OBSERVATIONS ON THE ULTRASTRUCTURE OF HUMAN TUMORS
WITH PARTICULAR REFERENCE TO THE ROLE OF
TRANSMISSION ELECTRON MICROSCOPY IN TUMOR DIAGNOSIS

Volume 2

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Tumors of blood cells and lymphoid organs

Tumors discussed in this section are certain lymphomas, monocytic leukemia, histiocytosis, and thymoma.

Lymphoma

There is now little call for electron microscopy as a diagnostic aid for tumors in this category but in the past it has provided interesting and sometimes significant information on the component cells of the various types of lymphoma. Some features that are evident by light microscopy can be demonstrated effectively by EM. The dramatic nucleoli in a Reed-Sternberg cell are an example (figure 169). Nuclear details are better appreciated in semithin sections of the EM tissue blocks compared to paraffin sections, and in low magnification electron micrographs: the irregular nuclear profiles of a convoluted cell lymphoma (figure 170) contrast with the rounder nuclei of a Burkitt’s lymphoma (figure 171).

Figure 169. Reed-Sternberg cell.
Figure 170. Convoluted cell lymphoma. x 3,200.

Figure 171. Burkitt's lymphoma. x 3,700.
In a small cleaved follicular center cell (poorly differentiated) lymphoma, the majority of the cells appeared vacuolated by light microscopy. Immunoperoxidase staining demonstrated that the cells contained IgG/k. EM showed that smaller vacuoles coalesced to form the large vacuoles which contained microvesicles.

Figure 172. Signet ring cell lymphoma. x 5,100.
In 1978, Kim et al.\textsuperscript{44} reported seven lymphomas containing signet ring cells. In four of the tumors, most of the cells were vacuolated and they contained monoclonal IgG; in the remaining three cases, the cells contained inclusions resembling Russell bodies of monoclonal IgM. The signet ring cells in our case are histologically similar to the four vacuolated cell tumors Kim et al. reported and they too contained IgG. Small vacuoles are often seen in the vicinity of the golgi complex of the cells, and the large vacuoles that form the signet ring structure (figure 172) are formed by progressive confluence of smaller vacuoles. Some microvesicles can often be found within the cavity of a large vacuole (figure 173). The basic defect in signet ring cells could lie in immunoglobulin transport and secretion from the cell. In the case we report, results of serum protein electrophoresis and quantitative immunoglobulin analysis were with normal limits.

Ten large cell lymphomas studied by EM were reported because on many cells there were slender peripheral projections that simulated microvilli.
Figure 174. Large cell lymphoma with microvillus-like projections. x 7,500.

Figure 175. Large cell lymphoma with microvillus-like projections which are seen to better advantage in this tumor because the cells are more separated. x 5,600.
The neoplastic lymphocytes bore moderate to numerous cytoplasmic projections (figure 174), seen best when the cells were not compressed (figure 175). My colleagues liked the term 'porcupine' lymphoma cells and it has been used occasionally. In tissues where the cells are compactly grouped, the peripheral projections form a compressed layer between neighboring cells. The cells were transformed lymphocytes and not sinus histiocytes, and 9 of the 10 cases we reported were of presumed B-cell lineage. Monoclonal cytoplasmic immunoglobulin was confirmed in four of the ten cases and stains for muramidase were negative in every case. To the unwary, the cell projections might be misinterpreted as evidence of glandular differentiation, especially when the cells are within the peripheral sinusoids of a lymph node: we drew attention to a sinusoidal pattern of involvement by large cell lymphoma in a report on 18 patients. The surface projections are also seen well in large cell lymphoma within splenic sinusoids.


Seven spleens surgically removed from patients with hairy cell leukemia and hypersplenism were examined by EM. The splenic cords were infiltrated by hairy cells and many of these cells were also evident in the sinuses. By EM, the cell projections tended to interdigitate to form syncytium-like aggregates, and compression of the cells within the cords flattened the projections against the cell bodies. EM has not resolved the cylogenesis of hairy cells. They showed no evidence of phagocytic activity, although macrophages in the cords were actively phagocytic.

Figure 176. Hairy cell leukemia. x 7,500.
Figure 177. Hairy cell leukemia. The cell contains ribosome-lamellar complexes. x 15,000.

Hairy cell leukemia is aptly named because the neoplastic cells have long cytoplasmic projections (figure 176) and they are further characterized by the presence in the cytoplasm of some cells of ribosome-lamellar complexes (figure 177) which are not unique to this entity, being found in various leukemic disorders.
Figure 178. Large cell lymphoma. The cells have nuclear pockets. x 14,000.

Figure 179. Plasma cell myeloma. The cells contain extensive granular endoplasmic reticulum. x 6,300.
Figure 180. Lymphoplasmacytic lymphoma from lung. The cytoplasm of every cell was packed with crystals. x 4,000.

Figure 181. Same tumor as figure 180 showing the crystals in greater detail. x 33,000.
The surface of some neoplastic lymphocytes has projections that enclose small areas of cytoplasm and they have been called nuclear pockets (figure 178). They may look like pseudoinclusions of cytoplasm within the nucleus but are bounded by a band of chromatin. They can occur in various tumors but when numerous are strongly suggestive of lymphoma or leukemia.

In a transformed lymphocyte, the granular endoplasmic reticulum proliferates and may come to occupy much of the cytoplasm and this is a typical finding in a plasma cell myeloma (figure 179). Immunoglobulin may accumulate within the cells\(^{248}\) and the crystals in figures 180 and 181 were present in every cell in a biopsy of a lymphoplasmacytic lymphoma that was forming diffuse lung infiltrates in an adult female.

**Monocytic leukemia**

Two case reports described unusual complexes in monocytic leukemia that involved the skin.


A case of pure monocytic leukemia in a 55-year-old female presented as multiple cutaneous nodules. The cells contained unusual cylindrical formations of rough endoplasmic reticulum that differed from classic ribosome-lamellar complexes.


A 28 year old woman developed leukopenia and slight cervical lymphadenopathy. Bone marrow aspiration and special stains established the diagnosis of acute monocytic leukemia. Following chemotherapy, complete hematologic remission was achieved. Seven months later, bilateral subconjunctival, perilimbal, grayish-pink infiltrates appeared. A conjunctival biopsy showed acute monocytic leukemia and 4 months later she developed cutaneous lesions on the face and chest wall. Muramidase was detected in the tumor cells supporting the diagnosis of monocytic leukemia. EM revealed that 80% of the leukemic cells contained two types of cytoplasmic complexes of rough ER that displayed both tubular and helical configurations.
In both patients with cutaneous involvement by monocytic leukemia, the neoplastic cells contained formations of the granular endoplasmic reticulum with a scroll-like configuration around a central core of cytoplasm. The layers of the wall were either concentric (figure 182 – TC) or helical (figure 182 – HC). These complexes differ in their composition by the incorporation of cisternae, and in their construction by the occurrence of a concentric architecture, from the ribosome-lamellar complexes of hairy cell leukemia.

**Histiocytosis**

Proliferative disorders of histiocytic cells vary in their appearance in neoplasms. The most distinctive by EM are those of Langerhans cells.


A 52-year-old Latin American male experienced swelling of the right thigh and recurrent skin eruptions. A punctate skin rash involved the scalp and legs and enlarged lymph nodes could be palpated in the right femoral and inguinal regions and demonstrated radiologically in the lower paraaortic region. A right inguinal lymph node was biopsied. Light microscopic sections showed nodular sclerosing Hodgkin’s disease together with a geographic, predominantly sinusoidal distribution of uniform round cells with reniform nuclei and many intermingled eosinophils, and EM revealed the presence of Langerhans granules in the round cells (shown in figure 186).
Figure 183. True histiocytic lymphoma involving dermis. The spaces within the cells are produced by distension of endoplasmic reticulum. x 6,800.

Figure 184. Langerhans cell histiocytosis from gingiva. The nuclear indentations are typical. x 5,300.
Figure 185. Langerhans cell histiocytosis from skin. The cells have sparse to moderate numbers of organelles but few lysosomes. x 11,700.

Figure 186. Langerhans granules in a cell from a lower extremity lymph node that was involved by Hodgkin’s disease. x 15,000.
Figure 187. Eosinophilic granuloma of soft tissues overlying the zygoma of an infant. A Langerhans granule at the surface of the cell is in continuity with the cell membrane. x 42,000.

The tumor in figure 183 was a true histiocytic lymphoma involving dermis and the spaces within the cytoplasm are a histiocytic feature, being formed by dilatation of the endoplasmic reticulum. There are very few attached ribosomes and only a small number of lysosomes. Marker studies confirmed that the cells were histiocytic. The patient was a 73-year-old white woman with cutaneous nodules that fluctuated in size and occasionally regressed, and the diagnosis was true histiocytic lymphoma. In contrast, the histiocytic cells in figure 184 have few or no lysosomes but they possess a distinctively indented nucleus that in section presents a mushroom-head profile, seen better in figure 185. A prominent golgi complex lies in or near the indentation, and Langerhans granules are present in the cytoplasm. When the granules contact saccules of the golgi complex, the distinctive racquet-like bodies are created (figure 186). Langerhans granules may be seen in contact with the cell membrane (figure 187), and this consistent with the concept that they are endocytotic organelles conveying material from surface receptors to the golgi complex. Langerhans cells may be associated with other neoplasms, as in lung adenocarcinomas, and in the case we reported they coexisted in a lymph node with nodular sclerosing Hodgkin’s disease. The clinical course of Langerhans cell histiocytosis ranges from spontaneous resolution to a chronic and sometimes lethal disease.
Thymoma

It is not usually difficult to identify a thymoma by light microscopy but the tumor may present in uncommon locations such as pleura or a lymph node, or it can have an atypical appearance, and on these occasions ultrastructural support for the diagnosis can be helpful.

A review based on EM study of 23 thymomas with correlated light microscopy including immunoperoxidase studies. Each patient had an anterior mediastinal mass and 12 were asymptomatic. Seven of the tumors were invasive as determined from the operative reports and examination of the resected masses, and of these seven patients, three developed recurrent disease.

Figure 188. Thymoma. At this low magnification, the contrast in size and fine structural appearance between the epithelial cells and lymphocytes is seen well. x 3,400.

An 83-year-old female was investigated for episodes of dizziness and painless swelling of the face and neck. Chest x-ray revealed a 5 cm mass in the upper and anterior mediastinum extending to the right and involving the region of the superior vena cava. An anterior mediastinotomy was performed to obtain biopsy tissue. Following surgery, marked decrease in size of the mass was achieved with radiotherapy.
Figure 190. Atypical thymoma from mediastinum. In this field, the epithelial cells have moderately electron dense cytoplasm which at higher magnification contained fine filaments. One lymphocyte is present. x 7,200.

Figure 191. Same tumor as figure 190. Frequent cells in this tumor were atypical with irregular nuclear profiles, considerable numbers of organelles, and diffuse fine filaments. x 8,800.
In the tumor case, the irregular nuclei, abundant cytoplasmic filaments, and groups of mitochondria were atypical features and the irregular contours of many of the mitochondria and variable cristal patterns were also unusual. Crystalloids were present in a number of the tumor cells. The desmosomes supported the diagnosis of thymoma since they were present in moderate numbers and were of mature construction with short tonofilament bundles.

In our study of 23 thymomas, most were encapsulated but one was invading an attached segment of the innominate vein and another infiltrated adjacent lung. Nine tumors were predominantly epithelial and two were almost exclusively composed of spindle cells. Nine showed an admixture of lymphocytes with epithelial cells by light microscopy and in five a predominance of lymphocytes almost obscured the epithelial cells. Thus the series of cases encompassed the spectrum of light microscopic morphology of thymoma. Although the tumor may be designated as epithelial or lymphocytic by light microscopy, an admixture of the two cell types can invariably be found by EM even though one or other cell type tends to predominate. Classification into lymphocyte rich, epithelial cell rich, or spindle cell type has not been found to be prognostically useful.

There is rarely difficulty identifying a thymoma at the ultrastructural level. Like normal thymic epithelial cells, the neoplastic counterparts are roughly four times the size of the lymphocytes and with their processes they occupy an even more extensive domain (figure 188). They are the true neoplastic cells and they do not usually exhibit a significant degree of cytologic atypia. Ultrastructurally, they present a bland appearance with smooth cell and nuclear margins, and where adjacent cells are apposed, there is little if any dovetailing of the cell margins or interdigitation of the plasma membranes. A small acinus-like space may contain a few microvillus-like projections from the surrounding cells. The nuclear chromatin is typically diffuse and fine. The cells may have considerable quantities of cytoplasm but organelles are limited to scattered mitochondria and a few slender profiles of rough endoplasmic reticulum. A small number of lysosomes and lipid droplets may be present. Occasionally a lymphocyte is detected within the cytoplasm of an epithelial cell indicating that emperipolesis is taking place. Cell attachments between the epithelial cells vary considerably in number but they are mature desmosomes with intermediate lines and attached tonofilaments: the latter can rarely be followed beyond the immediate vicinity of the desmosomes. The desmosomes are often long and they tend to group in series (figure 189) so they are usually conspicuous even at low magnifications, but in a few tumors the desmosomes are sparse and difficult to locate. Cytoplasmic extensions can usually be found although they vary in frequency, and their cytoplasm is identical to that of the cell body. A basal lamina is not always obvious but in three of our cases it was extensive. The tumor shown in figures 190 and 191 had typical areas but some cells had irregular nuclear profiles, unusually large numbers of cytoplasmic filaments, groups of mitochondria with irregular profiles and variable patterns of their cristae, and some crystalloids, and these were departures from the appearance of a classic thymoma, earning for the tumor an atypical designation.
Tumors of the soft tissues

The tumors discussed in this section are those of fibroblasts, fat cells, muscle cells, vasoformative cells, peripheral nerve sheath cells, and several that are of uncertain histogenesis.

