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The Big Eye in the 21st Century: The Role of Electron Microscopy in Modern Diagnostic Neuropathology

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Abstract. Electron microscopy (EM) remains a powerful and even essential tool in modern diagnostic neuropathology. Tumors are still encountered that defy histological or immunohistochemical classification, and EM can often provide answers in these cases. Tumors of the CNS for which EM is useful include unusual or atypical variants of meningioma, ependymoma, and schwannoma; oligodendroglioma-like tumors composed of small ''clear'' cells; and small ''blue cell'' tumors of childhood. EM is of great value in identifying site of origin for metastatic adenocarcinomas of unknown origin—an under-recognized and under-utilized potential for this technique. EM is useful in the diagnosis of peripheral nerve sheath tumors and gastrointestinal autonomic nerve tumors. EM is also important in the evaluation of certain congenital, inherited and metabolic diseases—including ceroid lipofuscinoses, CADASIL syndrome, certain myopathies, and mitochondrial encephalomyopathies—and of certain toxic and drug-induced peripheral neuropathies. An important application of EM is its utility in initiating a workup of an atypical tumor or metabolic condition, for which clinical and histological clues point in no obvious direction. In these situations, EM may provide either an answer outright (including answers to questions not asked) or important clues that guide further workup and narrow the range of diagnostic possibilities.

Key Words: Brain neoplasms; Central nervous system diseases; Diagnosis; Electron microscopy; Muscular diseases; Peripheral nervous system diseases.

INTRODUCTION

''The Big Eye'' it was called in the 1960s and 1970s. The ultimate diagnostic and research tool in anatomical pathology. Many of today's senior neuropathologists began their careers in the faint green glow of the electron microscope screen. By the mid-1980s, however, the glow was off, both figuratively and, increasingly, literally. The rise of immunohistochemistry provided insight into molecular expressions that rendered mere structure seemingly obsolete. And yet, as powerful as immunohistochemistry has proven to be, there remain areas of diagnostic pathology, and especially of diagnostic neuropathology, where electron microscopy (EM) provides diagnoses that cannot be achieved by immunohistochemistry or by any other technique. Today, pathology residents may receive only token exposure to the electron microscope and its potential. This, unfortunately, only further handicaps the use of this tool, as the most important element in ultrastructural diagnosis remains the knowledge on the part of the pathologist as to when EM can be helpful, as to what exactly EM can provide in the way of information, and as to how to prepare and submit tissue and to use this instrument to provide optimal, meaningful answers.

When and How to Use Electron Microscopy

Electron microscopy remains useful—and in some cases essential—in the identification of certain metabolic and inherited diseases of the nervous system, of certain myopathies, and in the diagnosis of certain diseases of peripheral nerves. EM is also useful, however, in tumor diagnosis, and this, in our experience, is the most common use of EM in modern diagnostic neuropathology.

Ultrastructural examination can be performed, if necessary, on almost any available tissue. A common misconception is that EM requires tissue specifically set-aside in exotic fixative (glutaraldehyde). Prompt glutaraldehyde fixation performed immediately upon receipt of a specimen provides excellent results and remains a routine procedure in many academic settings. However, tissue that has been routinely fixed in buffered formalin is entirely adequate for diagnostic EM. Even tissue retrieved from paraffin blocks, although less satisfactory, can be used for EM and can provide diagnostic answers in some situations. Promptness of fixation and neutral pH are far more important than other considerations, and tissue that has been fixed in buffered formalin soon after receipt is far preferable to tissue that has been held ''fresh'' for later glutaraldehyde fixation. Once fixed, fixed tissue is fixed tissue, and post-fixation of formalin-fixed tissue with glutaraldehyde serves no useful purpose.

An important step in an ultrastructural workup is histological review of a semi-thin (or ''thick'') plastic section prepared and stained for light microscopy. In some situations (especially peripheral nerve pathology), this preparation itself may provide the answers sought, obviating the need for EM. In all situations, review of thick sections by the submitting pathologist is essential in verifying that the tissue to be examined is representative of the tumor or lesion that has been identified histologically. It is also important that the electron microscopist (if different from the submitting pathologist) be aware of the

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relevant clinical and pathological features of the case, and of the histological differential diagnosis, prior to ultrastructural examination.

It is important to survey a large number of cells during ultrastructural examination, and, in tumor diagnosis, several areas of the tumor, if possible. As is the case in light microscopy, diagnoses based on single cells (or single organelles) should be avoided.

Ultrastructural Tumor Diagnosis

Elucidation of histogenesis is the cornerstone of modern tumor classification. The difference between a histogenesis-based approach to diagnosis and an approach based solely on pattern recognition is the basic distinction between a scientist and a technician. It is in the elucidation of histogenesis that EM can contribute insights that are, quite literally, invisible at the histological level. In contrast, EM is not useful in the determination of malignancy, which is better accomplished by light microscopy. Two decades after the advent of immunohistochemistry, only a handful of diagnostic markers have found routine use in the classification of nervous system tumors. And tumors for which both histological and immunohistochemical findings are inconclusive are still regularly encountered in any large neuropathology practice. EM is useful in many of these situations and, indeed, EM remains the gold standard of diagnosis for some tumor types, such as ependymoblastoma, choroid plexus carcinoma, and plexosarcoma.

EM is useful in the differential diagnosis of tumors with similar histological appearances. While well-differentiated tumors with classic histological appearances are generally easily identified on routine histological examination, there are often areas of overlap in the histological appearance of different tumors, yielding look-alikes. This problem is an especially thorny one with tumors of the spinal canal, where ependymomas and astrocytomas—and even schwannomas and meningiomas—can share histological features. Similarly, neurogenic tumors of the peripheral nervous system can be confused with other spindle-cell tumors. And both central neurocytoma and clear cell ependymoma can mimic the histological appearance of oligodendroglioma. In all of these situations, EM can be useful.

EM is useful is in the classification of atypical or poorly differentiated tumors. These include atypical examples of otherwise histologically obvious tumor types, such as meningioma, as well as tumors that are intrinsically undifferentiated at the histological level, such as small blue cell tumors. A third situation in which EM can be useful is a rare presentation of an otherwise histologically typical tumor (e.g. an intracerebral schwannoma). In these situations, EM can be useful in supporting a histological diagnosis that is at odds with clinical and gross pathological impressions.

Finally, an under-recognized and under-utilized application of diagnostic tumor ultrastructure is the identification of primary site for metastatic adenocarcinoma of unknown origin. Pulmonary, colonic, renal, and other adenocarcinomas have distinct ultrastructural appearances that often allow identification of their site of origin. Use of EM for this purpose has the advantages of being noninvasive (i.e. requiring no procedures beyond the already-performed biopsy) and being relatively inexpensive (in comparison with a full clinical workup).

This brief review focuses on tumors with common or overlapping histological appearances. For more thorough descriptions of particular tumors, the reader is referred to the original literature and to recent reviews (1, 2).

Tumors of the Central Nervous System and Skull

Ependymoma, Schwannoma, and Meningioma: Ependymomas, schwannomas, meningiomas and even astrocytomas may all show overlap in histological appearance, especially for examples arising in the spinal canal. Ultrastructurally, however, these tumors are all quite distinct and EM is thus often of practical use in differential diagnosis.

Ependymomas: Ependymomas (3) are readily identified by EM, even on casual inspection of random electron micrographs (Fig. 1A). These tumors form numerous small lumens complete with microvilli, cilia, and adjacent complex junctions. The rarity of such lumen formation (ependymal rosettes) in histological preparations is attributable not to their absence, but to the tiny size of these structures. In addition to these epithelial features, ependymomas show ultrastructural evidence of their glial origin in the form of filament-rich processes similar to those seen in astrocytomas. The presence of these dual epithelial and astrocytic features establishes a diagnosis of ependymoma.

