one or two basal bodies oriented toward the lumen were seen in each of the cells facing the rosette (Fig. 2). Thus, certain features of the tumor were indeed ependymal-like, but the characteristics were not those of most ependymomas. Could it be that the lack of cilia and microvilli, etc. were due to immaturity? To test this possibility we examined the neural tube of an eight-day-old chick embryo. Indeed, similar features were seen. Instead of small cylindical lumens the ventricular lumen in the chick were broad. Otherwise, the cytologi-

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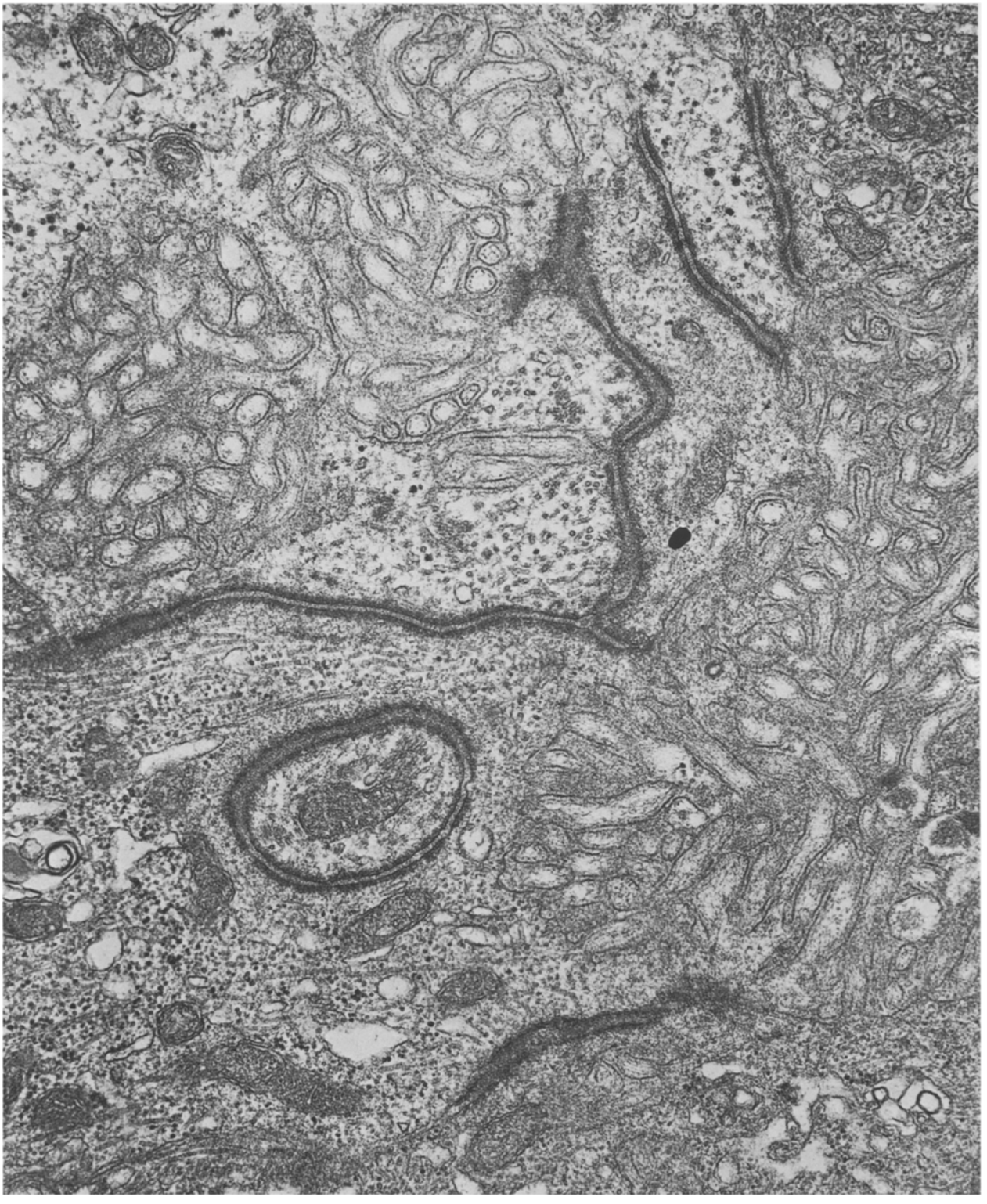

Fig. 1. An ependymoma showing the closely packed microvilli and well developed junctional apparatus. × 30 000

cal details of the cell apices were essentially identical. No microvilli or cilia had yet been formed but the junctional devices between adjacent cells were well developed and one or two basal bodies were seen in each cell. The diagnosis, then, in this case was ependymoblastoma, i.e., an immature ependymoma. The diagnosis could be confirmed when the patient died seven months after surgery and the tumor had spread along