Probably the greatest contribution of electron microscopy in the study of human tumors has been within the extensive category of soft tissue tumors. It is regrettable that the clinical relevance of the ultrastructural findings is extremely limited because the histologic typing of a soft tissue sarcoma does not usually influence choice of therapy: there are exceptions, but most are managed without regard to subclassification.

The enormous structural diversity encountered within this group of neoplasms contrasts with the unprepossessing appearance of cells of human mesenchyme.

Figure 192. Human mesenchyme. The cells have little cytoplasm and few organelles. x 3,800.
The cells of human mesenchyme have scanty elongated cytoplasm (figure 192), and a similar bland appearance is occasionally seen in a benign soft tissue tumor. There is some resemblance to mesenchyme in the cells of an intramuscular myxoma and the stroma is again virtually structureless with only scattered small groups of collagen fibrils (figure 193).

**Tumors of fibroblasts**

A spectrum of ultrastructure can be traced from reactive proliferations through malignant fibrous histiocytoma supporting the view that all the tumors in this group are of fibroblastic derivation. Dermatofibrosarcoma protuberans is a lesion in which the shape of the cells is not unlike that seen in a myxoma but the classic storiform pattern is well developed by light microscopy. Dr. Hugo Dominguez participated in the electron microscopy during the following study.


Thirty-eight specimens of dermatofibrosarcoma protuberans (DFSP) were examined by EM and immunohistochemistry. A fibroblastic/myofibroblastic origin was favored from the results of the studies.
Figure 194a. Dermatofibrosarcoma protuberans. The slender cells have thin processes. x 3,700.

Figure 194b. Dermatofibrosarcoma protuberans. The cytoplasm of the tumor cells resembles that of a fibroblast. The cytoplasmic extensions are typically slender. x 6,100.
Figure 195a. Dermatofibrosarcoma protuberans. A dendritic cell has radiating arms of cytoplasm and coarsely clumped chromatin. x 5,500.

Figure 195b. Pigmented dermatofibrosarcoma protuberans. The melanosomes are in macrophages. x 4,200.
In DFSP, the cells have slender but long cytoplasmic extensions (figure 194a) that run in parallel and curve in conformation with the storiform pattern that is conspicuous in light microscopic sections. The area shown in figure 194b shows some of the attenuated cytoplasm and it also illustrates the cytoplasm of a cell body which contains moderate numbers of flattened cisternae of granular endoplasmic reticulum. A few poorly defined cell junctions connect cells and processes with one another. Occasional so-called dendritic cells are interspersed among the fibroblast-like cells (figure 195a): their nuclear chromatin is more clumped and the radiating arms contain some lysosomes. Convoluted and multilobated nuclei are often seen. The histogenesis of DFSP continues to be controversial but EM does not show evidence of neural features and the ultrastructure is in keeping with fibroblastic origin. In the pigmented DFSP or Bednar tumor, the pigment is in melanosomes (figure 195b).

The next seven figures illustrate fibroblastic features in aggressive fibromatosis, atypical fibrous histiocytoma, and fibrosarcoma. The hallmark of a classical fibroblast is the copious granular endoplasmic reticulum that typically occupies most of the cytoplasm, and figure 196 shows this well in cells from a fibrosarcoma.

Figure 196. Fibrosarcoma. The extensive granular endoplasmic reticulum is forming elongated cisternae. x 6,200.
Figure 197. Aggressive fibromatosis. The fibroblast contains intracellular collagen fibrils. x 9,400.

Figure 198. Aggressive fibromatosis. The peripheral band of smooth muscle myofilaments marks the cell as a myofibroblast. x 10,000.
Figure 199. Aggressive fibromatosis. A slender anchoring filament extends from a myofibroblast into the surrounding collagen. x 9,700.

Figure 200. Aggressive fibromatosis. The cytoplasm of the fibroblast is moderately electron dense. x 3,800.
Figure 201. Same cell as figure 200. Detail of the perinuclear cytoplasm showing that the electron density is produced by diffuse fine filaments. $\times 14,500$.

Figure 202. Atypical fibrous histiocytoma of skin. Cisternae contain amorphous dense material. $\times 17,500$. 
A 47-year-old woman had a painless mass in her right axilla. It measured approximately 3.5 cm and was composed of loosely arranged spindle cells which were immunoreactive for smooth muscle actin. By EM, some of the cells contained peripheral bands of smooth muscle myofilaments establishing that they were myofibroblasts. There was sparse mitotic activity and no cytologic atypia, and by flow cytometry the tumor was diploid with a low s-phase. A diagnosis of myofibroblastoma was favored although the possibility of a low grade sarcoma could not be ruled out. The tumor did not recur during a short follow up period.

Dr. Chae Hong Suh participated in the electron microscopy during this study. EM was performed on 60 fibrosarcomas. Myofibroblastic differentiation was detected in 33 tumors but usually in scattered cells. Diffuse aggregates of nonspecific intermediate filaments were present in some of the tumors.

The basic feature of a fibroblast, extensive cisternae of granular endoplasmic reticulum occupying much of the cytoplasm, is evident in the fibrosarcoma in figure 196. Other features of the normal or reactive fibroblast are often seen in fibrosarcomas but they are more consistently found in fibromatoses. Intracellular collagen can be encountered in fibromatoses (figure 197) and the illustrated cell contains small wisps of filaments in the peripheral cytoplasm. The filaments are better developed in figure 198 where they form an orderly, longitudinally-oriented bundle in subplasmalemmal location and the patchy densities within the filament bundles show that they are smooth muscle myofilaments. In a view of parts of two cells from another aggressive fibromatosis (figure 199), smooth muscle myofilaments are present in the tapering tip of one cell, from which a thin filamentous strand extends out into the collagenous stroma.

Diffuse fine filaments are seen in some fibromatoses (figure 200) and they are fairly common in the better differentiated fibrosarcomas. The diffuse filaments are seen in more detail in figure 201: they are nonspecific in their appearance and seem to be randomly distributed. Rarely they are localized forming a spherical body similar to that seen in recurrent digital fibroma of childhood256, and I have observed these bodies in an aggressive fibromatosis from an adult patient. Dense material sometimes accumulates in cisternae in fibromatoses and fibrosarcomas, and it is more likely to be patchy than homogeneous. The atypical fibrous histiocytoma in figure 202 was suspected at low magnification to be harboring secretory granules.

It is probable that any fibroblast may in a suitable environment form smooth muscle myofilaments in order to acquire contractile capability257, and the slender strands that extend from the cell surface of a myofibroblast into the surrounding collagen provide a fulcrum through which the movement of the cell is transmitted to the adjacent stroma. The term fibronexus has been applied to this structure which was earlier known as a microtendon258. Singer259 in 1979 defined fibronexus as a transmembraneous association of external fibronectin-containing fibers and cytoplasmic 5 nm actin microfilaments within dense submembranous plaques.

It is not surprising that myofibroblasts should occur in neoplasms of fibroblastic derivation but their presence is an indication of differentiation and they would therefore be more likely to be found in benign and low grade malignant tumors. In my experience, this is the case. The tumor reported above was called a myofibroblastoma although a low-grade sarcoma
could not be ruled out. Myofibroblasts were detected in 33 of the 60 fibrosarcomas we studied but only in scattered cells and they were confined to the better differentiated tumors. Most of the reported myofibroblastic tumors have been benign \(^{26}\). EM is needed to confirm that myofibroblasts are present: separation of a myofibroblast from a smooth muscle cell is not always easy, but if it can be determined that the myofilaments are distributed throughout the cytoplasm rather than being confined to a subplasmalemmal band or pinocytotic vesicles are present, smooth muscle differentiation can be concluded. Subplasmalemmal plaques and possibly pinocytotic vesicles will provide corroborating evidence. A fibronexus would not be seen in a smooth muscle cell, and these structures are unusual in fibrosarcomas and rarely if ever occur in sarcomas. Like smooth muscle cells, myofibroblasts are typically positive for vimentin and alpha smooth muscle actin, and desmin is not a useful discriminant between smooth-muscle and myofibroblastic lesions \(^{28}\).

Malignant fibrous histiocytoma (MFH) continues to be a controversial tumor. In an EM study, we found a range of fine structure which overlapped to such a degree with that of fibrosarcomas that the specificity of the entity appears dubious and the reproducibility and relevance of subtyping are questionable. In this study, Dr. Chae Hong Suh participated in the electron microscopy and performed the morphometry.

**Suh CH, Ordonez NG, Mackay B. Malignant fibrous histiocytoma: an ultrastructural perspective. Ultrastructural Pathology 24:243-50, 2000.**

One hundred and fifty-seven tumors diagnosed by routine light microscopy as MFH were studied. The series included representative specimens of the various subtypes. Clinical data were reviewed and every tumor was examined by light and electron microscopy. Immunohistochemical stains were performed on 77 tumors.

![Figure 203](image_url)

Figure 203. Malignant fibrous histiocytoma. A multinucleated pleomorphic cell is surrounded by fibroblast-like fusiform cells. x 3,700.
Figure 204. Malignant fibrous histiocytoma. Slender cells with attenuated cytoplasm. x 3,400.

Figure 205. Malignant fibrous histiocytoma. Material within the cisternae forms a cribiform pattern. x 4,100.
Figure 206. Malignant fibrous histiocytoma. Epithelioid variant: the cells are plump and intimately apposed. x 3,600.

Figure 207. Angiomatoid malignant fibrous histiocytoma. The cells resemble fibroblasts. x 3,800.
Among the defined subtypes of MFH, the most frequent is the pleomorphic form in which multinucleated cells mingle with spindle cells (figure 203) that are often arranged in a storiform pattern. The cells have been referred to as fibroblast-like and histiocyte-like and likened to cells that are seen in chronic inflammation, but it seems reasonable to postulate that the larger, sometimes bizarre and often multinucleated cells are formed from the mononuclear spindle cells. There is clearly a spectrum with all manner of transitional forms, and the basic features of the cytoplasm are not sufficiently different to postulate that these are two separate cell types. In the storiform areas, the cells are often slender with long branching cytoplasmic extensions (figure 204), reminiscent of what can be seen in other fibroblastic tumors including DFSP. As is the case in some cells of fibromatoses and fibrosarcomas, dense material can accumulate within cisternae of MFH cells and condensation of this material in distended cisternae produces a cribriform appearance like that in figure 205. Some MFH are composed of round cells in compact sheets with intimate apposition of cell membranes and diffuse filaments throughout much of the cytoplasm (figure 206), and an epithelial tumor may then be suspected by light microscopy, an impression that might on occasion be supported by immunostaining since some MFH have been found to be keratin-positive. The figure was 6.5% in our study. This would appear to be comparable behavior on the part of neoplastic fibroblasts to the epithelioid type of change that is seen in some smooth muscle and vasoformative cell malignancies, and one could make a case for an epithelioid subtype of MFH.

A comparison between MFH and fibrosarcoma was attempted by examining electron micrographs of 100 fibrosarcomas from our files. Individual cells of the two tumors were often so similar at the EM level that it was impossible to assign the tumor to one or other category without seeing the light microscopic pattern. Evidence for true histiocytic differentiation, from the occurrence of phagocytosis or the presence of many lysosomes, was rarely found, though an example of erythrophagocytosis is described below, and lysosomes are present in the cells of the angiomatoid MFH in figure 207. Ultrastructural and immunohistochemical studies of angiomatoid MFH have failed to detect endothelial differentiation of the tumor cells. One study of 98 angiomatoid MFH indicated a myoid, primary myofibroblastic, phenotype. The term angiomatoid myosarcoma has been suggested.

The myxoid form is a well recognized subtype of MFH. The following case is unusual in that the tumor cells were vigorously phagocytosing erythrocytes.

A tumor forming multiple protuberant masses on the foot and leg of a 50 year old white male had the histopathology of a myxoid MFH and by EM the cells had some fibroblastic features. Extravasated erythrocytes were present throughout the tumor nodules and many had been phagocytosed by the tumor cells. Endothelial cell markers were negative.
Figure 208. Myxoid malignant fibrous histiocytoma. The tumor cell has phagocytosed an erythrocyte. x 5,700.