Tanycytic Ependymomas: Tanycytic ependymomas (4, 5) are composed of slender, elongated bipolar cells, with 1 pole showing a microvillus-bearing surface that may be crowded against an adjacent cell rather than lining an identifiable lumen (Fig. 1B). The opposite poles of the tumor cells generally abut a basal lamina, usually adjacent to a blood vessel. In addition to ''pure'' ependymomas, occasional complex tumors contain an ependymoma element that can only be recognized by EM (6, 7).

Schwannomas: Schwannomas (8) are also readily obvious on ultrastructural examination. A characteristic feature is long, often quite thin, cytoplasmic processes that show extensive intertwining with similar processes from adjacent cells (Fig. 1C). The overall impression in a two-dimensional electron micrograph is one of widely separated ''cells'' (actually nucleated perikarya), with the ''intercellular'' (interperikaryal) space filled with numerous thin cytoplasmic sheets or processes of indeterminate origin. A further feature, and one that distinguishes these

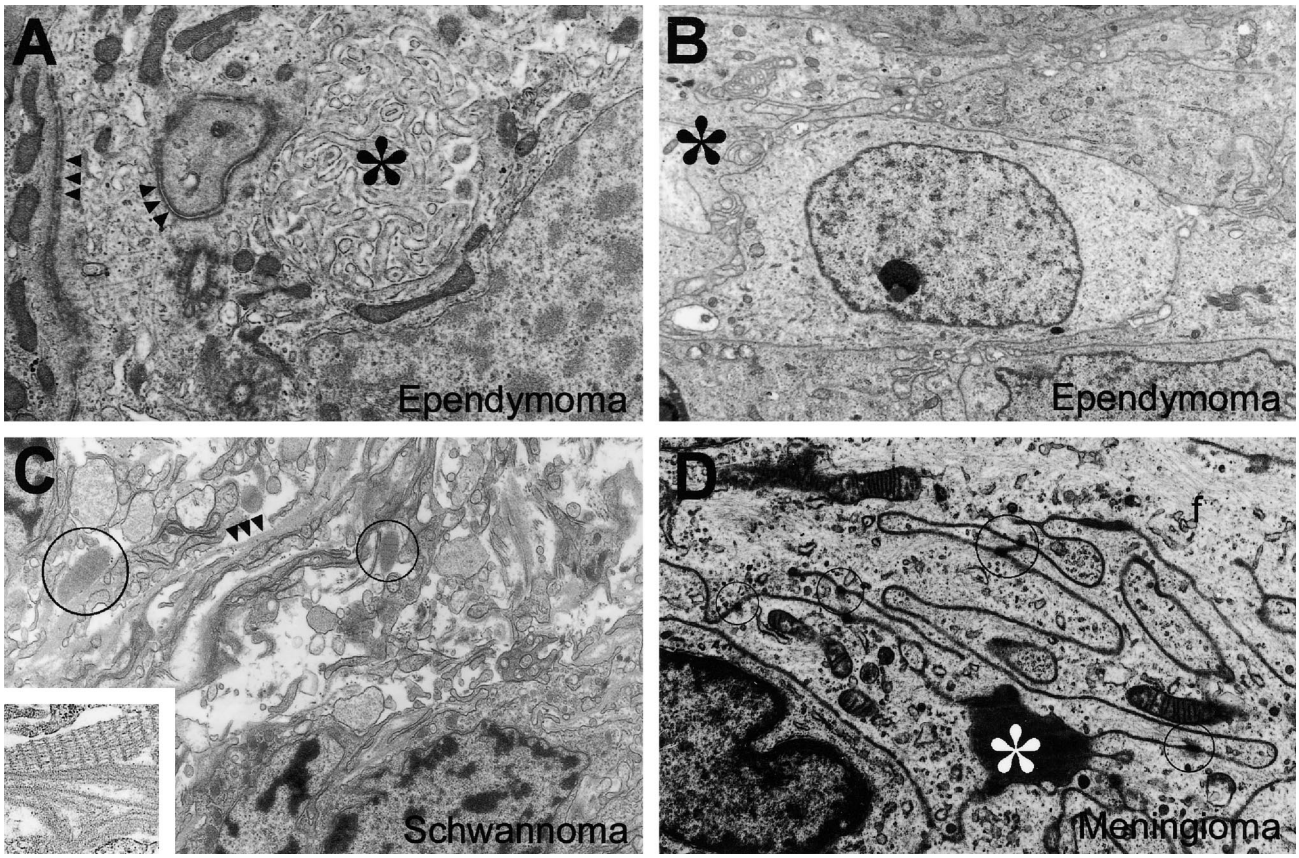

Fig. 1. Distinguishing features of ependymomas, schwannomas, and meningiomas. Most ependymomas (A) show miniature lumens (*) filled with microvilli and elongated intercellular junctions (arrowheads). Tanycytic ependymomas may show a ''stacked'' or ''brick-wall'' appearance (B), with complex processes, suggesting inspissated microvilli (*), at one end of tumor cells. Schwannomas (C) show numerous intertwining processes lined by basal lamina (arrowheads) and frequent ''Luse bodies'' (circles). Redundant basal lamina and a Luse body are shown at higher magnification in the inset. Meningiomas (D) show interdigitating cell processes and ''splotches'' of extracellular basal lamina-like material (*), but no true basal lamina. There are also cytoplasmic filaments (f) and numerous desmosome-type intercellular junctions (circles). From Mrak (1).

tumors from meningiomas, is the presence of basal lamina that completely encircles each tumor cell and is often reduplicated in the spaces between the intertwining processes. Schwannomas also quite commonly contain abnormal, thickened collagen fibers with unusually long periodicity to their banding structure (''fibrous long-spacing collagen'' or ''Luse bodies''). These are frequently florid in schwannomas, but they are neither specific nor necessary for diagnosis.

Meningiomas: Meningiomas show complex interdigitation of cell processes, in a manner superficially reminiscent of schwannoma (Fig. 1D). The interdigitating processes are broader than those in schwannoma, however, and basal lamina are lacking. Instead, meningiomas show scattered, electron-dense ''splotches'' of basal lamina-like material in intercellular spaces. Meningiomas also show frequent, well-formed desmosome junctions, again in contrast to schwannomas where such junctions are rare or absent. Cytoplasmic filaments are a prominent feature of meningiomas. These filaments are vimentin type, and usually have a loosely matted appearance that contrasts with the dense, homogeneous glial filaments of astrocytes and ependymal cells. Such glial filaments tend to completely fill cell processes and thus impart a homogeneous appearance to the cytoplasm at low magnification, an appearance that is generally not seen in meningiomas. The filaments of meningioma are further distinguished by attachment to desmosomes, a feature reminiscent of squamous carcinomas. *Fibroblastic meningiomas* may show greater amounts of extracellular basal lamina-like material, and lesser development of junctions and filaments. *Papillary meningiomas* may be particularly difficult to identify histologically. By EM, however, this malignant meningioma variant shows classic meningioma features and is easily diagnosed (9, 10).