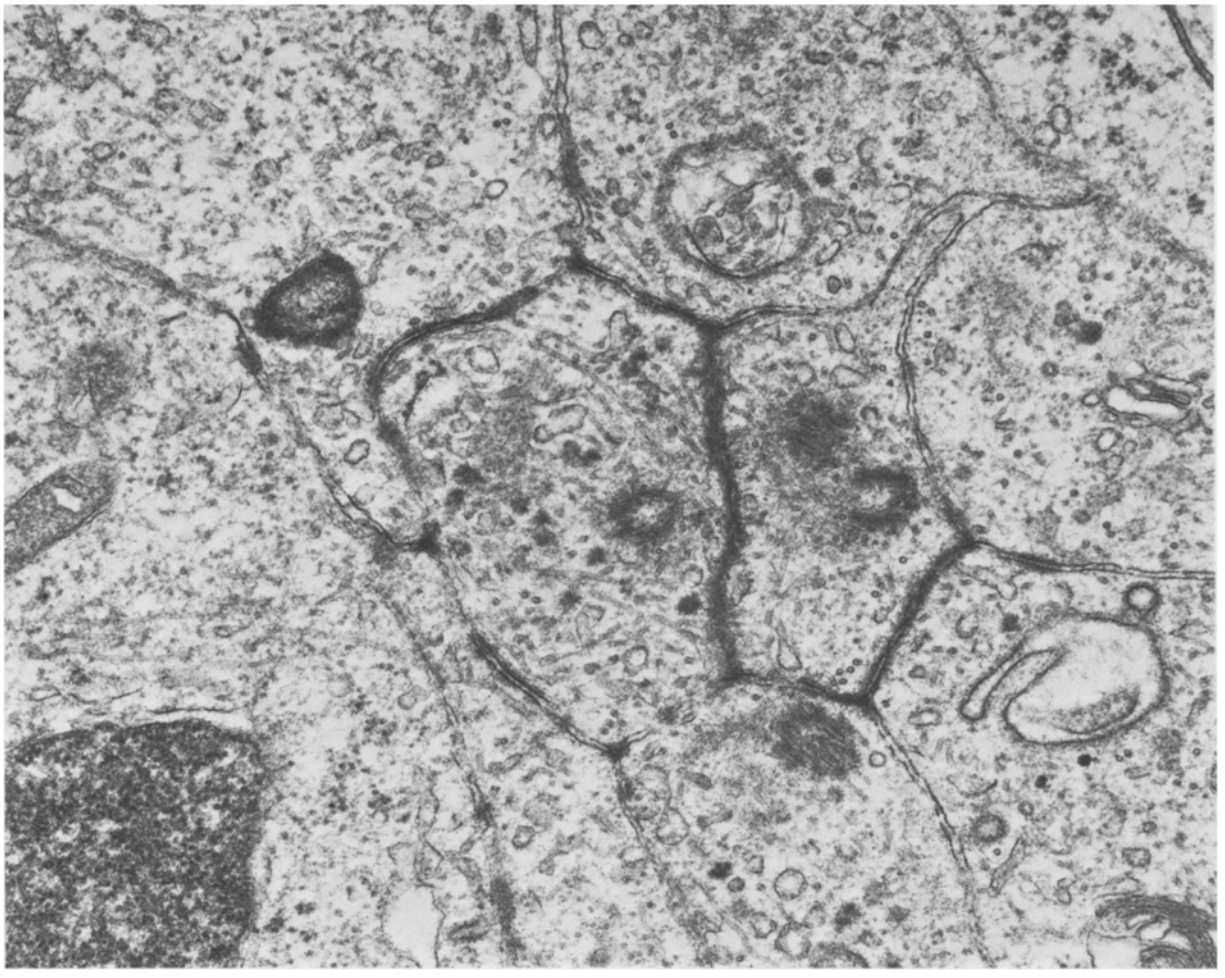

Fig. 2. An ependymoblastoma showing one or two basal bodies in several cells and the well developed junctional apparatus. Mirovilli or cilia are usually absent. × 30 000

the entire neuraxis showing the characteristic light microscopic features of an ependymoma in many areas.

The electron microscope has also served to differentiate a cyst which was thought to be of ependymal origin from one which was apparently derived from the endoderm. A 3-cm, intradural extramedullary cyst, adhering to the cord, was discovered at surgery of a 44-year-old man who had complained of back pain. The light microscope revealed a single layer of ciliated cuboidal cells lining the cyst and resting on a connective tissue stroma. The presence of cilia immediately brought to mind the possibility of an ependymal origin for the lesion (Wisoff et al., 1971). The presence of connective tissue, however, made the cyst unusual so we decided to examine its fine structure. The electron microscope confirmed the presence of cilia but also revealed certain features quite unlike ependymal cells (Hirano et al., 1971). First, two types of cells were present. In addition to ciliated cells, other, nonciliated cells with microvilli on their surface and containing secretory granules were also present. The apical surfaces of the secretory cells, including the microvilli, were coated with a fine granular-fibrillar material which is not present in the microvilli or cilia of normal ependyma (Fig. 3). Second, a basal lamina was uniformly present between the cuboidal cells and the connective tissues. In normal ependyma, except for the surface of blood vessels (Hirano and Zimmerman, 1967) and in part of the lamina terminalis, a basal lamina is absent. Other differences relating to the cell junctions and cytoplasmic contents were also noted. The fine structure of the cyst was therefore quite unlike anything seen in the normal central nervous system, and, despite the presence of cilia, not at all like ependyma. Instead, it was essentially identical to the epithelium of the upper respiratory tract, specifically, the trachea. Our own examination of the trachea of the hamster confirmed this fact. We therefore concluded that rather than an ependymal cyst, the lesion in question was an epithelial cyst of endodermal origin. Such