Figure 209. Same tumor as figure 208. Many erythrocytes lie free in the stroma and one has been almost completely engulfed by the tumor cell. x 7,000.
A characteristic of myxoid tumors, including myxoid MFH, is the relatively structureless stroma with wisps of collagen fibrils in a sea of ground substance (figure 208). This tumor had a deceptively innocuous appearance by light microscopy, resembling an area of fibrosis when it presented as a nodule in the foot, but it spread inexorably in spite of further resection and then amputation. The cells resembled fibroblasts but the nucleoli are unusual in their size and skein-like nucleonema (figure 209). There were many erythrocytes in the stroma and tumor cells were engulfing them: some cells contained several ingested red cells. This is an unusual example of phagocytic activity in cells of an MFH, and offers some, albeit meager, support for the existence of facultative fibroblasts with histiocytic properties. Erythrophagocytosis is a recognized characteristic of reactive histiocytes in the hemophagocytic syndrome associated with a variety of infections and it has been seen in tumor cells in malignant histiocytosis, some leukemias, lymphomas, and exceptionally in metastatic malignant epithelial cells in bone marrow and lymph nodes, and even in squamous cell carcinoma and melanoma.

My impression from our EM observations is that MFH falls within the general category of tumors of fibroblasts, lying towards the dedifferentiated end of the morphologic spectrum.

**Tumors of fat cells**

EM is rarely needed to diagnose tumors of fat cells but it can be useful as part of the documentation when the tumor presents in an unusual location.


A 54 year old man with progressive loss of voice and dysphagia of several weeks duration was found to have a lesion in his larynx. It was excised and diagnosed by light microscopy as a myxolipoma. Three years later, he developed progressive dysphagia and a recurrent mass, located in the right aryepiglottic fold, was biopsied. The diagnosis was well differentiated liposarcoma. A supraglottic laryngectomy with right upper and mid-jugular lymph node dissection was performed.

The next four figures show aspects of the ultrastructure of typical liposarcomas.
Figure 210. Myxoid liposarcoma. The tumor cell contains fat droplets that vary in size, and dense mitochondria. x 3,600.

Figure 211. Myxoid liposarcoma. One cell contains a single large aggregate of lipid. x 3,500.
Figure 212. Cellular myxoid liposarcoma. In this field, lipid is not evident and the cells are closely apposed. x 3,400.

Figure 213. Pleomorphic liposarcoma, metastatic in lung. Many lipid droplets are present. Linear condensations have formed within cisternae. x 9,800.
Two fairly consistent ultrastructural aspects of liposarcomas cells are the presence intracytoplasmic lipid droplets and mitochondria that are long and dense (figure 210). One or both of these features may be absent but they are overall common findings. The lipid droplets are typically of different sizes, in contrast to those seen in small numbers in some fibrosarcomas and MFH, and they progressively coalesce to produce a single large vacuole that occupies much of the cell (figure 211). The cellular myxoid liposarcoma from the thigh shown in figure 312 is an example with a paucity of lipid: it was present in some cells but many areas including the chain of cells illustrated contained little or no lipid. Although this was designated a myxoid tumor, the stroma in the area shown is quite collagenous. Like neoplastic fibroblasts, lipoblastic tumor cells can accumulate amorphous material within dilated cisternae that may condense to form linear aggregates, as in the lung metastasis of a liposarcoma in figure 213.

The cytophysiology of normal adipose tissue cells is reflected in liposarcomas. During embryonic development, and in areas of fat deposition, the immature cells are multilocular and the lipid droplets they contain vary in size, ultimately combining to form a single large droplet. All 15 liposarcomas studied by Rossouw et al.66 were composed of cells having ultrastructural components seen at some stage in lipoblastic differentiation, and the authors list them as lipid droplets, micropinocytotic vesicles, glycogen, external lamina, intermediate filaments, Golgi apparatus, rough and smooth endoplasmic reticulum, and mitochondria. They found that the nuclear-cytoplasmic ratio and nuclear pleomorphism were inversely related to the size and number of lipid droplets. Multivacuolated, mitochondria-rich lipoblasts resembling brown fat cells were also seen. Most of the tumors contained lipid-free, poorly differentiated mesenchymal cells that showed a continuum of morphologic differentiation to cells which closely resembled early lipoblasts and contained nonmembrane-bound lipid vacuoles. Dedifferentiated liposarcoma is an uncommon form in which the dedifferentiated component most often resembles either storiform MFH or myxoid MFH7, and the small sample taken for EM may not convey representative features of the dedifferentiated portion.

**Tumors of muscle cells**

I carried out an extensive EM study of smooth muscle tumors, most of them sarcomas but including some leiomyomas.


Observations using EM were made on over 200 smooth muscle tumors. Most were sarcomas but the series included 20 leiomyomas from various locations. Features of the tumors are described below.
Figure 214. Leiomyosarcoma, metastatic in lung. Large quantities of myofilaments are present. \( \times 7,300 \).

Figure 215. Same tumor as figure 214. The myofilaments are smooth muscle type. Long intercellular junctions with intermediate lines connect the cells. \( \times 16,600 \).
Figure 216. Leiomyosarcoma, metastatic in groin at a site of prior radiation therapy. An elongated tumor cell is shown in cross section to demonstrate the distribution of myofilaments throughout the cytoplasm. x 4,000.

Figure 217. Leiomyosarcoma. By light microscopy the tumor was poorly differentiated, but myofilaments are present in most of the cells. x 3,700.
Since the tumors in our series had been diagnosed as smooth muscle neoplasms by routine light microscopy, it was not a surprise to encounter myofilaments in all the primary soft tissue and uterine tumors. The appearance in the metastases was quite variable. The tumor in figure 214 originated in the lower thigh and spread to the lung, and in both locations the cells contained abundant myofilaments. The type of junction that can occur between neoplastic smooth muscle cells was well shown in this tumor: it consists of long paired subplasmalemmal densities with a distinct intermediate line (figure 215), resembling a giant desmosome without tonofilaments. A cross section through a leiomyosarcoma cell shows that the myofilaments are distributed throughout the cytoplasm and not confined to a peripheral band as in a myofibroblast (figure 216). Sometimes the cells and nuclei have a wavy contour (figure 217), but this is not a common finding. Small numbers of pinocytotic vesicles are often seen in leiomyoma cells but they are infrequent in the sarcomas.

Smooth muscle cells occasionally accumulate lysosomes within their cytoplasm and if they are sufficiently numerous a granular cell leiomyoma or leiomyosarcoma may be diagnosed. The tumor cells can become plump to rounded and qualify as epithelioid, and smooth muscle ultrastructural features may still be seen within them, unlike the situation in gastrointestinal stromal tumors where myofilaments were not seen in epithelioid variants. We have encountered smooth muscle tumors that were typical in their ultrastructure but manifested keratin immunoreactivity.

The following case was referred with a diagnosis of pulmonary carcinoid tumor but EM revealed that it was a glomus tumor, and additional clinical information confirmed that the lung tumor was a metastasis.


A 19 year old white male was found on routine physical examination to have a coin lesion in his left lung. At thoracotomy, two soft hemorrhagic 2.5 cm nodules were resected. Tumor was not detected in hilar lymph nodes. One month later, a small subcutaneous lump in the left buttock was noticed and it grew slowly over a period of four months, at which time it was excised and was found to be deeply infiltrating the gluteal musculature. In the course of the next few months, lesions from the scalp and left axilla were removed and the histology was similar in all locations. A CT scan of the abdomen revealed extensive retroperitoneal lymphadenopathy encasing great vessels and extending along the length of the abdominal aorta. Chemotherapy was initiated and produced moderate shrinkage of the abdominal lymph nodes but superficial nodules appeared on the forehead, right shoulder, and lateral chest wall. This tumor is shown in figure 219.
Figure 218. Glomus tumor of skin, benign. The cells are more or less round although they have irregular profiles with areas of intercellular contact. x 4,100.

Figure 219. Malignant glomus tumor, metastatic in lung. Numerous pinocytotic vesicles can be seen the cell surfaces. x 18,500.
Cells in the wall of the main anastomotic vessel of the Suquet-Hoyer canal have the ultrastructural features of vascular smooth muscle. The vessel is surrounded by numerous small nerve endings with synaptic vesicles but these are not a feature of the tumor cells although their surfaces are often in contact. The myofilaments in cells of a glomus tumor are poorly oriented and they may be sparse (figure 218). The lung metastasis described in the case report is shown in figure 219. As in the benign tumors, many sub-plasmalemmal vesicles are typical. Slender sheets of collagenous stroma intervene between the cells at many points and encircle cell clusters. Glomus tumors can occur in unexpected sites and EM is useful for confirmation of the diagnosis.

A glomus tumor may be locally aggressive. One in the nasal region of a 32-year-old woman recurred six times over a period of 14 years following the initial excision but did not metastasize. Gould et al. reported six locally aggressive glomus tumors and noted that they were larger and more deeply located than the conventional glomus tumor: three tumors recurred locally but none metastasized. The tumor we reported was clearly malignant, and the subcutaneous lump in the buttock which was found at surgery to be infiltrating deeply into the gluteal muscles was presumed to be the primary location. Authentic malignant glomus tumors are rare and require EM for confirmation of the diagnosis.

A retroperitoneal neoplasm was composed of spindle cells with smooth muscle differentiation but they also contained keratin filaments.


An abdominal mass in a 36 year old man was thought from radiological studies to be a renal neoplasm but at surgery the kidney was not involved. The 9.5 cm retroperitoneal tumor was totally resected together with an attached portion of psoas muscle, and it was encapsulated and appeared by light microscopy to be benign. The pattern of the spindle cells suggested a cellular schwannoma but immunohistochemistry and EM did not support this impression and instead revealed epithelial and smooth muscle features compatible with a myoepithelioma.
Figure 220. Spindle cell tumor, psoas region. The cells are long and thin and they have rather dense cytoplasm. × 3,900.

Figure 221. Same tumor as figure 220. Smooth muscle and keratin filaments are present in the same cell. × 6,200.
The diagnosis of this bland spindle cell tumor was uncertain by light microscopy, and at low magnification with the electron microscope (figure 220). Detailed scrutiny by EM coupled with the results of immunohistochemistry led to a diagnosis of myoepithelioma, based on the presence of both smooth muscle and keratin in many of the long fusiform cells (figure 221). A smooth muscle tumor with keratin immunoreactivity must be considered a possibility but the light microscopy did not suggest a leiomyoma, smooth muscle myofilaments were not abundant and pinocytic vesicles were lacking, and I have not seen keratin filaments by EM in a keratin-positive leiomyoma. Outside the salivary glands and breast, pure myoepithelial tumors are uncommon, and particularly so below the diaphragm.

Skeletal muscle myofilaments are easily recognized by electron microscopy but as the next series of figures illustrate, they can present a variety of appearances.

Figure 222. Rhabdomyoma. The extensive cytoplasm contains glycogen and numerous mitochondria. x 3,700.
Figure 223. Rhabdomyoma. Many small fragments of sarcomeres with exaggerated z-band components are present, and part of a flattened satellite cell is visible. x 7,900.

Figure 224. Rhabdomyoma. Peculiar membranous matrical inclusions can be seen in mitochondria. x 16,000.
Figure 225. Rhabdomyoma. Large round densities are present in some of the mitochondria. x 18,600.

Figure 226. Rhabdomyosarcoma, well differentiated, from the bladder of a young boy. The sarcomeres are well formed. x 4,900.
Figure 227. Myofiber invasion by a sarcoma of the chest wall. The rim of cytoplasm of the invaded cell contains z-band material and is bordered by a basal lamina. x 7,200.

Figure 228. Embryonal rhabdomyosarcoma. The nuclear profiles are quite irregular. Myofilaments are not present in this view. x 5,300.
Figure 229. Same tumor as figure 228. A cell is rich in skeletal muscle myofilaments that are predominantly of myosin type. x 7,600.

Figure 230. Rhabdomyosarcoma. The cytoplasm contains many sarcomere fragments. x 14,000.
Figure 231. Rhabdomyosarcoma. There is minimal differentiation, limited to the presence of a few aggregates of z-band material with sparse actin filaments. x 17,000.

Figure 232. Rhabdomyosarcoma. Seen in cross section, the filaments are clearly of skeletal muscle type: a hexagonal array of actin filaments surrounds each myosin filament. x 28,000.
Figure 233. Rhabdomyosarcoma. Ribosomes are lined up between myosin filaments in primitive sarcomeres. x 21,000.

Figure 234. Rhabdomyosarcoma. A tangled mass of myofilaments. x 15,000.
Figure 235. Rhabdomyosarcoma. An even denser cluster of myofilaments than in the previous figure but individual filaments can be resolved at the periphery. x 15,000.

Figure 236. Rhabdomyosarcoma. Stellate bodies are an uncommon type of filament aggregate. x 8,800.
Figure 237. Rhabdomyosarcoma. These aberrant forms of sarcomeres have been called zebra bodies. x 14,700.

The large cells of the rhabdomyoma in figure 222 contain zones of glycogen and many organelles. Extracardiac rhabdomyomas have been divided into adult, fetal cellular, and fetal myxoid types279, 280, and skeletal muscle myofilaments form fragments of sarcomeres that are usually short in the adult variety. The sarcomere fragments may be scattered randomly but are more often arranged in rows. In figure 223, their grouping is haphazard and z-bands are unusually prominent. At the edge of this figure, there is a crescentic cell intimately applied to the rhabdomyoma cell in the manner of a satellite cell and they are a common finding281. Some of the organelles and inclusions in a rhabdomyoma are bizarre, and mitochondria can contain membranous structures (figure 224), crystalline material, or large dense bodies (figure 225).