Oligodendroglioma-like Tumors: Oligodendrogliomas, central neurocytomas, and clear cell ependymomas are classic histological look-alikes. Neuron-specific immunohistochemical markers are often useful in identifying central neurocytoma, but there are no specific markers

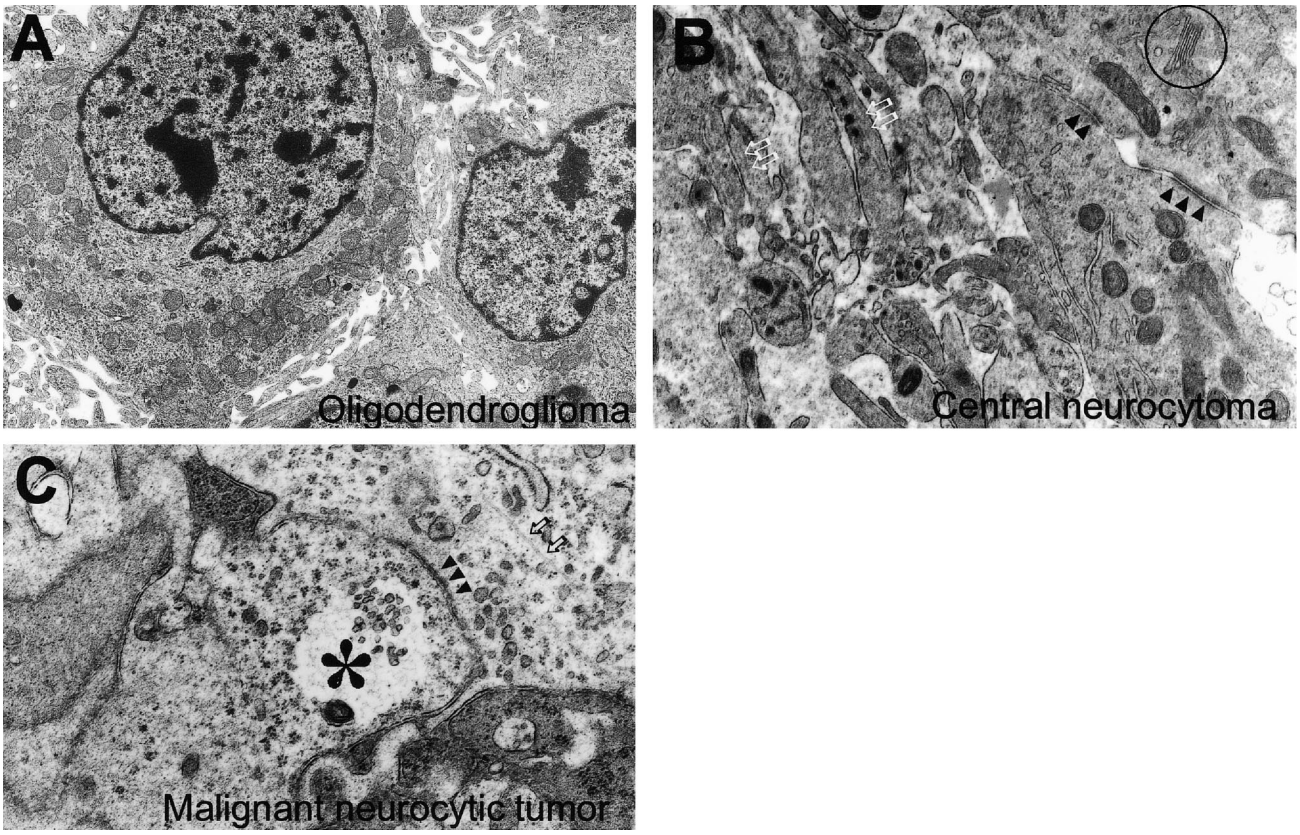

Fig. 2. Distinguishing features of oligodendroglioma-like tumors. Oligodendrogliomas (A) are ultrastructurally undistinguished cells containing polyribosomes and a few mitochondria. Central neurocytomas (B) show well developed neuronal features such as synapse-like intercellular junctions (arrowheads), Nissl-like stacks of endoplasmic reticulum (circle), and microtubules within neuritic cell processes (arrows). Neuronal features are also seen in malignant neurocytic tumors (C). Note synapse-like structure consisting of a vesicle-containing process (*) and membrane ''thickening'' in the adjacent tumor cell perikaryon (arrowheads). Note also microtubule (arrows) in the tumor cell cytoplasm. From Mrak (1, 14).

for oligodendroglioma or clear cell ependymoma, and EM can be useful in differentiating these tumors (11).

Oligodendrogliomas: Oligodendrogliomas are ultrastructurally undistinguished tumors that are composed of small round cells that may have a few, short, branching processes (Fig. 2A). The tumor cell cytoplasm generally contains only sparse organelles, although occasionally there are abundant mitochondria (12), microtubules, ribosomes, or even lysosomes (13). This diagnosis is, to a large extent, one of exclusion.

Central Neurocytomas: Central neurocytomas, in contrast to oligodendrogliomas, show striking neuronal differentiation by EM, including microtubule-containing cell processes (neurites), and synapse- and synaptic bouton-like contacts between cells (Fig. 2B). These ultrastructural features easily establish the correct diagnosis. A malignant counterpart of the central neurocytoma has been described (14) that shares the ultrastructural features of central neurocytoma (Fig. 2C).

Clear Cell Ependymomas: Clear cell ependymomas resemble oligodendrogliomas histologically, but are readily distinguished ultrastructurally by their ependymal features: lumens, microvilli, cilia, and complex junctions, as described above.

Central Nervous System Small Cell Tumors of Childhood: Few entities encountered in surgical neuropathology are the source of as much confusion and controversy as are those tumors composed of small, round, ''blue'' cells. These tumors may be truly primitive or undifferentiated (medulloblastomas and primitive neuroectodermal tumors), or may show convincing neural (neuroblastoma) or even ependymal (ependymoblastoma) features. EM has proven to be of great value in classifying (or in some cases even in defining) these small round cell tumors.

Medulloblastomas and Primitive Neuroectodermal Tumors: Medulloblastomas of the cerebellum (15) and primitive neuroectodermal tumors of the cerebrum (16) are composed of rather unremarkable, undifferentiated cells with a paucity of distinguishing cytoplasmic features (Fig. 3A, B). Intercellular junctional devices, usually simple in type, are a common feature of medulloblastomas,