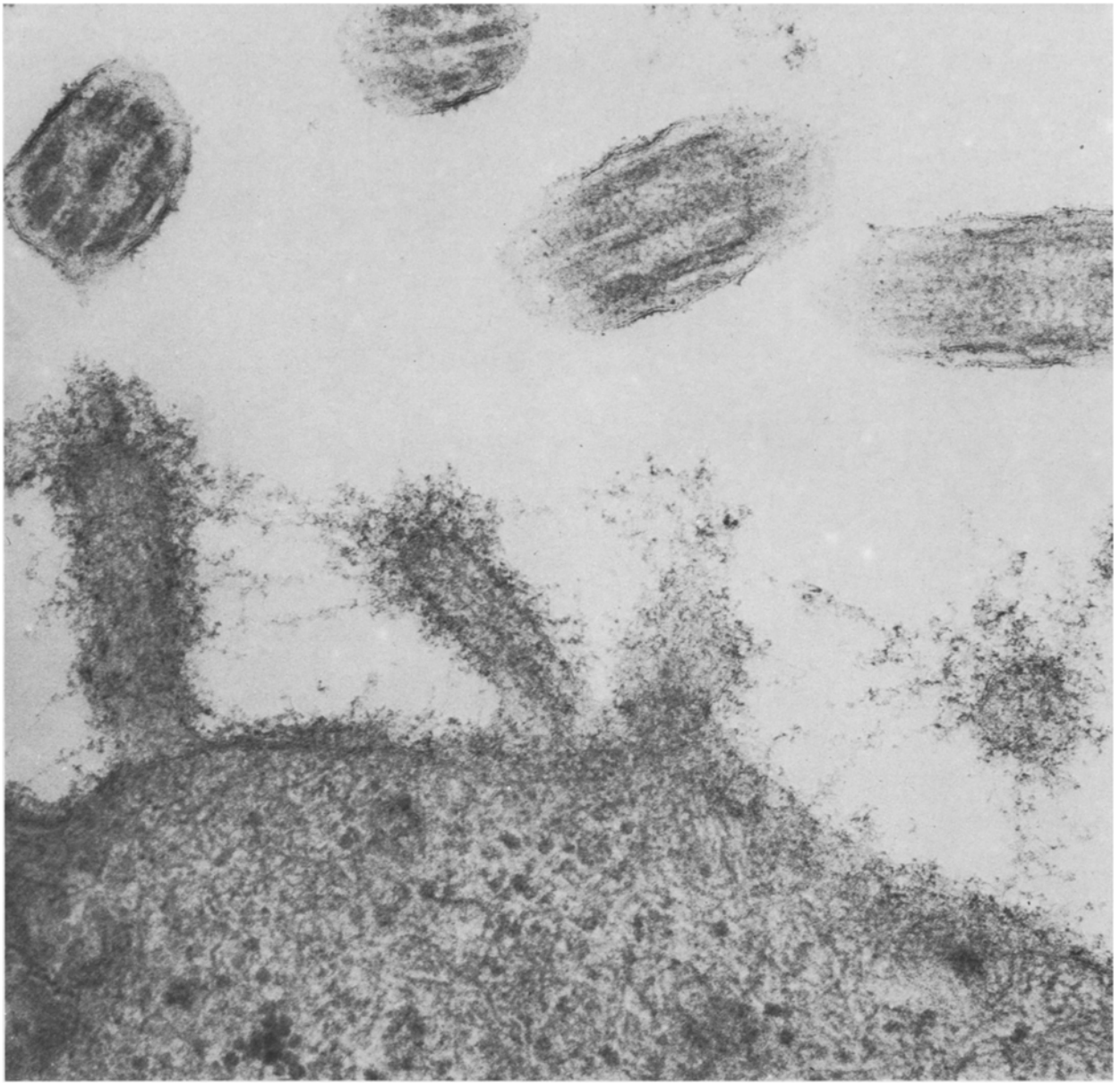

Fig. 3. The internal surface of an epithelial cyst. Microvilli and the surface of the nonciliated cell is coated with a fine fibrillar-granular material. The ciliary surfaces are smooth. ×96000

enterogenic cysts are well known to occur along the neuraxis of the central nervous system (Russell and Rubinstein, 1977; Leech and Olafson, 1977).