A well differentiated rhabdomyosarcoma is represented in figure 226 by a sarcoma botryoides from the bladder. The segments of sarcomeres are separated but they contain the normal complement of bands. The flattened cell on the surface of the aggregate in figure 227 contains skeletal muscle filaments but it is part of the cytoplasm of a non-neoplastic myofiber that was invaded by a sarcoma, classified by light microscopy as an MFH. Nuclei in an embryonal rhabdomyosarcoma are often quite irregular in profile (figure 228). There are no myofilaments in this field but search of the EM grid led to the discovery of scattered cells rich in myofilaments (figure 229), mostly of the myosin type. Commonly, fragments of sarcomeres in a rhabdomyosarcoma cell are slender and poorly oriented (figure 230). When they are difficult to find, a clue to the diagnosis may be the observation of small densities which on scrutiny are recognized to be fragments of z-bands (figure 231). There is rarely difficulty establishing that filaments are of skeletal muscle type, especially when they display the cross sectional appearance in figure 232 in which hexagonal arrays of actin filaments surround each myosin filament. Sarcomere formation is often rudimentary and z-bands may not be formed, but the structure in figure 233 is nevertheless evidence of skeletal
muscle differentiation: rows of ribosomes between the ends of the filaments create a distinctive picture. A dense clump of filaments is sometimes formed and in figure 234 it is easy to see individual filaments at the periphery, confirming that this is a rhabdomyosarcoma cell. Though it is more difficult with the dense clump in figure 235, filaments can still be identified at the edge of the aggregate. Filaments radiating from a dense central zone (figure 236) are unusual, but the so-called zebra bodies are seen occasionally (figure 237). Among other features that have been recognized in rhabdomyosarcomas, lipid rich cells are uncommon, but neural elements are sometimes identified. EM is useful to reach or verify a diagnosis of clear cell and pleomorphic forms of rhabdomyosarcoma, and to distinguish between a rhabdoid tumor and a true rhabdomyosarcoma.

Tumors of vasoformative cells

Some unique features that are seen by EM in vascular neoplasms reflect the potential of the proliferating cells to fashion the elements of vessel walls.


A report of a tumor that arose in the soft tissues of the neck of an 18-year-old female. EM showed cells containing diffuse nonspecific intermediate filaments, pinocytotic vesicles, and intracytoplasmic lumens, and scattered pericytic cells were identified. A diagnosis of epithelioid hemangioendothelioma arising in soft tissue was made.

Figure 238. Epithelioid hemangioendothelioma. The large lumen in the cell simulates a capillary. The darker cell is pericytic. x 4,500.
A hemangioendothelioma is regarded as a low-grade malignancy of cells with vasoformative potential. The spindle cell variety is considered by some to be a non-neoplastic lesion. The morphologic spectrum of vasoformative cell tumors is broadened by the tendency of neoplastic endothelial cells to assume an epithelioid appearance. Epithelioid hemangioendothelioma is a unique tumor of adult life which is characterized by its component of "epithelioid" or as they have also been called, "histiocytoid" endothelial cells, and this type of transformation may be observed in benign and malignant neoplasms. They can occur in lung or soft tissues, and infrequently in liver, and our experience has been that the ultrastructural features are similar in the different locations. Since the tumor is of low grade, it can be anticipated that its cells will manifest at least some of their capability to form the structural components of blood or lymphatic vessels, and an example is the production of intracytoplasmic lumens (figure 238). The darker cell in this figure is pericytic, and the characteristic shape of the pericytic cell with its radiating arms of cytoplasm encircling the more electron-lucent tumor cells is seen in figure 239. Pericytic cells were identified by EM in a report on three cases from the liver.

Mackay B, Ordonez NG, Huang WL. Ultrastructural and immunohistochemical observations on angiosarcomas. Ultrastructural Pathology 13:97-110, 1989. Forty-seven angiosarcomas were studied by light microscopy, immunohistochemistry, and EM. Neoplastic endothelial cells did not display consistent ultrastructural features but certain aspects of their fine structure and arrangement were found to be useful to establish or confirm the diagnosis. Experience with the common endothelial cell markers as diagnostic aids was briefly reviewed.
Figure 240. Angiosarcoma. The spaces within the cluster of cells may represent crude attempts at lumen formation. x 8,200.

Figure 241. Angiosarcoma. A similar appearance to that seen in figure 240. There are frequent clefts amidst the groups of tumor cells. x 5,300.
Figure 242. Angiosarcoma. Membrane-limited spaces in a tumor cell represent intracytoplasmic lumen formation. x 7,100.

Figure 243. Angiosarcoma. Many erythrocytes were present within the groups of tumor cells. x 5,400.
Clefts within groups of angiosarcoma cells could be artefactual but they are so frequent that their presence suggests crude attempts at lumen formation (figure 240), though cells adjacent to the clefts do not show surface development such as formation of tight junctions (figure 241). The cell clusters may be bordered by a basal lamina but when present it is invariably incomplete. Intracellular lumens are occasionally found in the tumor cells (figure 242). Some cells contain large zones of diffuse, non-specific filaments. The cells of an angiosarcoma frequently show an association with erythrocytes (figure 243), manifested by the present of numbers of them within clusters of tumor cells. This could reflect an affinity of the tumor cells for the erythrocytes, comparable to what is often seen in early Kaposi’s sarcoma, but large numbers of extravasated red cells are not always present in the surrounding stroma, and actual phagocytosis is unusual. Conceivably the groups of tumor cells could be establishing communication with a nearby capillary.

I have not seen Weibel-Palade bodies in angiosarcoma cells, and this was also the situation in a study of 80 cases in which EM was performed on 12 of the tumors\(^ {292} \). The authors noted the presence in tumors from their series of basal lamina, pericytes, intercellular and intracellular lumens with or without red blood cells, whorls of intermediate filaments, and pinocytotic vesicles.


A mass was noticed on the right side of the neck of a 3-day-old boy. Ultrasound examination revealed both cystic and solid components with areas of calcification. Because of the rapid growth of the mass, it was resected when he was 4 months old and was found to be a large multicystic growth infiltrating the trapezius and sternomastoid muscles and occupying the whole posterior triangle of the neck. It was completely resected with removal of portions of the neck musculature. Postoperative recovery was uneventful and there was no evidence of recurrence during a 4-year follow up.
Figure 244. Hemangiopericytoma from the neck of an adult. The tumor cells occupy the stroma between normal-appearing small vessels. x 3,600.

Figure 245. Hemangiopericytoma. A cell contains Weibel-Palade bodies. There is no well-defined basal lamina. 14,000.
The cells in the congenital hemangiopericytoma were closely packed but their fine structure was identical to that of adult tumors that we have examined and the latter are illustrated. Cells in the hemangiopericytoma in figure 244 are rather loosely distributed throughout the stroma amidst thin-walled vessels that are structurally unremarkable (figure 244). The cytoplasm of a hemangiopericytoma cell in figure 245 contains Weibel-Palade bodies (there is no well-defined basal lamina to suggest that this might be a normal vessel wall). The antivon Willebrand factor has been localized to Weibel-Palade bodies. Hemangiopericytoma cells tend to be ovoid to crescentic with nuclei that are often folded, and fine filaments occupy much of the cytoplasm. Small cell junctions can be found but they are primitive in construction. Dardick described variations among the cells of hemangiopericytomas but complicated the terminology by classifying tumor cell differentiation as pericytic (32%), myoid (8%), nondescript (48%), fibroblastic (4%), and histiocytic (8%). The distribution of the neoplastic cells among normal appearing small vessels is itself distinctive, and it is seen particularly well in sinonasal hemangiopericytomas which are often viewed as being distinct from the soft tissue variety, in part because of their improved behavior. In this location, the tumor cells again show bundles of filaments, tapered cytoplasmic extensions, and intercellular junctions.

Figure 246. Kaposi's sarcoma. Elongated cells resembling fibroblasts are present in a collagenous stroma and some contain engulfed erythrocytes. x 3400.
An early stage in the development of Kaposi’s sarcoma is illustrated in figure 246, and spindle cells are present which have some resemblance to fibroblasts in their complement of distended cisternae. Bosman et al.\textsuperscript{297} agree that the spindle cells in Kaposi’s sarcoma have a fibroblast-like aspect by EM. The cells in figure 246 are partially in contact and one has phagocytosed erythrocytes. Those in figure 247 are forming a crude lumen, devoid of basal lamina or cell junctions, that has become filled with red blood cells, and one red cell is sitting in a breach where the wall has not been completely formed (figure 247). Intracytoplasmic lumen formation also occurs. Weibel-Palade bodies could not be identified at this early stage. Erythrophagocytosis was noted in an EM study of skin biopsies from 24 patients of Kaposi’s sarcoma\textsuperscript{298} and the ultrastructural appearance was correlated with hyaline bodies seen by light microscopy.
Tumors of schwann cells

Neural differentiation in neoplastic cells can create striking appearances at the ultrastructural level. They are seen to good advantage in most benign peripheral nerve sheath tumors but are only evident in a minority of the sarcomas.

Figure 248. Schwannoma. Most of the cell extensions are seen in longitudinal section. x 2,900.
Figure 249. Same tumor as figure 248, from an area where the processes are cut transversely. x 2,900.

Figure 250. Schwannoma. The cell is exhibiting mesaxon formation. x 8,200.
Figure 251. Neurofibroma. Tumor cell processes are invested in basal lamina and they contain longitudinally-aligned microtubules. x 6,400.

Figure 252. Perineurioma. Curving whorls of cells with greatly attenuated cytoplasm. x 2,900.
Long extensions of the cytoplasm of the tumor cells dominate the appearance of a benign schwannoma (figure 248). Stroma is present but the large number of processes is evident when they are transversely sectioned (figure 249). A schwannoma cell may manifest mesaxon formation by forming tongue-like extensions of its peripheral cytoplasm that extend like pincers to enclose a few collagen fibrils or a small zone of ground substance, and the cell will then proceed to line the space with a basal lamina (figure 250). In a neurofibroma, the cells and their processes of the cells are similar to those of schwannomas (figure 251), and they are bordered by basal lamina and contain longitudinally aligned microtubules and a few lysosomes and mitochondria. In the so-called cellular schwannoma, the organization of the neoplastic cells is irregular and specific features are more difficult to detect299. A cellular schwannoma lacks Verocay bodies300 and may require EM for confirmation of the diagnosis300. Perineurial cells often coexist within a schwannoma but if they are the only cell type found, the tumor is a perineurioma. The cells have long sheets of cytoplasm that form broad curving parallel bundles and the cell bodies with their nuclei are interspersed among the layers (figure 252). The cytoplasmic extensions of the perineurioma cells lack the smooth profiles and intact external lamina seen in normal perineurial cells302, and the branch frequently (figure 253). Melanotic303, granular301, and epithelioid304 variants of schwannoma have been described. In a glandular schwannoma, the cells of the epithelial component have microvilli with core rootlets305. Heterologous elements such as foci of rhabdomyoblastic differentiation may be detected in a peripheral nerve sheath tumor by immunostaining or EM306.
A diagnosis of malignant peripheral nerve sheath tumor can often be made by routine light microscopy, and it is aided if the patient is known to suffer from neurofibromatosis, or if the tumor is positive with immunostaining for S-100 protein. The tumor illustrated in figure 254 is an unusual variant in that it had extensive myxoid stroma, but the diagnosis was not in doubt after EM study. The long processes of benign peripheral nerve sheath tumors are often visible in the better differentiated malignant tumors and they tend to run in parallel and may be apposed, but they contain few or no microtubules and have at most fragments of basal lamina. A multitude of slender processes is less common but distinctive (figure 255). Comparing the number of processes with the frequency of nuclei, it is clear that many processes must have arisen from one cell either directly or by branching of primary processes. Mesaxon formation was occurring (figure 256), and the cells formed a basal lamina within the enclosed spaces which in this figure contain collagen fibrils. A remarkable amount of mesaxon formation is being displayed by the malignant peripheral nerve sheath tumor in figure 257.

![Figure 254. Myxoid neurosarcoma. The stroma is relatively structureless. Much of the cytoplasm in the figure is made up of closely apposed cell processes. x 3,500.](image-url)
Figure 255. Same tumor as figure 254 illustrating the many slender cell processes. x 7,600.

Figure 256. Same tumor as figure 254. Two sites of mesaxon formation are present. x 7,200.
Figure 257. Neurosarcoma (EM tissue block provided by Dr. Samuel Hammar, Bremerton, Washington). The tumor is showing a marked degree of mesaxon formation. x 3,300.

Variants of malignant peripheral nerve sheath sarcomas occur. Perineurial cells are sometimes present, and some tumors are composed predominantly or entirely of nests of round cells. An example of the latter is described.