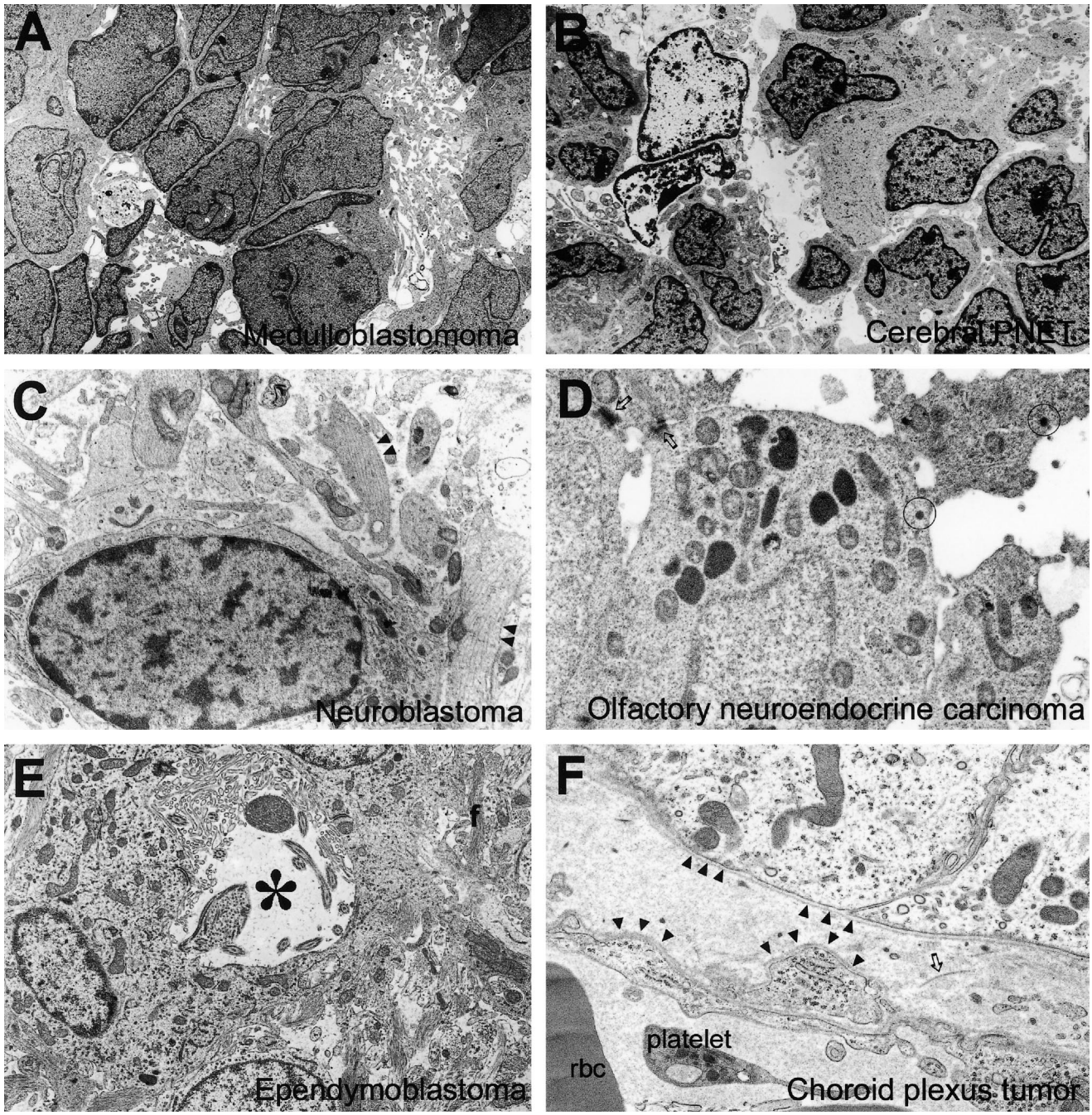

Fig. 3. Distinguishing features of central nervous system small-cell tumors. Cerebellar medulloblastomas (A) and cerebral primitive neuroectodermal tumors (B) are composed primarily of primitive, featureless tumor cells with high nuclear-to-cytoplasmic ratios. Neuroblastomas (C) show neuronal features such as microtubule-containing processes (arrowheads). Classical esthesioneuroblastomas show features similar to those seen in panel C, while the neuroendocrine carcinoma variant (D) shows more rudimentary neuronal features (neurosecretory granules, circles) together with fair numbers of intracellular junctions (arrows). Ependymoblastomas (E) show ependymal features that include lumen formation (*) with apical microvilli, cilia, and gap junctions, as well as fibrillated cell processes (f). Choroid plexus carcinomas show ependymal-type epithelial features, but also show a fibrovascular core (F), consisting of basal lamina (arrowheads) lining both the tumor cells (top) and the capillaries (bottom) with an intervening stroma containing collagen (arrow). rbc = red blood cell. From Mrak (1).

but are apparently less frequent in cerebral primitive neuroectodermal tumors. While focal or rudimentary neuroblastic, or even ependymoblastic, differentiation is not unusual in primitive neuroectodermal tumors, well-developed and widespread neuroblastic differentiation should prompt re-classification of a tumor.

Neuroblastomas: Neuroblastomas are small blue cell tumors with ultrastructural evidence of neuroblastic differentiation that occur in both the cerebrum (17) and the cerebellum (18). The salient ultrastructural features include neuritic processes (Fig. 3C) and dense-core, neurosecretory-type vesicles. The processes are generally slender, of more-or-less constant diameter, and are apparent in longitudinal or cross section. They fill the space between adjacent tumor cell perikarya. Occasionally such masses of processes form an ''intercellular'' area large enough to be discerned histologically as a Homer Wright rosette. The processes may contain longitudinally oriented microtubules and variable numbers of dense-core neurosecretory-type granules, although such granules are generally more frequent in the cell perikarya. Some tumors show synapse-like contacts between processes or between processes and perikarya, sometimes complete with small, clear vesicles reminiscent of synaptic vesicles. The presence of characteristic neuritic processes in combination with any of the other distinguishing features is strong evidence of a neuroblastic histogenesis.

Specialized variants of neuroblastoma include retinoblastoma, olfactory neuroblastoma (esthesioneuroblastoma) and pineoblastoma. *Retinoblastomas* show characteristic evidence of photoreceptor differentiation in the form of rosettes, within which there are 1) elongated apical mitochondria-filled cytoplasmic extensions (presumably developing inner segment ellipsoids) protruding into the lumen, 2) apical junctional complexes that include tight junctions, reminiscent of epithelial-type terminal bars, and 3) apical cilia with a 9 + 0 microtubule doublet pattern typical of photosensory neurons. These specialized rosettes have been called ''fleurettes.'' In contrast to the ultrastructural specialization of retinoblastomas, *olfactory neuroblastomas* are similar to nonolfactory neuroblastomas, with the exception of the *neuroendocrine carcinoma* variant (19). These latter tumors are found in older patients (mean age 50 yr) than are classic olfactory neuroblastomas (mean age 20 yr) and they resemble paragangliomas. They lack the neurites found in olfactory (and other) neuroblastomas, but do contain neurosecretory granules in addition to well-developed intercellular junctions (Fig. 3D). *Pineoblastomas* may appear similar to medulloblastomas or primitive neuroectodermal tumors or may show evidence of rudimentary photoreceptor differentiation.

Ependymoblastomas: Ependymoblastomas (20, 21) may be poorly differentiated, medulloblastoma-like tumors by light microscopy, or may show ependymoma-type perivascular pseudorosettes or even true ependymal rosettes. Ultrastructurally, these tumors show minute intercellular lumens with apical basal bodies and adjacent well developed apical junctional specializations (Fig. 3E). Microvilli and cilia are seen in the better-differentiated cases.

Choroid Plexus Carcinomas: Choroid plexus carcinomas (22) may present as solid tumors composed of small anaplastic cells without distinctive histological pattern. In these cases, electron microscopy can be useful in demonstrating epithelial features such as specialized intercellular junctions and apical microvilli. These tumors can be distinguished from ependymoblastomas by the relationship between the tumor cells and the component blood vessels. Ependymal tumors have a gliovascular core in which tumor cells abut directly upon the basal lamina that encircles the capillary, and the capillary endothelium is ''neural'' in type, without fenestrations. In contrast, choroid plexus tumors have a fibrovascular core that contains collagen and has blood vessels with fenestrated endothelium. There is a basal lamina that separates the tumor cells from this core, and a second basal lamina that surrounds the capillary itself (Fig. 3F).

Metastatic Tumors

Amelanotic Melanomas: Amelanotic melanomas are diagnostic problems wherever they are found. The usefulness of EM in such cases rests on the presence of melanocytic differentiation (i.e. the presence of cytoplasmic melanin granules or melanosomes) that is not detectable by the light microscopy or immunohistochemistry. The failure of histological methods to find these structures may result from a paucity of melanosomes or from the presence of unduly small melanosomes (less than 250 nm). In addition, spindle cell variants of melanoma may not express immunohistochemical ''markers'' such as HMB-45 and MART-1. The diagnostic feature is the intermediate stage melanosome, characterized by a filamentous (or perhaps membranous) internal structure with a characteristic (and diagnostic) transverse banding. Normal melanosomes are round or oval with longitudinally oriented filaments, but neoplastic melanocytes may contain pleomorphic melanosomes that have coils or spirals of filaments (Fig. 4A). Melanosomes must be distinguished from the granules of mast cells and basophils, from the rod-shaped microtubulated bodies (Weibel-Palade bodies) of vascular endothelial cells, and from tertiary lysosomes. True melanosomes may also occur, rarely, in tumors other than melanoma. These include pigmented schwannomas (8) and melanotic neuroectodermal tumors of infancy.