Similar fine structures were encountered in colloid cysts of the third ventricle (Hirano and Ghatak, 1974; McKeever and Brissie, 1977). Again, two types of cells, ciliated and nonciliated, were evident and a basal lamina was present among other distinctive features. Recently, in colloid cysts, Ghatak and his associates described a third type of wedge-shaped cell which is apparently the immature precursor of the ciliated and non-ciliated epithelial cell (Ghatak et al., 1977). Similar cells were also present in the epithelial cyst of the cauda equina described above and are normal features of the tracheal epithelium. On these bases one must then begin to

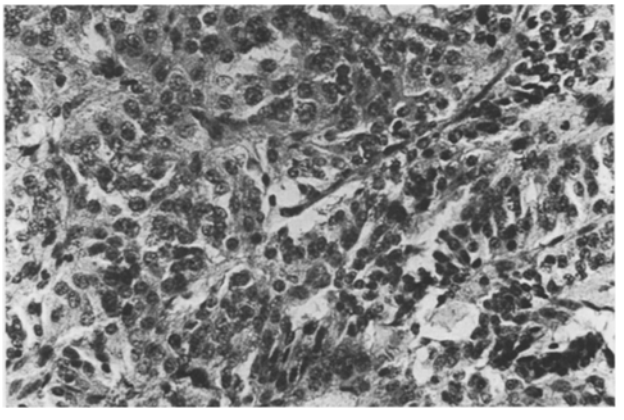

Fig. 4A. Light micrograph of a paraganglioma in the caudal region. ×195

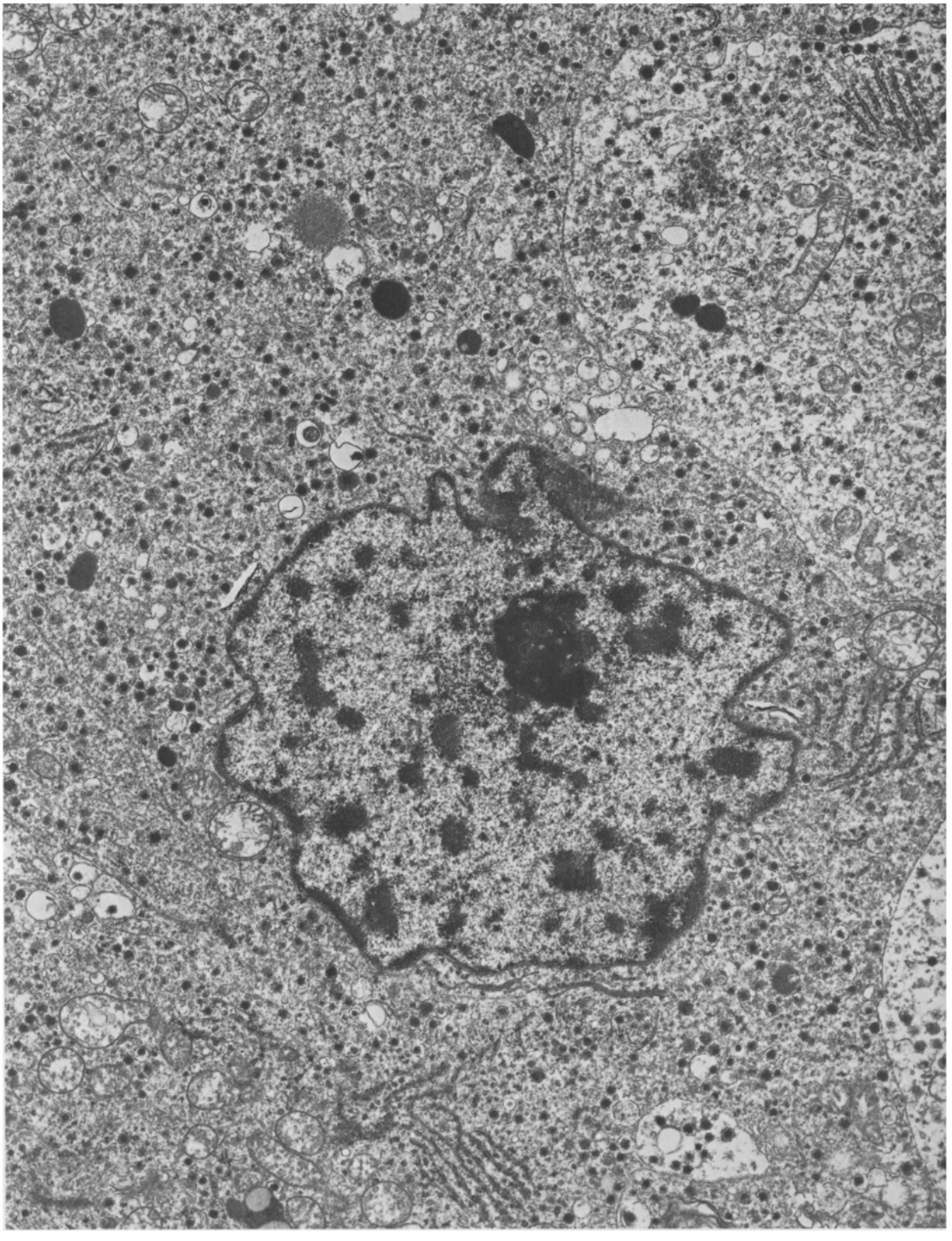

Fig. 4B. Tumor cells in a paragangioma. Numerous dense core vesicles are visible. × 12000