A swelling had been present on the dorsum of the right foot of a 43-year-old male for three years and it had been growing slowly for six months. The mass was excised and postoperative radiotherapy was given but two small nodules appeared during therapy, one below the knee and the other below the inguinal ligament, and they increased in size. The upper nodule was biopsied and showed similar histology to the primary. Tumor recurred in the vicinity of the scars on the foot and in the inguinal region, and ten months after the first surgery, a right superficial groin dissection was performed. Metastatic tumor was present in 2 of the 8 lymph nodes in the specimen, and also in the subcutaneous nodule from the lower leg which was excised at the same time. Radiation therapy and chemotherapy were continued but tumor spread within the leg and a right hemipelvectomy was performed. Multiple foci of tumor were present in the subcutaneous tissue of the thigh and inguinal region, and in soft tissues of the popliteal space and thigh. A nodule from the chest wall removed at the same time was also metastatic tumor. Despite further therapy, the patient expired 40 months from the time of the initial surgery on his foot.
Figure 258. Malignant peripheral nerve sheath tumor, epithelioid variant. The tumor cells are forming irregular clusters. Hematoxylin and eosin.

Figure 259a. Same tumor as figure 258. The cells are forming a compact group. x 4,200.
Figure 259b. Same tumor as figure 258. A slender cell process is snaking through the stroma. x 4,000.

Figure 260a. Same tumor as figure 258. A loose collection of processes cut in different planes of section. x 8,000.
This is an example of the epithelioid variant of malignant peripheral nerve sheath tumor\textsuperscript{308-310}. The light microscopic appearance of the tumor is shown in figure 258. Nest of small round cells are irregular and interconnecting, and in some areas, the cells formed short curving and branching cords. Within a cluster, cells were closely apposed (figure 259a) and a few primitive cell attachments could be found. The cells and their nuclei were small and round to ovoid and there was not a continuous basal lamina. A single neural process can be seen emerging from a cell cluster and extending through the stroma in figure 259b. The processes sectioned in different planes in figure 260 do not show a tendency to form parallel bundles. Microtubules and small dense-core granules were present (figure 260b), and were it not for the processes, the tumor could not be identified by EM as a neural neoplasm. As the case report shows, this was an aggressive tumor that responded poorly to combination therapy.

**Soft tissue tumors of uncertain histogenesis**

For a number of defined soft tissue tumors, the cell of origin is still uncertain, and EM can always contribute some enlightenment in this situation even though it may not provide a final answer. Five such tumors are discussed here.

**Synovial sarcoma**

Despite the name, there is little ultrastructural support for synovial differentiation in either the monophasic or the biphasic varieties of this tumor.

Thirty-nine primary synovial sarcomas (15 biphasic, 24 monophasic) and 19 metastatic synovial sarcomas were studied with a battery of antibodies, and 22 of the primary and 18 metastatic tumors were also studied by EM. The results with epithelial markers were significant. Epithelial and/or spindle cells in every biphasic tumor, primary and metastatic, stained positively for keratin and EMA, but only six primary tumors (five biphasic and one monophasic) showed weak reactivity for CEA which, in the biphasic tumors, was confined to the epithelial component. Of the monophasic tumors, 16 primary (83%) and two metastatic (25%) stained for keratin whereas seven primary (29%) and two metastatic (13%) tumors reacted for EMA. Only one primary monophasic synovial sarcoma stained for CEA. Ultrastructural features of tumors from the series are shown in the figures that follow.

Figure 261. Monophasic synovial sarcoma. The cells are closely packed but thin sheets of collagen intervene at some point. x 3,400.
Figure 262. Same tumor as figure 261. When the spindle cells are cross-sectioned, the distribution of the thin bands of stroma are seen more readily. x 3,200.

Figure 263. Biphasic synovial sarcoma. The full thickness of the wall of a gland-like aggregate of tumor cells is shown with basal laminar material and stroma below and lumen above. x 3,200.
Figure 264. Biphasic synovial sarcoma. The cells surround a small lumen. There are very few microvilli. × 3,900.

Figure 265. Same tumor as figure 263. Where two adjacent cells border the lumen, a junctional complex is formed. × 12,500.
The fusiform cells in a monophasic synovial sarcoma (figure 261) are oriented in parallel and tightly packed and only a little stroma is visible. Nuclei are smooth-surfaced, the chromatin is fine, and nucleoli are small. When the cells of the same tumor are viewed in a plane of section perpendicular to the long axis of the cells, the thin trabeculae are seen intervening between small groups but probably no group is completely isolated from its neighbors (figure 262). The cells that form the wall of a gland-like structure in a biphasic tumor are columnar and they rest on a basal lamina (figure 263). A small number of irregular microvilli project from the apical surfaces of the cells into the lumen (figure 264), and the cells are joined at the lumen margin by tight junctions and lower down by small desmosomes (figure 265).

A monophasic synovial sarcoma has some resemblance to a well differentiated fibrosarcoma by light microscopy and a herring-bone pattern can be well developed. By EM, monophasic synovial sarcoma cells have bland nuclei with smooth outlines and fine chromatin, and the nuclei appear to be close together because there is so little cytoplasm. A fibrosarcoma in contrast typically displays some nuclear irregularity and chromosome clumping, and at least a moderate amount of long, tapering cytoplasm that contains considerable numbers of cisternae.

Spindle cells in the stroma of a biphasic synovial sarcoma are not identical in structure to the cells of a monophasic tumor, nor do these stromal cells closely resemble the epithelial cells that form the glandular areas of a biphasic tumor. Despite differences, it is not possible to state that the cells are unrelated. The descriptions are based on classic areas and among the tumors there is some variation in each of the cell types, while the sharp demarcation between epithelial and spindle cells in a typical biphasic tumor is not always seen. In the biphasic tumors, spindle and epithelial cells both stain with epithelial markers although the staining in the spindle cells is weak and patchy. The epithelial cells in the well-defined glandular structures are connected to their neighbors by desmosomes and junctional complexes, but microvilli on these cells are thin and irregular and they do not have microfilament cores. It may well be, as some have suggested\textsuperscript{311,312} that epithelial differentiation occurs within spindle cell areas of the biphasic tumors. Some tumors show marked epithelial predominance\textsuperscript{313} and they could represent the epithelial counterpart of the monophasic spindle cell tumors.

EM has not revealed the histogenesis of synovial sarcomas, but it casts doubt on assertions that they are displaying synovial differentiation. In an examination of eight specimens of normal human tendon sheath and synovium obtained from amputation specimens\textsuperscript{314}, we could not detect a close similarity in fine structure between the cells of the normal tissues and those of the tumors. The lining cells in synovium and tendon sheath do not form an epithelial layer. Instead, they create a loosely arranged stratum several cells thick that does not rest on a basal lamina. With this construction, replacement of surface cells from underlying connective tissue precursors can be easily effected. I have seen a similar multilayered arrangement around breast implants.
Epithelioid sarcoma

The histogenesis of this unusual tumor has not been determined. Electron microscopy has been informative but it has not indicated the cell of origin.


Sixteen tumors diagnosed as epithelioid sarcomas by light microscopy were studied using immunohistochemistry and electron microscopy. Eleven were accepted as classical in that they fulfilled Enzinger's light-microscopic criteria and conformed in anatomic site and behavior. The remaining five were atypical clinically or by light microscopy, and to some degree in their ultrastructure.


A slowly enlarging nodule in the palm of a 24-year-old male had been present for 4 years before it was excised. Three years later, it recurred and was again excised. After a further year, multiple nodules were palpable at the site and the involved area of skin and subcutaneous tissue was resected.

Figure 266. Epithelioid sarcoma, recurrent in the palm. Nuclei tend to be pushed to the edge of the cell. x 3,700
The history of the case is classical for epithelioid sarcoma and the histology was also typical. The nodules of tumor were round and well circumscribed by light microscopy, but by EM, cells within the nodules showed a marked tendency to lose cohesion. The homogeneous cytoplasm and eccentric nuclei are shown in figure 266. Most of the cytoplasm is filled with fine filaments and the few organelles are peripheral and barely visible (figure 267). As in our initial group of cases, tumors that have been called epithelioid sarcomas by light microscopy have occurred in locations other than the distal extremities of young adults. In these situations, it would be desirable whenever feasible to obtain confirmation that the EM appearance is compatible with the diagnosis. Epithelioid sarcoma does not closely resemble any other defined soft tissue tumor in the combination of its clinical features, light microscopy and ultrastructure. Probably the closest similarity by EM is with the epithelioid variants of some soft tissue sarcomas, MFH and angiosarcoma are examples, but the cellular details of epithelioid sarcoma are far from identical with those of any other tumor.
Granular cell tumor

When Abrikosoff described this lesion in 1926, he saw a resemblance between the granular cells and skeletal muscle cells and coined the term myoblastoma, but EM has shown that this is a misnomer. I encountered the first case during my residency in pathology. The second case report describes a malignant example.

A 45-year-old woman underwent surgical exploration for a mucocele of her gall bladder and a granular cell tumor was discovered in the wall of the cystic duct. The lesion measured 7 x 15 mm and was pale, homogeneous and unencapsulated. In light microscopic sections, the tumor cells appeared to merge with the intermuscular connective tissue of the duct wall.

A 7 cm mass on the thigh of a 59-year-old man had been present for at least several months and was gradually enlarging, and left inguinal adenopathy could be palpated. Local excision of the thigh lesion revealed an irregular, grayish-yellow mass within the subcutaneous tissue. A left inguinal lymph node was biopsied and it contained a 1 cm metastatic nodule. Chemotherapy was given. Ten months later, a 5 cm tumor nodule was removed from the left iliac region of the pelvis.
Figure 269. Granular cell tumor. The profusion of lysosomes is illustrated. x 5,300.

Figure 270. Granular cell tumor. Some of the compartments contain lysosomes. Others have few or none. x 4,400.
Figure 271. Granular cell tumor. This view shows that the compartments are often elongated. x 4,400.

Figure 272. Granular cell tumor. Some of the processes contain longitudinally aligned microtubules. x 13,500.
An individual granular cell appears lozenge-shaped by light microscopy and it is relatively large. EM reveals that it is made up of many compartments, each of them membrane limited, with several nuclei scattered throughout the structure (figure 268). When the lysosomes which create the granular appearance are numerous, as is usually the case, the compartments are obscured (figure 269). At the edge of the granular cell in figure 270, lysosomes are sparse and the compartments can be seen without difficulty. Their shape depends on the plane of section, and in figure 271 they are elongated producing an appearance vaguely reminiscent of a peripheral nerve bundle. The similarity to peripheral nerve is heightened by the presence of longitudinally aligned microtubules within the compartments (figure 272). A malignant granular cell tumor (figure 273) has markedly irregular nuclei and it is difficult to identify the membranes around compartments.

Granular cell tumors have been reported from virtually every region of the body including the biliary tree (319). The diagnosis is usually a simple one, but lysosomes can accumulate in other tumors including leiomyoma (320), so EM may be useful for confirmation. I have seen two examples located in the superficial tissues of the breast that were considered clinically and radiologically to be carcinoma and were only recognized as granular cell tumors on frozen section examination. The histogenesis of the lesion is still uncertain but immunohistochemistry and EM favor a neural tumor. I have encountered focal accumulation of lysosomes in an otherwise normal peripheral nerve, and a benign granular cell tumor has arisen from a digital nerve (321). Only a small percentage of granular cell tumors are malignant (322) and EM is then required to establish the diagnosis.
Alveolar soft part sarcoma

The cell of origin of this enigmatic neoplasm has not been determined but EM has narrowed the list of possibilities.


Fourteen alveolar soft part sarcomas (ASPS) were studied using EM and a battery of immunohistochemical procedures in order to assess the extent to which these methods could explain the histogenesis of the tumor. EM clarified the mode of formation of the distinctive cytoplasmic crystals which were detected in all 14 tumors.

Figure 274. Alveolar soft part sarcoma. Crystals are intermingled with round membrane-bound dense bodies, and some small dense vesicles are present in the upper corners of the figure. x 6,800.
Figure 275. Alveolar soft part sarcoma. There are many small vesicles with dense cores in the immediate vicinity of the golgi complex. x 8,600.

Figure 276. Alveolar soft part sarcoma. Crystal formation can be perceived within the dense bodies. x 6,700.
Figure 277. Alveolar soft part sarcoma. Dense bodies between the nucleus and the Golgi zone contain forming crystals. x 13,000.

Figure 278. Alveolar soft part sarcoma. Some of the mature crystals have a limiting membrane. x 7,200.
Figure 279. Alveolar soft part sarcoma. Occasionally, a tumor cell contains many mitochondria with unusual cristal patterns. x 8,400.

Figure 280. Alveolar soft part sarcoma. Zones of smooth endoplasmic reticulum also occur. x 12,000.
The cells in all 14 ASPS had extensive cytoplasm, and the cell membranes of neighboring cells within a group were closely apposed. Spaces at the center of cell clusters are seen in paraffin sections but they do not occur in EM preparations. Some small, uniform lipid droplets were a common finding. Intermediate filaments were sparse and specific myofilaments were never detected.