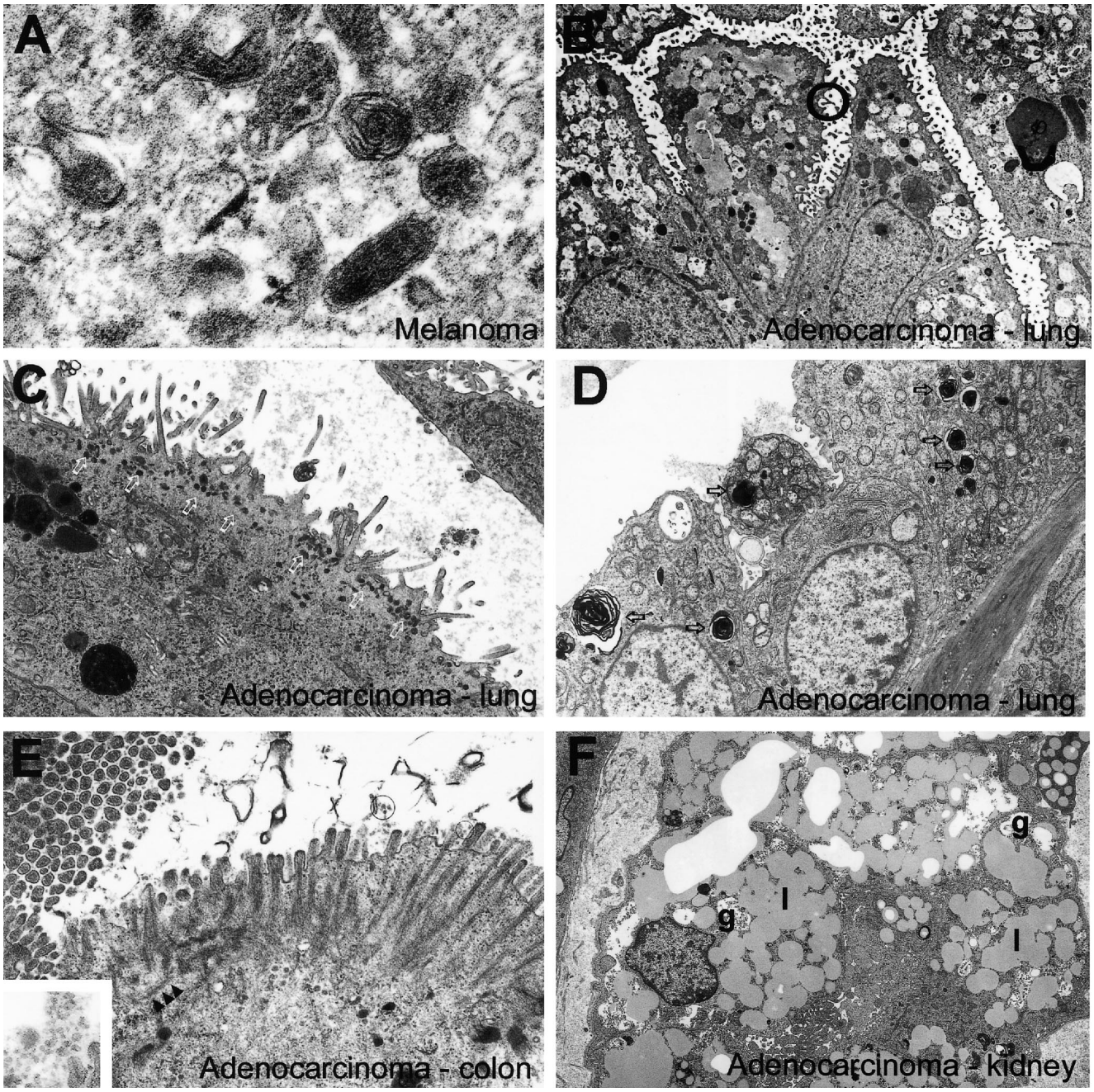

Fig. 4. Ultrastructure of metastatic tumors. Melanomas (A) contain diagnostic intermediate melanosomes, with longitudinal or circular filamentous structures, transverse periodicity, and an enclosing unit membrane. Pulmonary adenocarcinomas (B–D) may show deep ''gullies'' between tumor cells with abundant, irregular, occasionally branching (circle) microvilli (B); may show small subapical irregular dense bodies (Clara cell granules, arrows, C); or may show cytoplasmic membranous whorls reminiscent of surfactant production (arrows, D). Colonic adenocarcinomas (E) show well-formed, uniform microvilli with filamentous cores extending down into the underlying cytoplasm (core rootlets) and filaments running perpendicular to these rootlets (terminal web formation, arrowheads). Glycocaliceal bodies—small ring-like structures in the overlying glycocalyx—are not well developed in this example (circles) but are shown at higher power in another tumor in the inset. Renal cell carcinomas (F) contain abundant cytoplasmic glycogen (g) and lipid (l). From Mrak (1).

The differentiation of *metastatic renal cell carcinoma* from *hemangioblastoma* presents another classic look-alike problem in diagnostic neuropathology. This is a distinction for which immunohistochemistry can be useful, as renal cell carcinomas often show immunoreaction for epithelial membrane antigen and for cytokeratins. Furthermore, both tumors are immunoreactive for GLUT1, but differ in their patterns of immunoreactivity, making this antibody a useful

distinguishing tool (23). In cases for which these studies are inconclusive, these tumors are readily distinguished by EM. Hemangioblastomas show lipid-containing stromal cells filling the spaces between capillaries, while renal cell carcinomas show epithelial features such as microlumen formation and apical microvilli.

Metastatic adenocarcinomas of unknown origin are seen fairly regularly in neurosurgical material. In about a third of patients with adenocarcinoma metastatic to the brain, this lesion is the first manifestation of a systemic cancer. Among patients for whom the primary tumor is known or subsequently discovered, the common primary sites are lung, kidney and colon. Carcinoma of the breast commonly metastasizes to the brain, but only rarely does so in the absence of an obvious primary lesion. Prostatic adenocarcinomas rarely present as brain metastases but may present as spinal or spinal cord metastases. Other, less common sources of metastatic adenocarcinoma include upper gastrointestinal tract, pancreas and thyroid gland.

Well-differentiated and moderately differentiated adenocarcinomas frequently (and poorly differentiated adenocarcinomas occasionally) retain ultrastructural features that allow identification of their site of origin, and as many as 80% to 90% of such metastatic tumors can be traced to a specific anatomic site of origin on the basis of their ultrastructural appearances (24, 25). EM, then, is a potentially useful tool in the evaluation of patients with metastatic adenocarcinomas of unknown origin.

Pulmonary Adenocarcinomas: Pulmonary adenocarcinomas make up the largest group of metastatic adenocarcinomas to the central nervous system (25), and also show the greatest ultrastructural diversity. Ultrastructurally, pulmonary adenocarcinomas are composed of 1 or more of 3 basic cell types: mucus-secreting cells, Clara cells, and alveolar (type II pneumocyte) cells (26). These ultrastructural categories do not correspond with recognized histopathological categories. Pulmonary adenocarcinomas may also contain cells showing squamous or neuroendocrine (oat cell) differentiation. The ultrastructural appearances of metastatic pulmonary adenocarcinomas are thus quite diverse, but useful ultrastructural features include the presence of divergent lines of cellular differentiation, circumferential branching microvilli without filamentous cores or glycocaliceal bodies, subapical Clara cell granules, and surfactant-type lamellar cytoplasmic bodies (Fig. 4B–D).