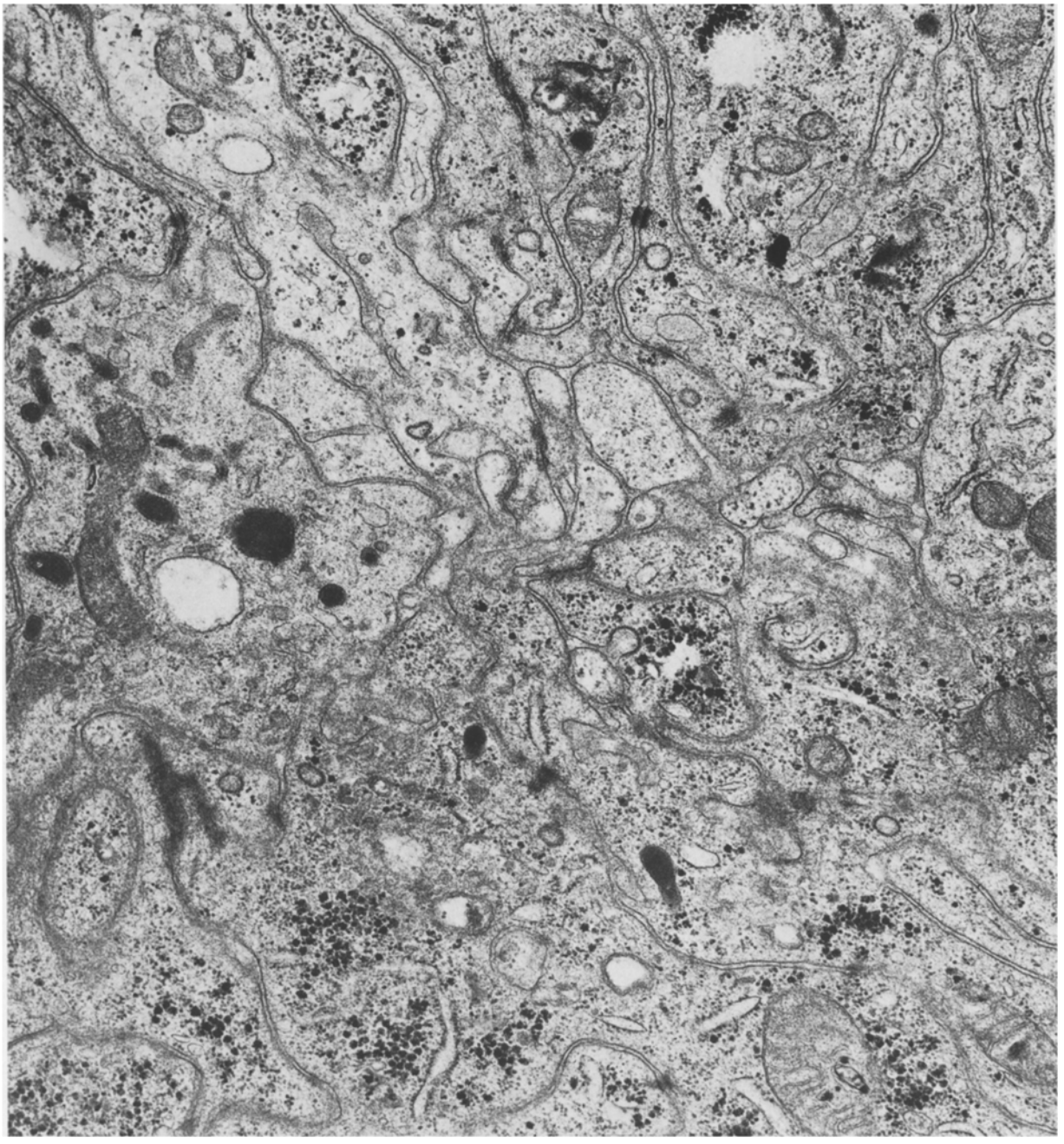

Fig. 5. A meningioma showing the elaborate interdigitation of the closely packed cell processes often joined by desmosomes. × 30 000

question the presumed neurogenic origin of colloid cysts.