Cytoplasmic crystals are usually present in at least some cells of an ASPS but they can be scarce and occasionally they are not found in EM sections. In these instances, light microscopic examination of multiple PAS-stained sections may be needed before one is located. Many crystals are rhomboid but some are hexagonal or irregular (figure 274) and they fuse to produce more complex shapes. If crystals are not found, the diagnosis should still be possible from EM study. The golgi complex is extensive, and within and near it, small dense vesicles are always present (figure 275). Numbers of larger membrane-bound dense bodies also lie near the golgi zone (figure 276) and crystal formation can often be detected within them (figure 277). The cell in figure 278 contains small but mature crystals in company with a number of the rounder densities, and several of the crystals have limiting membranes. An occasional finding is a cell with many mitochondria and some of them have peculiar cristal patterns (figure 279). Zones of cytoplasm can be occupied by anastomosing tubules of smooth endoplasmic reticulum which may be in continuity with the cell membrane (figure 280).

Eight of our 14 tumors stained for vimentin, 8 for desmin, 2 for muscle-specific actin, and 1 for fast myosin. No reactivity was detected with other antibodies. These results suggest a myogenic phenotype, and Christopherson raised the possibility in 1952 that the tumor might arise from muscle spindles. Intrafusal fibers have been shown to contain different types of myosin. Any explanation for the origin of the tumor must take into account the cytoplasmic crystals and they are not a feature of skeletal muscle cells, but we have observed crystals in a rhabdomyoma so the myogenic hypothesis should not be discarded.

**Small cell tumor with divergent differentiation**

Examination of ten tumors with EM produced confusing results.

Ordonez NG, El-Naggar AK, Ro JY, Silva EG, Mackay B. Intra-abdominal desmoplastic small cell tumor: a light microscopic, immunohistochemical, ultrastructural, and flow cytometric study. Human Pathology 24:850-865, 1993. In this clinical, light microscopic, and immunohistochemical study of 22 tumors, ten were examined by EM. With the exception of a scrotal neoplasm, all the tumors originated within the abdomen or pelvis and multiple peritoneal nodules were typical. Five tumors also involved the retroperitoneum. Nine of 11 tumors were diploid, one tetraploid, and one aneuploid. 16 patients died of widespread metastases 8 to 50 months (mean 25 months) from the time of diagnosis, and five were alive with known evidence of disease. Immunoreactivity for both keratin and desmin was demonstrated in every tumor although the intensity and pattern of the staining varied. The range of ultrastructure was broad and a specific group of features could not be defined.
Figure 281. Small cell tumor with divergent differentiation. The typical appearance of solid nests of cells within a fibrous stroma. Hematoxylin and eosin.

Figure 282. Small cell tumor with divergent differentiation. A small lumen within a nest of cells. Hematoxylin and eosin.
Figure 283. Small cell tumor with divergent differentiation. A compact nest of tumor cells. x 2,800.

Figure 284. Small cell tumor with divergent differentiation. Some cells contain zones of filaments. x 4,200. The inset shows small desmosomes with short tonofilaments. x 15,000.
Figure 285. Small cell tumor with divergent differentiation. Part of a perinuclear aggregate of filaments. x 25,000.

Figure 286. Small cell tumor with divergent differentiation. A lumen contains some irregular microvilli. x 4,800. The inset shows a tiny lumen plugged by microvilli, with tight junctions connecting the surrounding cells. x 8,200.
Figure 287. Small cell tumor with divergent differentiation. A number of cytoplasmic processes are present in this tumor and one is arising from a tumor cell (arrow). x 4,500.

Figure 288. Small cell tumor with divergent differentiation. A cross-sectioned process contains small dense-core granules and microtubules. x 18,000. The inset shows an obliquely sectioned process with similar contents. x 8,000.
Various appearances can be seen in this tumor by light microscopy but the two most common patterns in our cases were solid nests of closely packed cells sharply etched against a fibrous background (figure 281), and small lumens bordered by layers of similar cells (figure 282). As the electron micrographs demonstrate, a wide variety of fine structure was encountered. A small nest of cells is shown in figure 283, with part of a cluster in figure 284, and the cells have irregular nuclear profiles, fine filaments, and closely apposed cell membranes connected by desmosomes (figure 284–inset). Hyaline zones within the cells are produced by the presence of diffuse nonspecific filaments that can be sufficiently plentiful to displace organelles and nucleus to the edge of the cell (figure 285). A small lumen in figure 286 contains some irregular microvilli, and in the inset a small lumen is filled with microvilli and tight junctions connect the surrounding cells. Some cells have processes that may be sufficiently numerous to form a small bundle (figure 287). Occasional processes contain small granules of neuroendocrine caliber (figure 288) and the process in the inset contains granules and a few microtubules.

The term intraabdominal desmoplastic small round cell tumor was introduced by Gerald et al.\textsuperscript{329} in 1991. Immunostaining for both keratin and desmin is the evidence for divergent differentiation. It is an uncommon neoplasm and study of more cases may clarify the histogenesis, but EM failed to indicate a specific cell of origin and instead indicated that several lines of differentiation including neuroendocrine were being displayed, presenting an argument for derivation from a multipotential cell.
Tumors of cartilage and bone

The tumors discussed in this section are small cell osteosarcoma, variants of chondrosarcoma, parachordoma, and Ewing's tumor.

Requests for assistance in the diagnosis of the common tumors of cartilage and bone, other than small cell tumors, have been infrequent in our EM diagnostic service. The first four figures show typical features of osteosarcoma and chondrosarcoma.

Figure 289. Osteosarcoma. Mononucleated and multinucleated cells were mingled and both contained cribriform cisternal densities. x 4,200
Figure 290. Small cell osteosarcoma. An indication of the size of the cells can be obtained by comparing them with the erythrocytes. x 5,800.

Figure 291. Chondrosarcoma. The cells are within lacunae in the matrix. They have moderate numbers of organelles including slightly dilated cisternae, and a few short filopodia. x 4,200.
Figure 292. Chondrosarcoma. The cells are larger than those in figure 291 and the cisternae are markedly dilated. Electron lucent material occupies much of the cytoplasm. x 4,000.

Although the appearances that are illustrated are typical, a range of fine structure is seen among osteosarcomas depending in part on the line of differentiation, whether fibroblastic, chondroblastic or osteoblastic. One EM study of 27 osteosarcomas identified seven tumor cell types (chondroblast-like, fibroblast-like, histiocyte-like, myofibroblasts, osteoclast-like, malignant multinucleated osteoblast-like, and atypical primitive mesenchymal cells), and endothelial cells and pericytes were also present. Clearly EM has little constructive value in the diagnosis of a tumor with so many variants. Immunohistochemistry also has its limitations in this area. The osteosarcoma cells in figure 289 have dilated cisternae that contain cribiform patterns of amorphous material, and a similar appearance occurs in some tumors of fibroblasts and fat cells. Small cell osteosarcoma cells are relatively small but not particularly uniform, and they are often in contact but have at most only a few primitive cell junctions. They do contain moderate numbers of organelles (figure 290), which distinguishes them from most Ewing’s tumors, but not from mesenchymal chondrosarcoma. Small cell osteosarcoma has been described as having three patterns: Ewing’s like, lymphoma-like, and spindle cell, but most show osteoid formation and the stromal changes are important in differential diagnosis. Evidence of early osteoid production in the stroma is the most helpful ultrastructural criterion in the differential diagnosis of small round cell tumors of bone. Although matrical densities can be seen in chondrosarcomas, their presence in a small cell tumor involving bone should suggest small cell osteosarcoma.
Variants of chondrosarcoma

The typical chondrosarcoma cells (figures 291, 292) are illustrated to contrast them with the variants described below.

Tetu B, Ordonez NG, Ayala AG, Mackay B. Chondrosarcoma with additional mesenchymal component (dedifferentiated chondrosarcoma). II: An immunohistochemical and electron microscopic study. Cancer 58:287-298, 1986. Chondrosarcomas which contained a second, non-cartilaginous mesenchymal component were studied by routine light microscopy, immunohistochemistry, and EM. Cells of the non-chondroid portion stained for alpha-1-antichymotrypsin in 12 of 20 cases and these areas had been classified by light microscopy as fibrosarcoma or MFH. Staining for S-100 protein was consistently negative in the additional component whereas the chondrosarcoma portion stained in 14 cases. Six tumors stained for desmin and 4 of the 6 were positive for myoglobin and 2 for smooth muscle myosin. In 4 tumors, a rhabdomyosarcomatous component was identified in hematoxylin/eosin sections. EM was performed in ten tumors and there was good correlation between the immunohistochemical and ultrastructural findings. The results of this study support the view that the tumors are formed by the synchronous differentiation of two separate clones of cells.

Figure 293. Dedifferentiated chondrosarcoma. A band of filaments surrounds the nucleus. x 4,400.
With the availability of immunoperoxidase methods, EM is not essential in the evaluation of a dedifferentiated chondrosarcoma, but it can serve to confirm the nature of the mesenchymal component. The filaments encircling the nucleus in figure 293 are seen at higher magnification (figure 294) to be of skeletal muscle type and they are forming fragments of sarcomeres.


Eleven examples of extraskeletal myxoid chondrosarcoma from three institutions were studied. Five of the tumors contained intracisternal microtubules, and one of the five was presented in detail. The 4.5 cm mass was from the thigh of a 70-year-old woman and it was well demarcated with a firm consistency and a glistening cut surface. Finger-like extensions of the tumor into surrounding fat could be identified by light microscopy.
Figure 295. Extraskeletal myxoid chondrosarcoma. Much of the cytoplasm is occupied by clear material. x 4,400.

Figure 296. Extraskeletal myxoid chondrosarcoma. A spindle cell is transversely sectioned. The cytoplasm contains a few mitochondria and lysosomes and large numbers of microtubules. x 3,500.
Figure 297. Same tumor as figure 296. Microtubules were confined to the cisternae. A few have solid interiors. x 32,000.

Figure 298. Mesenchymal chondrosarcoma. The small cells are closely apposed in small clusters. They have moderate numbers of organelles including some enlarged mitochondria. x 5,300.
The microtubules were straight, parallel, closely-packed, and evenly spaced, but they did not form geometric arrays. Aggregates of clear material were present in the cisternae as well as the surrounding cytoplasm, but microtubules were never found outside the cisternae. The presence of the intracisternal microtubules that occur in a proportion of extraskeletal myxoid chondrosarcomas can not be anticipated from routine light microscopy or the common immunoperoxidase procedures. They may be only be seen in small numbers (figure 295) but at the other extreme they can occupy much of the cell (figure 296). The only other tumor in which intracisternal microtubules are present in such profusion is malignant melanoma and then only in a small minority of cases. In the melanoma cells that I have studied, the microtubules form geometric arrangements that have not been seen in the chondrosarcomas. As in the melanomas, some of the microtubules have a solid dense core and a thin fuzzy investment is present around each microtubule (figure 297). The tumor cells have been shown to possess tubulin immunoreactivity. I have not observed them in a mesenchymal chondrosarcoma and cisternae are usually short and slender in this variant (figure 298).

**Parachordoma**

Information on the ultrastructure of chordomas proved useful in characterizing the rare parachordoma.

*Figure 299. Chordoma. Within the cytoplasm there is a small intracytoplasmic lumen containing microvillus-like projections. x 4,300.*
A nodule had been present in the soft tissues of the posterior calf of a 20-year-old woman for approximately one year. It was slowly enlarging and was excised. The resected tumor was elongated measuring 5.5 by 1.5 cm, and it appeared to be covered by a thin fibrous capsule. The cut surface had a cystic appearance with a central space into which fronds of tumor projected. A wider excision was performed with removal of at least 1 cm of soft tissue around the previous site, and microscopic groups of tumor extended to the margin of this resection. Additional tissue was taken at the involved sites. EM findings on this tumor and parachordomas from 5 additional patients were described.
Figure 301. Parachordoma. A small lumen contains microvillus-like projections. x 11,400.

Figure 302. Same tumor as figure 301. The cell is bordered by an incomplete basal lamina. x 14,600.
Chordoma cells vary in size and in the number and type of their cytoplasmic contents but many cells have extensive cytoplasm and it always contains vacuoles. Some of the larger spaces are intracytoplasmic lumens bordered by microvilli, and neighboring cells can be connected by desmosomes. A small internal lumen containing a few microvilli is visible in figure 299. Irregular projections of the peripheral cytoplasm can occupy gaps between neighboring cells (figure 300). The lumen with a microvilli in figure 301 may not be intracellular. The nests of cells in this tumor were bordered by an incomplete basal lamina (figure 302).

Dabska used the term parachordoma in 1977 when she reported ten cases that had been collected over a 26-year-period at the Institute of Oncology in Warsaw. She notes that the term has been known since 1951, and that five of the tumors had been included earlier by Laskowski in a Polish textbook. Earlier cases were probably called extraskeletal myxoid chondrosarcomas or given the names of other soft tissue myxoid neoplasms. Povysil examined a tibial parachordoma with EM and agreed that tumors similar to chordoma may occur at sites other than the axial skeleton. The case we reported is significant in that it arose in the calf of a 20-year-old female, and unsuspected small groups of tumor cells located some distance from the main tumor mass were present in wider excision specimens. The cells are keratin-positive as is staining for S-100, and either method provides a useful aid in detecting small foci of tumor within skeletal muscle or other soft tissues. We found that cells that resembled histiocytes and endothelial cells in the hematoxylin/eosin-stained sections could be recognized as neoplastic when they were highlighted with keratin immunostaining.