Adenocarcinomas from the Colon and Rectum: Adenocarcinomas from the colon and rectum are generally readily identifiable ultrastructurally by their distinctive apical cell surfaces. These are lined by well-organized, nonbranching microvilli with prominent filamentous cores that extend deeply into the underlying apical cell cytoplasm (''core rootlets'') (Fig. 4E). Overlying this there is a prominent glycocalyx containing minute, round, ring-like structures (''glycocaliceal bodies''). In addition to these features, mucin production is usually evident. Occasional mucous pulmonary adenocarcinomas may show scattered foci of (and rare pulmonary tumors may show widespread evidence of [27]) similar-appearing microvilli. The distinction rests upon the extent of such microvillous specialization, as well as upon the presence or absence of distinguishing pulmonary features.

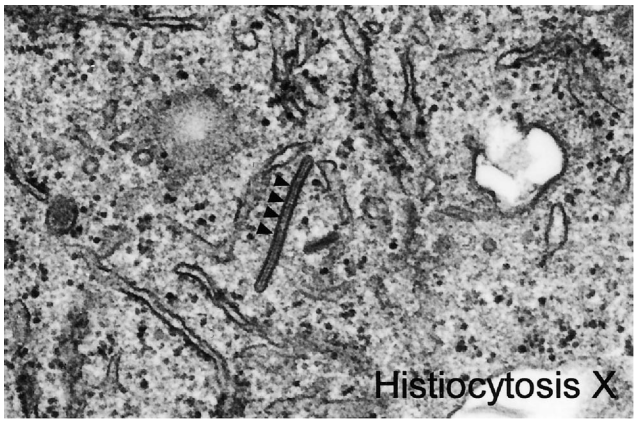

Fig. 5. Zipper-like Birbeck granule (arrowheads) in a case of histiocytosis X. From Mrak (1).

Renal Cell Adenocarcinomas: Renal cell adenocarcinomas are clear-cell carcinomas identified ultrastructurally by the presence of glycogen and lipid in copious amounts and by the absence of mucin (Fig. 4F). They are distinguished from pulmonary clear cell carcinomas by the presence of lipid and by the nature of their lumenal surfaces. Renal cell carcinomas form small, tight intercellular lumens that contrast with the ''hobnail'' (or even circumferential) microvillus-lined surfaces of pulmonary adenocarcinoma cells (Fig. 4B). Renal cell carcinomas may also contain granular cells. These granular cells contain generous numbers of cytoplasmic mitochondria, but cytoplasmic lipid and glycogen are generally still evident.

Other adenocarcinomas metastasize to the central nervous system with less regularity. *Mammary adenocarcinomas*, *prostatic adenocarcinomas*, *gastric adenocarcinomas*, and other adenocarcinomas all have distinct ultrastructural appearances that are reviewed elsewhere (1, 24, 25, 28).

Histiocytosis X: Histiocytosis X generally presents in neuropathology practice as a skull lesion. Histiocytosis X cells are identified by the ultrastructural demonstration of a unique cellular organelle, the Langerhans cell granule, or Birbeck granule (Fig. 5). Birbeck granules are cytoplasmic rod-like structures of variable length but constant width that appear as 2 closely apposed membranes with a central parallel line between them. In addition, there may be an enlarged vesicle at one end imparting a ''tennis racquet'' appearance to the granule. These structures identify the component cells of a lesion as Langerhans

cells and thus establish a (nonspecific) histiocytic lesion as a (specific) histiocytosis X lesion.

Tumors of the Peripheral Nervous System

The distinction between peripheral nerve sheath tumors and other spindle cell tumors is a common histopathological problem, and one for which EM has proven useful. Peripheral *schwannomas* (8) are ultrastructurally similar to the central schwannomas discussed above, with intertwining cell processes and redundant basal lamina. Smooth muscle tumors (but not fibroblastic tumors) may also have basal lamina, but the long intertwining cell processes are (in this context) unique to Schwann cell tumors. *Cellular schwannomas* (29) and *epithelioid schwannomas* (both benign [30] and malignant [31]) are generally similar to ''classic'' schwannomas ultrastructurally. *Pigmented (melanotic) schwannomas* contain melanosomes and pre-melanosomes in addition to showing features of schwannoma (8). *Granular cell schwannomas*, in contrast, show less well-developed schwannian features (8). In particular, cell processes are not well developed and the basal lamina often encircles groups of cells rather than individual cells. The striking ultrastructural feature of these tumors, of course, is the myriad secondary lysosomes that fill the cytoplasm, a feature that these tumors share with other non-schwannian, granular cell tumors (32). Rare schwannomas may show histological features resembling neuroblastoma or primitive neuroectodermal tumor, but these show typical schwannian features by EM (33).

Perineuroma: Perineuroma (Pacinian neurofibroma) (34) is an unusual peripheral nerve sheath tumor that also shows abundant, long, thin cytoplasmic processes—a feature that distinguishes it from ''usual'' neurofibromas. Perineuromas are distinguished from schwannoma by a relative absence of basal lamina and by the presence of frequent junctional complexes and numerous surface vesicles. Malignant forms of this tumor also occur (35).

Malignant Peripheral Nerve Sheath Tumors: Malignant peripheral nerve sheath tumors show the same features as benign schwannomas, but these features are less well developed. Cell processes are broader and sparser, and basal lamina may be present only as occasional pericellular fragments or extracellular scrolls or whorls. EM can be essential in the diagnosis of these tumors (36).

Plexosarcoma or Gastrointestinal Autonomic Nerve Tumor: Plexosarcoma, or gastrointestinal autonomic nerve tumor (37, 38), originates from neural elements of the enteric plexus. By light microscopy these tumors may appear as bland spindle cell tumors similar to leiomyomas, but this belies a clinical course characterized by widespread metastases and short survival. Ultrastructurally, the component cells of plexosarcomas have intertwined cell processes (neurites) containing both dense-core and empty synaptic-type vesicles, as well as filaments (Fig. 6). These cells do not have the smooth muscle features of basal lamina, filaments with dense bodies, or attachment plaques.

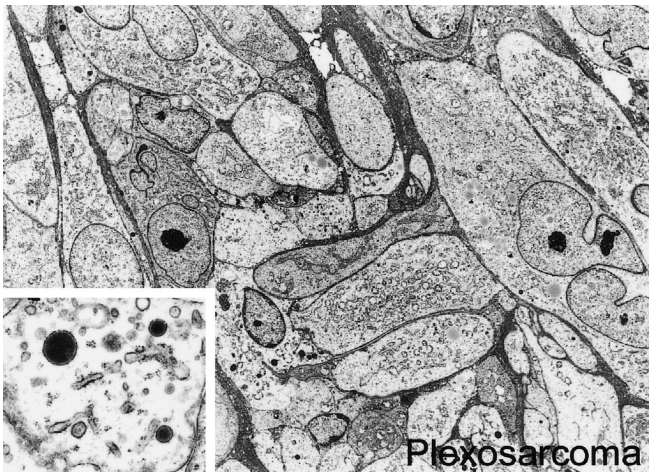

Fig. 6. Plexosarcoma with numerous cross- and longitudinally sectioned cell processes between perikaryal areas and dense-core, neurosecretory-type granules (inset). From Mrak (1).