Tumors Containing Dense Core Granules

In addition to revealing specific surface specializations, the electron microscope can often help in the diagnosis of differentiated tumors by allowing us greater insight into the nature of various cytoplasmic inclusions. A large, sausage-shaped, 8-cm-long solid tumor was removed from the cauda equina of a 41-year old man who had a 6-month history of cauda equina syndrome. The clinical impression had been one of Schwannoma, hemangioma, meningioma or ependymoma. However, the tumor turned out to be intradural and extramedullary and unattached to either the dura mater or spinal cord unlike meningioma or ependymoma. Light microscopic study revealed a highly vascularized tumor with cuboidal tumor cells compactly arranged in nests intercalated into the vascular network (Fig. 4A). The

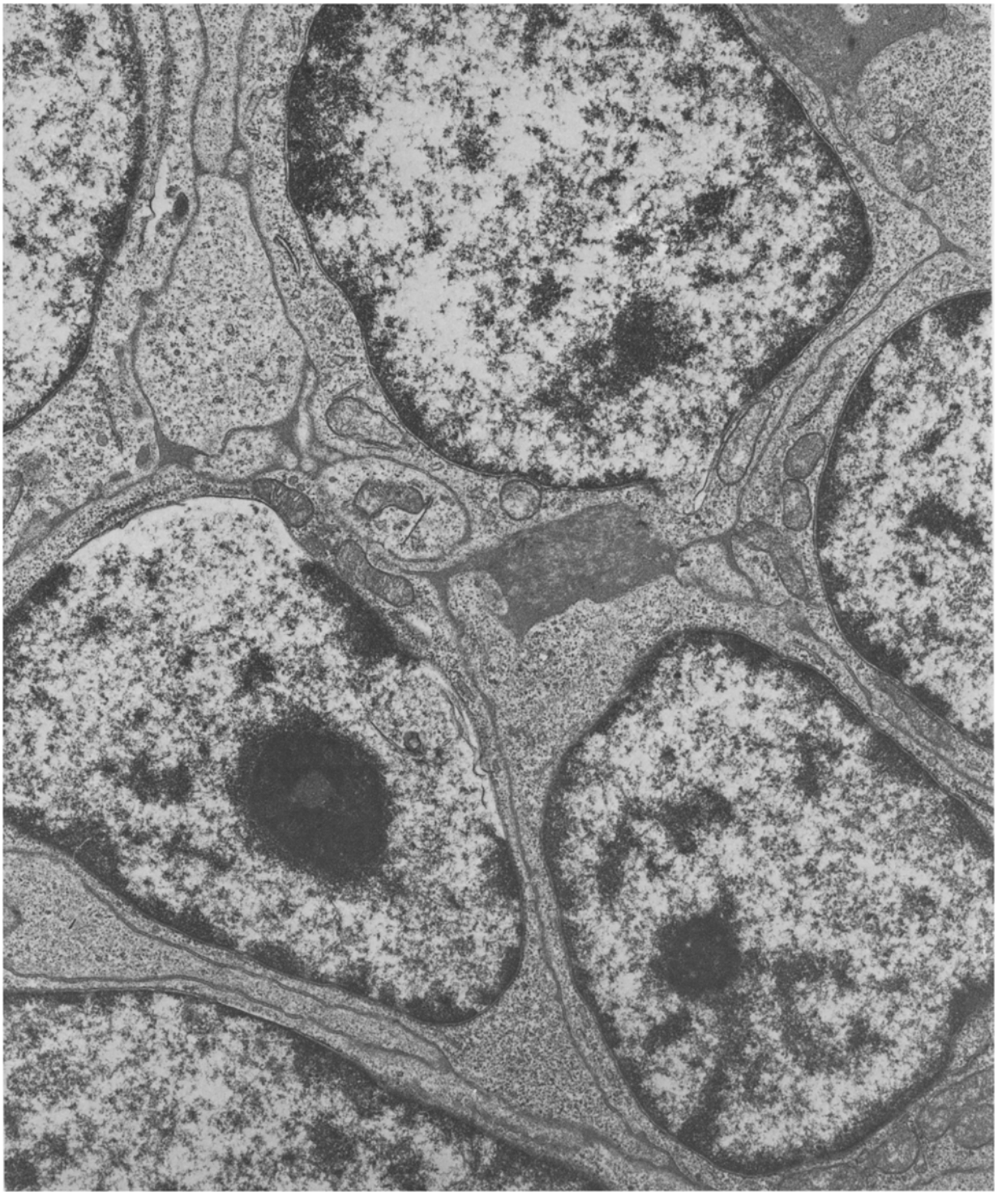

Fig. 6. A primary lymphoma in the central nervous system. The closely packed cells are free of junctional devices. × 15000

histology was unlike any of the tumors suspected on clinical grounds and the diagnosis remained uncertain.

The electron microscope revealed two significant features. The tumor cells contained numerous, small, dense core vesicles (Fig. 4B) and the blood vessels were fenestrated. The fine structure was, therefore, consistent with a diagnosis of paraganglioma. The same features have been described in paragangliomas outside the central nervous system (Reyes et al., 1977) and, on rare occasions, even in the cauda equina

(Lerman et al., 1972; Horoupian et al., 1974; Russel and Rubinstein, 1977).