Parachordoma cells tend to form short chains and nests, and neighboring cells are closely apposed or separated by slender clefts bridged at intervals by sites where the cell surfaces make contact, and small and usually primitive cell junctions can be found. Irregular filopodia project into some of the clefts and from free cell surfaces. Filaments are not obvious within the cytoplasm but small zones or tiny wisps of fine filaments, nonspecific in their appearance, can be detected in some cells. Wavy bands of keratin have not been found in any of our cases. The cells are mostly round but a few are more elongated, and the majority have moderate to considerable amounts of cytoplasm. Vacuoles may contain amorphous or focally condensed material similar to that seen in the adjacent stroma. Lysosomes and lipid droplets are generally present but never plentiful. Small lakes of glycogen can be found in some cells. A basal lamina was present in every tumor we studied but it was invariably incomplete and was absent around most of the cell groups. A thin band of collagen fibrils fringed the fragments of basal lamina but the surrounding stroma was largely structureless.

Comparing the parachordomas with chordomas, there is more variation in size among chordoma cells and many are coarsely vacuolated (physaliferous). Smaller vacuoles are more usual in parachordoma cells. Cell junctions are present in both tumors but they have been primitive in the parachordomas I have examined. A discontinuous basal lamina and small lumens containing filopodia can be found in both tumors, often after some searching. Intracisternal tubules were found in scattered cells of two chordomas and not in the parachordomas. These differences are of degree only and are influenced by the paucity of ultrastructural studies on the rare parachordoma.

An important aspect of the case described above is that small clusters of tumor cells detached from the main tumor mass may go unsuspected by routine light microscopy. Awareness of the possibility should prompt a diligent search for minute foci of tumor in surrounding soft tissues. I detected them in frozen sections of this case during intraoperative consultation. The value of keratin immunostaining has been pointed out. Three of Dabska's ten parachordomas recurred, one of them 19 years after it had initially been excised. Almost all
of the reported parachordomas have been in the lower extremities, and one tumor from another hospital that I was given to present at a national pathology symposium was an elongated mass a few centimeters thick that extended down the femoral canal into the popliteal fossa. The notochordal plate develops in the 3rd week of intra-uterine life and becomes enclosed within the developing vertebral column during the 5th week, and it is possible to envisage that in the early embryo, precursor cells might be drawn from the sacrococcygeal notochord as the limb bud develops and elongates.

**Ewing's tumor**

James Ewing was uncertain of the nature of the tumor that bears his name, calling it an endothelioma of bone in 1921, and three years later, an endothelial myeloma. In recent years, immunohistochemical, molecular genetic, and ultrastructural studies have clarified our understanding of this neoplasm.

**Schmidt D, Mackay B, Ayala AG. Ewing's sarcoma with neuroblastoma-like features.** *Ultrastructural Pathology* 3:143-151, 1982.

Four tumors with the clinical and light microscopic features of Ewing's sarcoma contained cells that possessed to varying degrees ultrastructural features suggestive of neuroblastoma. The tumors were considered to be Ewing's sarcoma of bone by radiologic examination and on clinical grounds and the light microscopy was consistent with the diagnosis in every case.

**Suh CH, Ordonez NG, Hicks J, Mackay B. Ultrastructure of the Ewing's sarcoma family of tumors.** *Ultrastructural Pathology* 26:67-76, 2002.

Dr. Chae Hong Suh participated in the electron microscopy during this study and performed the morphometry. Specimens of 47 tumors diagnosed by routine light microscopy as Ewing's tumor of bone, and 5 histologically similar soft tissue tumors (extraskeletal Ewing's sarcomas), were examined by EM. Immunohistochemistry was performed on all the tumors, and pre- and post-therapy specimens from five patients were compared. Cell and nuclear areas were assessed in 41 tumors by morphometry performed using low magnification electron micrographs. DNA ploidy was determined by static cytometry on 51 of the tumors. None of these methods revealed differences between the bone and soft tissue tumors. The ultrastructural spectrum extended imperceptibly from the typical forms to variants with irregular cells and nuclei, and was much broader than could be anticipated from light microscopy. Neural features were observed but they were not common. Morphometric comparison of the Ewing's sarcomas with a group of other small round cell tumors (rhabdomyosarcoma, neuroblastoma, small cell carcinoma) using the same procedure showed that the areas of the cell bodies and nuclei are similar and therefore not of value in attempts to distinguish among them.
Figure 303. Ewing's tumor. The cell is from a fine needle aspiration biopsy. x 3,600.

Figure 304. Same specimen as figure 304 showing a lake of cytoplasmic glycogen. x 9,700.
Figure 305. Ewing's tumor. A typical field with close apposition of the cells and rare primitive cell junctions. Nuclei have dispersed chromatin and small nucleoli. Organelles are sparse. x 3,600.

Figure 306. Ewing's tumor. The cells contain considerable amounts of glycogen, and they have dendritic processes that are cut in various planes of section. x 4,200.
Figure 307. Same tumor as figure 306. Small granules are visible in one of the processes. x 16,000.

Figure 308. Same tumor as figure 306. Dendritic processes are cross-sectioned. The largest process is connected to the cell body by a primitive cell junction. x 9,800.
The cells from a Ewing’s tumor in figures 303 and 304 were obtained by fine needle aspiration biopsy and are evidence of the good preservation that can be obtained when the aspirate is processed correctly, using the procedure described earlier (page 10). Cells and nuclei typically have smooth surfaces and scanty cytoplasm with few organelles. Discrete lakes of glycogen are common though the amount ranges from copious to scanty, and it may be absent (more likely in treated cases). In a solid tumor, the cells are as a rule closely packed with intimate apposition of the cell membranes (figure 305) and scattered minute attachment densities. A minority of the tumors contain cells with moderate numbers of organelles, little or no glycogen, and irregular cell and nuclear profiles, and atypical Ewing’s tumor is sometimes diagnosed by light microscopy.

When the manuscript describing coexistence of features of Ewing’s tumor and neuroblastoma in small cell tumors was submitted for publication, a reviewer pertinently asked if these tumors were primarily neuroblastomas or Ewing’s tumors. I responded that the diagnosis of Ewing’s tumor of bone had been made in each of our cases on clinical and radiologic grounds. A distinction on the basis of routine light microscopy alone would be difficult although an occasional primary small cell bone tumor has an obvious rosette pattern that will strongly suggest a neural tumor. In these four cases, the neural features were not well developed in comparison with typical neuroblastomas, and the ultrastructural features were more in keeping with Ewing’s tumor. The cells contained cytoplasmic lakes of glycogen (figure 306) which are uncommon in a true neuroblastoma, and they had in addition irregular cell margins, and, relative to neuroblastoma, only occasional slender cytoplasmic processes containing small dense-core granules and some microtubules (figure 307). The largest process in figure 308 is connected to the adjacent cell surface by a primitive junction.

The collective findings from this study are in keeping with the currently favored view that Ewing’s sarcoma and peripheral primitive neuroectodermal tumor are at the extremes in a morphologic continuum within which neural differentiation ranges from absent to prominent. The so-called Askin tumor may fall within this spectrum. I found that a peripheral neuroectodermal tumor of the chest wall in a 19-year-old female had irregular dendritic processes with rare dense-core granules, and others have made a similar observation. It now appears likely that the Ewing’s sarcoma family of tumors is derived from pluripotent neural crest cells that synthesize acetylcholine transferase and lack adrenergic features, characteristic of primitive cells associated with the parasympathetic autonomic nervous system. All Ewing’s sarcomas share several unique chromosomal translocations, involving the EWS gene located on the long arm of chromosome 22 and several other chromosomes, resulting in chimeric protein production that leads to malignant transformation.
Tumors of the central nervous system and meninges

Glial cell tumors are commonly diagnosed and graded by light microscopy. In an early study, we recognized that some ultrastructural features are common to all gliomas and are thus of limited value in subclassification. More recent publications have demonstrated the breadth of the ultrastructural spectrum, and shown that EM is still of value, particularly in view of the morphologic diversity of astrocytomas, and because rare examples occur in uncommon sites such as the leptomeninges, the cerebellopontine angle, or even outside the neuroaxis. Tumors of ependymal and meningeal cells show highly distinctive features at the ultrastructural level and they are discussed in this section.

A common finding among astrocytomas is the presence of cell processes and they can be numerous and highly irregular as in the glioblastoma in figure 309. In contrast, oligodendroglioma cells are typically smaller and more or less round with few organelles but they often contain large quantities of concentrically oriented filaments (figure 310).

Figure 309. Glioblastoma. The cells are extremely irregular but a suggestion of process formation is conveyed. x 4,100.
Ependymoma

Among primary brain tumors, ependymoma has probably the most unique combination of ultrastructural features.


Twenty-six ependymomas were studied by light microscopy and EM. The acellular zones seen around small vessels by light microscopy are made up of large numbers of closely packed, filament-rich cytoplasmic processes encircling the narrow band of collagenous stroma that surrounds the vessels. Lumens were consistently present in the tumors, many of them small, and they contained slender, curving microvilli and varying numbers of cilia. The cells bordering lumens were connected by unusually long tight junctions.
Figure 311. Ependymoma. Cilia, microvilli and a long tight junction are shown. x 7,800.

Figure 312. Ependymoma. The cells have intracytoplasmic lumens. x 3,000.
Figure 313. Ependymoma. A small lumen is filled with microvilli and bordered by cells with long tight junctions. x 11,000.

Figure 314. Ependymoma. Filament-rich processes terminate on a convoluted basal lamina near a small vessel. x 5,200.
Ependymomas are relatively frequent tumors of the central nervous system, accounting for 6% of all intracranial gliomas and for approximately 60% (though there is much variation among reported figures) in the spinal cord\(^\text{356}\). The vicinity of the 4th ventricle is the most common single site and 9 of the 26 tumors in our series occurred there. Two of our cases were extra-axial tumors: one presented as a pelvic mass involving the right adnexa, and the other was a midabdominal mass centered on the mesentery. EM can be useful to establish or confirm a diagnosis of ependymoma when the light microscopic histology is atypical or when the tumor arises in an unusual location. The embryonic heritage of the ependymal cell confers the potential for epithelial and/or glial expression within its neoplasms. The presence of perivascular pseudorosettes is characteristic, but true rosettes in the form of small glands or tubules were only found in 35% of our cases by light microscopy and they were often sparse, requiring examination of multiple sections for their detection. We did not include ependymoblastomas or subependymomas in our study but the ultrastructure of these neoplasms has been described\(^\text{357, 358}\). Ependymoma cells contain varying numbers of glial filaments and they can occupy much of the cytoplasm. Glycogen is sometimes plentiful\(^\text{359}\). Mucin\(^\text{360}\) and melanin\(^\text{361}\) are unusual components.

Cilia, many of them with abnormal axonemes, were found in 73% of the 26 tumors we studied (figure 311), in some instances after considerable searching but the two intra-abdominal tumors contained many cilia. Microvilli greatly outnumber the cilia, and they tend to curve and intertwine. Some of the lumens in figure 312 are probably truly intracellular\(^\text{362}\), and signet ring cells have been described\(^\text{363}\). The junctions that unite the apical margins of adjacent cells around a lumen are unusual. They suggest unusually long tight junctions but the densities on the apposed cell membranes are separated by a slender gap (figure 313) and they are clearly different from the zonulae occludentes (tight junctions) of epithelial tumors. Lumens were found in 96% of our cases by EM and most of them were too small to be resolved in paraffin sections, particularly since they were to some extent obscured by being packed with microvilli. The lumens presumably reflect an attempt by the tumor cells to recapitulate the normal ependymal cavity. Conversely, the glial phase of ependymal differentiation is manifested by the formation of numerous cell processes containing a profusion of glial filaments, and the acellular zone created by a layer of closely packed processes around a small vessel is a distinctive diagnostic feature by both light and electron microscopy (figure 314). The processes vary in number and they can be sparse and hard to find, but they dominate the histology of the so-called tanycytic ependymoma. In this variant, true ependymal rosettes are absent, and perivascular rosettes are inconspicuous, so misinterpretation as schwannoma or astrocytoma is a diagnostic problem\(^\text{364}\). Microvilli and cilia are present in myxopapillary ependymomas of the filum terminale and conus medullaris or subcutaneous tissues of the sacrococcygeal region, and some of the cells covering the papillary cores contain mucin.
Meningioma

Some meningiomas present in extracranial sites and EM can be useful to confirm the diagnosis. Three case reports are summarized.