Non-Neoplastic Diseases of the Nervous System

Ultrastructural analysis, usually of non-neural tissues such as skin, conjunctiva, muscle, rectal mucosa, liver, or peripheral blood lymphocytes (39–41), is a classic diagnostic tool in the evaluation of inherited metabolic disorders, most of which have either diagnostic or characteristic ultrastructural findings (40, 42). Modern enzyme analyses and genetic tests have narrowed the range of lysosomal and peroxisomal disorders for which EM is required for diagnoses, but these new tests have not eliminated this need. Moreover, these rare metabolic diseases may present with incomplete, atypical, or nondiagnostic clinical and metabolic findings, leaving the clinician and the pathologist at a loss as to where to begin a workup. In these cases EM can be particularly useful, first in establishing that there is or is not a ''storage'' disorder present, and secondly in either establishing a diagnosis outright or at least suggesting specific disease possibilities to focus further workup (43). In addition, ultrastructural analysis remains the only method of establishing the diagnosis of some variants of neuronal ceroid lipofuscinoses, and these diseases are among the more common in this category (44). These diseases are characterized by ''storage'' of lipofuscin-like material. This material often has unique ultrastructural features, which vary from lamellar forms to collections of undulating ''curvilinear'' membranes (Fig. 7A) to ''fingerprint'' patterns to more ordinary tertiary lysosome-like inclusions. EM is useful in the evaluation of mitochondrial encephalomyopathies

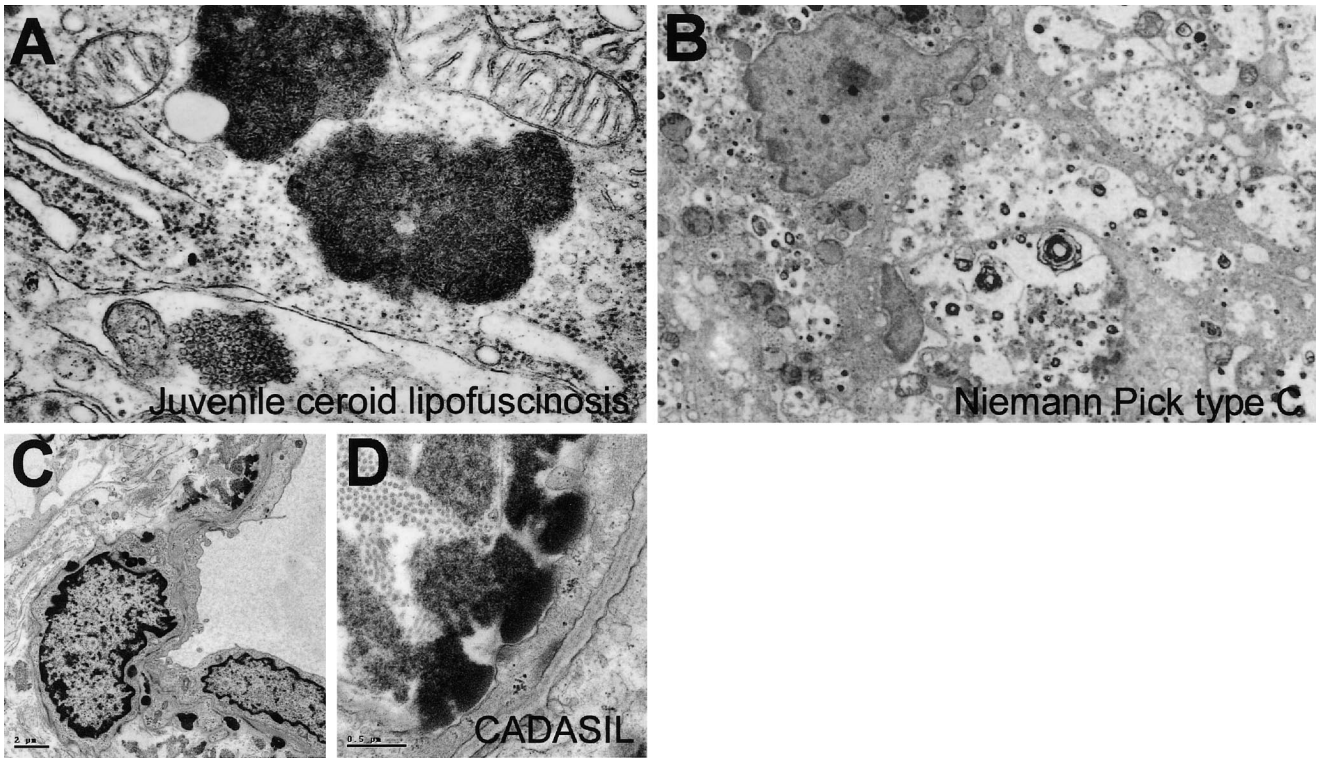

Fig. 7. Metabolic diseases of the nervous system. A: ''Curvilinear'' lipofuscin deposits in a brain biopsy from a 20-yr-old woman with juvenile ceroid lipofuscinosis. B: Niemann-Pick type C disease in an infant with hepatosplenomegaly and ''suspected genetic/metabolic disease.'' A liver biopsy showed characteristic pleomorphic, electron-dense, finely lamellar bodies admixed with granular material that suggested the diagnosis, and this was subsequently confirmed by genetic testing. C, D: CADASIL in a 53-yr-old woman with progressive dementia and diffuse white matter lesions. This unsuspected diagnosis was made by electron microscopy of a brain biopsy, and subsequently confirmed by genetic demonstration of a Notch3 mutation. Note electron-dense deposits adjacent to the vascular smooth muscle cell and its processes.

(see below under Diseases of Muscle), and in the evaluation or diagnosis of Pompe's disease, Anderson's disease, Krabbe's disease, Niemann-Pick disease (Fig. 7B), adrenoleukodystrophy, and other genetic and metabolic diseases.

The recently described CADASIL syndrome is another example of a disease with diagnostic ultrastructural findings in biopsies of non-neural tissues (45). Arterial and arteriolar blood vessels, both within the brain and in peripheral organs such as skin and muscle, show numerous electron-dense, granular deposits (resembling immunoglobulin deposits) along both the luminal and abluminal surfaces of vascular smooth muscle cells (Fig. 7C, D). These deposits, known as granular osmiophilic material deposits, often lie within small infoldings of the smooth muscle cell.

There are few areas in which routine paraffin embedding shows greater limitations than in the evaluation of peripheral nerve biopsies. Modern pathological analyses of these diagnostic specimens are absolutely dependent