Hematogenous Tumors

In addition to serving as a useful diagnostic method, fine structural studies of brain tumors, has, on occasion, allowed us greater insight into significant morphological features of both normal and neoplastic tissue which may at first be inconspicuous in the light microscope. For example, meningiomas are common tumors of the central nervous system and have been extensively studied with both the light and electron microscope for many years. The fine structural feature we wish to emphasize here is the extraordinary elaboration of cell processes which they display (Poon et al., 1971; Toga et al., 1976). When viewed in thin sections these processes appear elongated rather than circular implying a sheet-like, rather than cylindrical, shape of the process (Fig. 5). This is not surprising in view of the function of the normal meningeal cell which is to serve as a covering or wrapping of the outer surface of the central nervous system. In keeping with this function also, is the presence of cell junctions between both normal and neoplastic meningeal cells. Indeed, because of the compact arrangement of the cells within the tumors the junctions are even more conspicuous. The tendency of the cell to form sheet-like processes is reflected in the whorl-like formations seen in meningiomas. The adjacent lamellae of the whorls are knit together by numerous cell junctions.

In distinct contrast to tumors such as meningiomas are the myeloid or lymphoid tumors which sometimes involve the central nervous system as the first clinical manifestation. A large, 5-cm, tumor involving the frontal dura mater was removed from a 37-year old male butcher with only a one month history of increased intracranial pressure (Llena et al., 1977). Despite the short clinical course and an eosinophilia of 20% the tumor was considered a probable meningioma on both clinical and radiological grounds as well as gross examination. Histologically, however, the diagnosis of granulocytic sarcoma was made. The tissue closely resembled neoplastic bone marrow with very young blastic cells and nucleated red cells. Other synonyms for this same lesion are myeloblastoma or chloroma. Electron microscopically, in addition to complicated cytoplasmic features the cells were characterized by the marked paucity of cell processes and the absence of cell junctions. Unlike the cells of meningiomas or gliomas, the cells of the granulocytic sarcoma are not fixed and they show the morphological characteristics associated with that trait. Seven months after surgery the patient died with myeloblastic leukemia.

Similar findings were made in a fine structural study of primary lymphoma of the central nervous system. A temporal lobe tumor was removed from a 44-year old patient with a 5-week clinical history of an expanding intracranial mass (Hirano et al., 1974). Unlike the previous case, granulocytes and red blood cells were absent but otherwise the essential features, i.e., a lack of cell processes and cell junctions, were also characteristic of the immature lymphocytes (Fig. 6).

Other small cell tumors of the central nervous system such as ependymomas, ependymoblastomas, medulloblastomas, and certain undifferentiated metastatic carcinomas in some ways superficially resemble the lymphomas. These, however, are characterized by their tendency toward junction formation (Hirano, 1975).

Neuroblastoma

The cerebellum is a relatively common site for various neoplasms. Among these are a number of neoplasms whose cell of origin is obscure due to the lack of specific features visible in the optical microscope. A large, lobulated, solid, midline cerebellar tumor was removed from the roof of the fourth ventricle from an 18-month old boy with hydrocephalus (Shin et al., 1978). In the light microscope the tumor appeared lobulated and the tumor cells were closely packed, often in rows separated by an acellular fibrillary material (Fig. 7A). The lobules were separated by reticulin-rich leptomeningeal infoldings which were heavily infiltrated by the tumor cells. The diagnosis varied depending on the neuropathologist.

The matter was resolved by use of the electron microscope. The small cells showed parallel, cylindical processes which were compactly arranged. Unlike medulloblastoma the processes contained small, clear vesicles (Fig. 7B) and, while uncommon, post-synaptic