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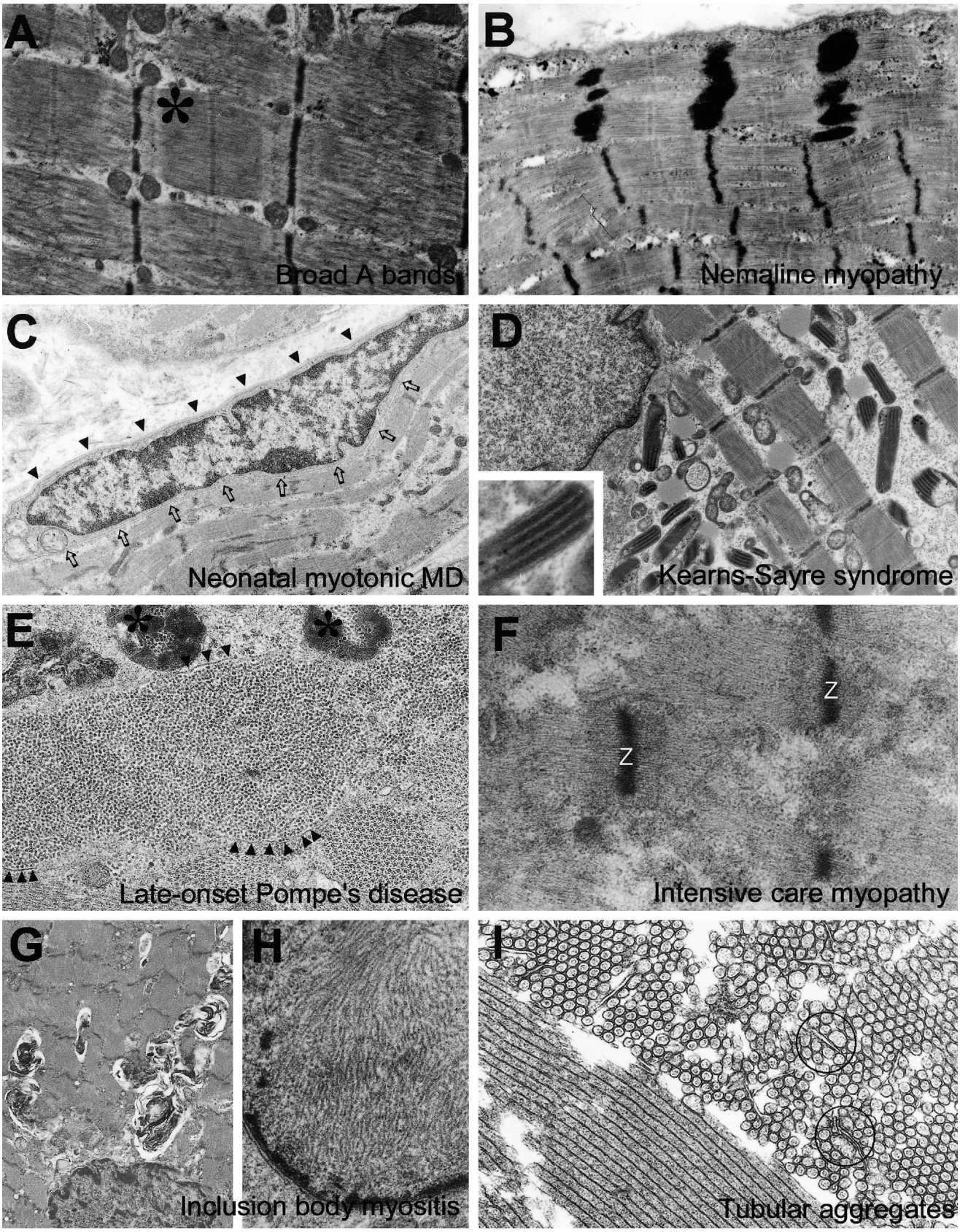
Fig. 8. Diseases of muscle. A: Subtle myosin disarray with broadening of the A-band in a congenital myopathy (50). Compare disorganized sarcomeres with the adjacent normal sarcomere (*). B: Early rod formation in nemaline myopathy. Classic rods were evident elsewhere in this biopsy. Note origin of rods from Z-lines. C: Neonatal myotonic muscular dystrophy. A satellite cell lies beneath the myofiber basal lamina (arrowheads) but is separate from the muscle fiber (arrows). These cells are markedly increased in number in neonatal myotonic muscular dystrophy. D: Kearns-Sayre syndrome with abnormal muscle mitochondria containing paracrystalline inclusions. One of these is shown at greater magnification in the inset. E: Glycogen ''storage'' both within lysosomal residual bodies (*) and within membrane-lined early lysosomes (arrowheads) in a case of late-onset Pompe's disease. F: Loss of myosin (thick) filaments in intensive care myopathy. Note preservation of Z-line (Z) and attached actin (thin) filaments. G, H: Inclusion body myositis, with vacuoles containing membranous ''whorls'' (G) and intranuclear paired helical filaments, or ''tubulofilaments'' (H). I: Tubular aggregates, seen in both transverse and longitudinal section, in a muscle biopsy from a patient with hypokalemic periodic paralysis. Note triad-like associations with transverse tubules (circles), suggesting origin



from the sarcoplasmic reticulum. Panel E is from Slonim et al (56). Panel F is courtesy of Dr. Jonathan Fratkin, University of Mississippi. Panel H is courtesy of Dr. Cheryl Palmer, University of Alabama at Birmingham. Panel I is from Mrak (60).

upon the preparation of plastic-embedded, semi-thin (''thick'') sections for histological examination. While this technique does not require an electron microscope, these sections are usually prepared in an electron microscope laboratory where plastic embedding and semi-thin sectioning is routine. In addition to the utility of semithin sections, however, there are diseases of peripheral nerve that do require full ultrastructural workup and examination for diagnosis. These include a variety of congenital or inherited neuropathies, certain drug-induced (e.g. amiodarone, chloroquine), toxic (e.g. hexane, methyl n-butyl ketone, acrylamide), and immunoglobulin (IgM)-associated neuropathies, and (as always) unusual and atypical diseases and disease presentations (46). Amiodarone toxicity produces characteristic polymorphous inclusions with concentric membranous lamellae in Schwann cell cytoplasm. Somewhat similar lamellar intralysosomal inclusions are seen in chronic chloroquine toxicity. Peripheral neuropathies associated with Waldenström's macroglobulinemia and other IgM gammopathies show a diagnostic widening of the intraperiod line of outer myelin lamellae that is attributable to IgM deposition within the lamellae (47).

Diseases of the Muscle

Electron microscopy remains a useful and even essential tool for the diagnosis of certain congenital and acquired myopathies. Congenital myopathies, in particular, are a heterogenous group of uncommon diseases that are defined and classified on the basis of their pathologic features (48), and some of these diagnostic features are discernible only by EM (49–53) (Fig. 8A). In other cases, EM is required for diagnosis in more subtle cases for which the histological features are inconclusive (54) (Fig. 8B). Neonatal myotonic dystrophy is characterized by an increased number of muscle satellite cells that are discernible only by electron microscopy. In the example shown in Figure 8C, this unsuspected diagnosis was suggested by EM and subsequently confirmed by genetic testing.

Metabolic diseases for which EM is useful include mitochondrial disorders (55) and glycogen storage disorders. Muscle biopsies from patients with mitochondrial encephalomyopathies may show excessive proliferations of normal-appearing mitochondria, enlarged mitochondria with disorganized cristae, electron-dense deposits within mitochondria, and/or striking condensation of mitochondrial cristae into ''paracrystalline'' or ''parking lot'' configurations (Fig. 8D). Atypical or late-onset cases of Pompe's disease (acid maltase deficiency) may be diagnosed by the EM finding of membrane-bound (i.e. lysosomal) glycogen storage within muscle (56) (Fig. 8E).

EM is also useful in certain acquired myopathies, as in the demonstration of the characteristic loss of myosin fibers in acute myopathy of intensive care (57) (Fig. 8F) and, in some cases, in the demonstration of diagnostic vacuoles with membranous contents and ''tubulofilaments'' in inclusion body myositis (58) (Fig. 8G, H).

Tubular aggregates are a striking ultrastructural finding in muscle (Fig. 8I). These structures were first described in hypokalemic periodic paralysis, but are now known to occur in a variety of inherited and acquired disorders, where they are useful in differential diagnosis (59).

Summary

Electron microscopy remains an important and, at times, essential tool in modern diagnostic neuropathology. EM can differentiate tumors with overlapping histological appearances, can provide diagnoses for poorly differentiated tumors that lack specific histological or immunohistochemical features, and can provide information on site of origin for metastatic adenocarcinomas. EM is highly effective as a first step in diagnosing or at least focusing the workup for childhood metabolic diseases that present without specific clinical or histological features and, moreover, EM is the only available method of diagnosis for some non-neoplastic diseases of the central and peripheral nervous systems and muscle.

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