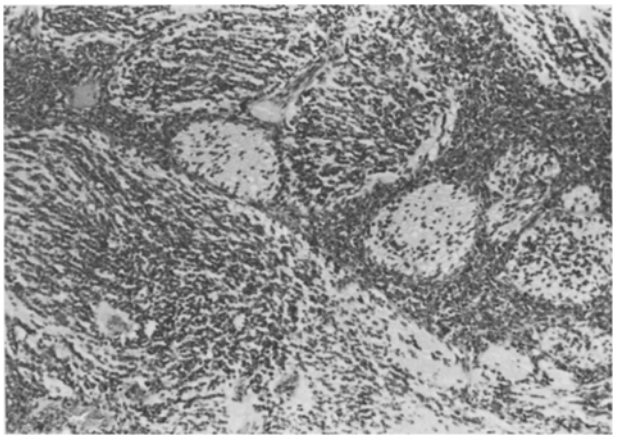

Fig. 7A. Light micrograph of a cerebellar neuroblastoma. × 95

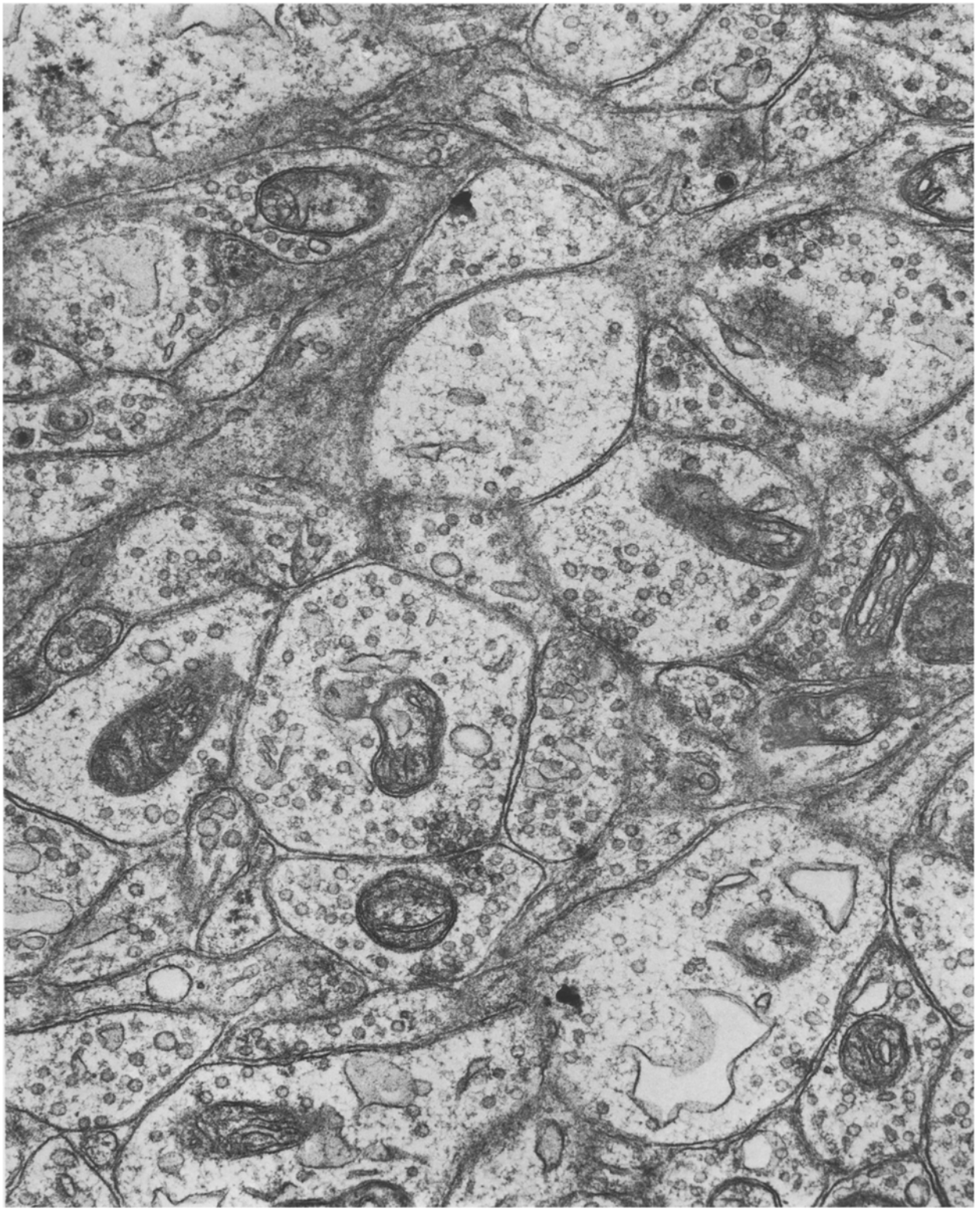

Fig. 7B. Closely packed, circular cell processes in a cerebellar neuroblastoma. Numerous clear synaptic vesicles may be seen in almost all the processes. Some vesicles show peripheral aggregates without a corresponding postsynaptic density. × 36 000

elements were sometimes present completing occasional synapses. These findings permitted a fairly straightforward diagnosis of cerebellar neuroblastoma similar to that reported by Ermel and Brucher in 1974.

In addition to the diagnosis that was made possible the fine structural findings also led to some interesting insights regarding synaptogenesis in the cerebellum. Based on their size and morphology the tumor cells were most likely derived from a granule cell precursor. These cells are the major afferent of the Purkinje cells and, under normal conditions, develop presynaptic terminals in association with the Purkinje cell dendritic spine. In the present instance, however, neither Purkinje cells nor spines were present within the tumor tissue. The occasional postsynaptic elements seen were dendritic shafts or cell somas and not spines. Thus it seems that under neoplastic conditions, at least, structures entirely similar to the presynaptic elements of normal tissue can develop without benefit of the immediate influence of a dendritic spine on a one-to-one basis. This conclusion is keeping with similar findings seen in the young "staggerer" mouse as reported by Sotelo in 1973. In this murine mutant the Purkinje cells are apparently unable to form dendritic spines at the proper age but the granule cells go on to form the presynaptic specialization despite the absence of their normal postsynaptic mates. Eventually, the granule cells degenerate.

Conclusion

In the present report we have presented a number of cases of neoplasm of the central nervous system in which the diagnosis on the basis of clinical and light microscopic grounds was uncertain and in which the electron microscope proved useful in resolving the issue. It is not our intention to imply that the electron microscope is required for the diagnosis of the great majority of brain tumors. However, its use should not be disregarded in selected cases and the value of the fine structural studies can sometimes be valuable from the point of view of the underlying biological processes.

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