A 19-year-old male complained of a burning sensation in the right eye and his eyelid was seen to be drooping. On clinical examination, he was found to have Horner's syndrome and a large firm mass could be felt behind the ramus of the right mandible. Complete paralysis of the right hemilarynx was observed. On surgical exploration, the firm mass was found to extend to the base of the skull and was densely adherent to the carotid vessels. It was resected with sacrifice of the external carotid artery and parts of the 9th, 10th and 11th cranial nerves and sympathetic chain. Psammoma bodies were present, and EM confirmed the diagnosis of meningioma.

A 44-year-old woman was investigated for a seven month history of intermittent headaches, nausea, explosive vomiting, episodes of diplopia with transient loss of vision, progressive hearing loss, and pain in the right ear. The inferior half of the bony portion of the external auditory canal was deformed by a soft tissue mass that could be felt under the right sternomastoid muscle and was fixed to the temporal bone. Tomograms showed erosion of the right jugular foramen and adjacent bone. A biopsy was performed and the initial diagnosis from light microscopy was carotid body tumor, but the clinical findings rendered this diagnosis suspect and the decision was made to operate. At surgery, a rather soft encephaloid tumor was found involving the lower portion of the tympanic cavity and the floor of the external auditory canal and extending down into the soft tissues of the neck within the internal jugular vein. The diagnosis was still uncertain until EM was performed. The tumor was encased by the wall of the internal jugular vein.

A nodule from the occipital region of the scalp in a 14-year-old boy had been present for approximately one year. It appeared to be growing slowly and a biopsy was obtained. The diagnosis was difficult by light microscopy but after EM study a diagnosis of meningioma was made and the tumor was then widely excised. It extended down to the periosteum but bone was not penetrated. The tumor recurred after several months, and after two years metastasized to lung and pleura, and one year later to chest wall.
Figure 315. Meningioma. The spaces between cells are bridged by branching extensions of cytoplasm. x 3,500.

Figure 316. Meningioma. The boundaries of the closely packed cells are delineated by the curving cell membranes. x 4,700.
Figure 317. Meningioma. Slender processes are joined by desmosomes with short tonofilaments. x 12,000.

Figure 318. Meningioma. In this extracranial tumor, the cells had extensive, elaborately curving extensions. x 7,600.
Figure 319. Meningioma. Cells from a tumor within the jugular vein have extensive cytoplasm, undulating cell membranes, and small desmosomes. x 8,600.

Figure 320. Malignant meningioma. There are many diffuse filaments in the tumor cell cytoplasm. x 12,000.
An ultrastructural comparison of human arachnoid villi and meningiomas365 showed that
common features are plasmalemmal interdigitations, desmosomes, and cytoplasmic
filaments. The most characteristic aspect of the tumor cells is the undulating cell surface
from which many curving extensions of cytoplasm extend into the surrounding stroma
(figure 315). The processes are compressed when the cells are closely packed but their
presence contributes to the infoldings and interdigitations of the apposed cell surfaces (figure
316). The cytoplasm of the cells in this figure have a uniform electron density because they
contain large numbers of filaments, and the presence of intermediate filaments is a frequent
finding in the cytoplasm of meningioma cells. Two-D gels revealed vimentin and vimentin
breakdown products as the only intermediate filament present and these findings were
verified by immunoblots369. Cells and processes are connected by mature desmosomes with
wisps of attached tonofilaments (figure 317).

The three case reports are examples of meningioma occurring in sites other than within the
cranial cavity. In the first case, the tumor in the neck appeared by light microscopy to be an
extracranial meningioma because of its architecture and the presence of psammoma bodies,
and ultrastructural detection of scroll-like extensions of the cell cytoplasm confirmed this
impression (figure 318). The diagnosis in the other two cases was not reached by light
microscopy but was apparent when the tumors were examined by EM. Many cells in the
tumor within the jugular vein were filled with filaments (figure 319) and they filled the
cytoplasm of plump epithelioid cells in the malignant meningioma shown in figure 320.
Papillary meningiomas have a similar fine structure367. I have not seen oncocytic
transformation of a meningioma but it has been reported and was found to be an aggressive
tumor368.
Electron microscopy of tumors in a department of pathology

While the electron microscope was being developed and for a time after it had been introduced, some scientists and pathologists were optimistic about the diagnostic potential of the instrument, believing that it might find an extensive role in service work. Initial enthusiasm had to be tempered as it became evident that most of the activities of routine surgical pathology could still be performed adequately by pathologists using the conventional light microscope. The benefits of EM were in some instances significant but they were restricted to a few areas of surgical pathology.

More than one kind of electron microscope was developed, but the transmission instrument, in which the beam of electrons passes through a thin slice of a specimen, is the only type that has had substantial value in diagnostic pathology. The scanning electron microscope can provide graphic and often visually impressive images of the surface of a specimen, but even with the use of specialized methods to prepare a specimen, such as freeze fracture techniques, the amount of information it provides on the interior of cells is limited, and virtually all the data that it provides can also be seen and often more clearly visualized from thin sections studied with the transmission instrument. Analytical electron microscopy yields information on the elemental composition of material within tissues and its use is rarely indicated in the routine study of human tumors. Immunoelectron microscopy allowed the precise localization of cellular and subcellular products within tissues and it is a valuable research tool but is not practical in routine tumor diagnosis since clinically relevant information can be obtained more rapidly and conveniently using immunoperoxidase methods and paraffin sections.

Range of contributions

The electron microscope was found to be of some benefit in the diagnostic evaluation of a small number of non-neoplastic specimens, and it continues to have an application in the interpretation of glomerular pathology. In oncology, it has been used with some success as a method for detecting and quantitating cardiac damage in patients receiving Adriamycin and other cardiotoxic drugs: my experience has been described in a number of publications. The most extensive role of transmission electron microscopy in surgical pathology has been in the study of human tumors, and the selection of observations in this thesis demonstrates the variety of the contributions it has made in this field.

Laboratory equipment and organization

The size of an electron microscope laboratory and the amount and type of equipment it contains vary greatly among institutions. Both are related to the size of the hospital and the number of specimens that are handled. A self-contained unit in a large hospital or medical center will be equipped and staffed to accession specimens, process the material, section and stain semi-thin and thin sections, evaluate specimens and issue reports on the findings, and perform a full range of black and white photographic services. In addition to these routine activities, the staff will often be called upon to participate in instructing residents, fellows, and histology technicians who are training in the department. In a smaller hospital, some of the equipment may be shared with other sections or departments, and photography may be performed in a central facility though there are distinct advantages to doing it within the pathology department.

Within the EM laboratory, a designated area should be assigned for the logging-in of specimens. Mechanisms will already be in place for conveying to the pathology department tissues for light microscopy from various sites in the hospital and from outside the hospital.
for review or consultation, and it is likely that specimens for EM can be transported in the same way and delivered to the EM laboratory. A designated area should be dedicated to gathering, logging in, and processing the tissue specimens and it should include a bench with a cutting block on which the tissue is diced, an adjacent sink, a nearby storage area for reagents and embedding media, an embedding oven, and a fume hood: the latter is mandatory when post-fixation with osmium tetroxide is part of the procedure.

In a small department, a single ultramicrotome may be used to cut both semi-thin and thin sections. Larger laboratories will often utilize an older ultramicrotome and use glass knives to cut semi-thin sections of the plastic-embedded tissue blocks for light microscopic evaluation, while one or more good ultramicrotomes are reserved for thin-sectioning with diamond knives and they are ideally are located on vibration-free tables in enclosed spaces free from air currents or rapid temperature changes.

It is good practice to set aside a small covered area to stain the thin sections. A cabinet with a folding glass front and arm holes can be constructed, but an inverted cardboard box will serve the purpose if nothing better is available. The frequent problem of contamination of the grids from dust in the laboratory air is then minimized.

Photographic film must be loaded into the electron microscope and exposed film has to be developed and the negatives printed. Depending on the model of electron microscope, it may be convenient to have a small light-proof cubicle adjacent to the microscope room where film cassettes are loaded and emptied. In all but the smallest laboratories, the photography is customarily performed within the pathology department, and many EM units have their own darkroom. There are obvious advantages in this arrangement. While a verbal opinion can often be rendered by the pathologist evaluating a particular specimen, it is sometime desirable to wait until micrographs have been printed, and this process is slowed considerably, and the cost escalated, if the negatives have to be sent to a separate photography section outside the pathology department. To expedite the preparation of micrographs on diagnostic cases, an automatic film processor is an asset. A Polaroid camera is sometimes used to provide rapid prints of EM observations but larger micrographs are preferable.

Currently, digital processing and storage of electron micrographs is being developed and expanded and I regret that I did not have the benefit of this technology.

**Documentation of specimens**

Tissue taken for EM from a larger specimen can be identified using the accession number assigned by the department, but it is advisable to assign an EM accession number and cross reference it to the pathology number. Even in a large department, the EM number is not likely to exceed five digits and it is therefore more convenient to use on EM tissue blocks, semi-thin section glass slides, petri dishes for thin-sections, negative envelopes, and photographic prints. Reports of the EM must include the pathology accession number and the EM number.

**Diagnostic procedure**

In some EM laboratories, a technician takes a number of photographs of a thin sections of a specimen, often of random areas, and prints are given to the pathologist who draws diagnostic conclusions by perusing them. This practice can lead to erroneous and missed diagnoses. Even an experienced technician cannot substitute for a trained pathologist who is
familiar with the clinical situation and has examined the semi-thin sections of the plastic-embedded tissue, verifying that they are representative and forming a mental image of the architecture of the area of the specimen that will be seen with the electron microscope. The last point is important since it is not always easy or even possible to correctly identify non-neoplastic components of a section from their ultrastructural appearance when they are mixed with neoplastic cells. Mesenchymal cells can readily be mistaken for sarcoma, plump endothelial cells may mimic adenocarcinoma, and inflammatory cells are readily misinterpreted as belonging to a lymphoma or leukemia. With prior knowledge of the clinical differential diagnosis and the histopathology, and awareness of the composition of the tissue within the thin section, these mistakes can be avoided. The pathologist performing the EM will draw diagnostic conclusions during the course of the study, rejecting some possibilities from the differential diagnosis and possibly introducing another idea. It is often possible to give a verbal opinion on a case as soon as the EM is performed, without having to wait for photographs to be printed, though in some instances the latter is necessary and the pathologist should not be rushed by pressure from an understandably impatient clinician anxious to institute therapy.

Value and limitations in diagnosis

There are situations in which the electron microscope provides a firm diagnosis of a tumor that could not be obtained by other means. These are satisfying but not frequent. More commonly, the ultrastructural findings serve to eliminate some possibilities that could not be ruled out by light microscopy or immunohistochemistry, thereby narrowing the differential diagnosis, and often weighting the remaining possibilities to the point where one is favored. It is always necessary to correlate the findings from EM with the available clinical information including results of radiologic studies, and of course with the routine light microscopy, and a pathologist trained in EM is the one physician with sufficient breadth of expertise to assess all the data.

The greatest contribution of EM in the diagnostic evaluation of tumors is with those that appear undifferentiated by light microscopy. Small round cell tumors devoid of any architectural pattern are an example and EM has made great strides with these neoplasms in pediatric patients and adults. Some pleomorphic poorly differentiated tumors do not possess structural features that will allow their classification by EM and distinction of an epithelial from a mesenchymal neoplasm is sometimes difficult. Among epithelial malignancies, a diagnosis of adenocarcinoma may be possible but when it is metastatic there is not always a clear indication of the primary site. Squamous differentiation is readily recognized when the tumor is at least moderately differentiated, but if metastatic, the primary site can not be determined as the tumors look the same regardless of the site of origin. Minimal differentiation may be detected by EM when it is not visible in paraffin sections, but such slight maturation can have little or no therapeutic relevance, as in the case of glandular structure in a large cell undifferentiated lung carcinoma. Soft tissue neoplasms tumors display a broad range of ultrastructure and subclassification of a sarcoma can often be achieved but the information may not influence management of the patient. These negative aspects should not deter the electron microscopist from striving for precision in the diagnostic assessment. The data may be extremely useful in subsequent clinicopathologic studies of series of cases.

EM reports

When EM is the only diagnostic study performed on a specimen, a report on the findings is issued by the pathologist responsible for, and hopefully performing the ultrastructural study. Generally, however, there is also tissue for routine light microscopy, and
immunohistochemistry may be performed. The electron microscopic findings must then be correlated with those from light microscopy, therefore a report of the EM, with selected micrographs, must be sent to the pathologist who is performing the light microscopy so that he/she can incorporate the findings in the initial or a supplemental report. The terminology that is used in a report to describe the ultrastructural findings should be kept as simple as possible, particularly if as is likely it will be read and interpreted by a clinician. Opinions from the electron microscopist on the possible diagnostic implications of the ultrastructural findings will often be helpful, but they should not be given independently to a clinician by the electron microscopist when other material is also being evaluated by a pathologist who is responsible for the entire case.

A variety of formats can be used for the EM report. A free-form report generated by a word-processing program is convenient and flexible, and the data can be transferred to a regular pathology report form if it is to be issued alone rather than being incorporated within the report of the entire pathology specimen. The EM reports can be stored in a computer within the EM Unit, and if the electronic record is reliably backed up, storage of hard copies may be dispensed with.

Data storage and retrieval

Particularly in a large department, a mechanism for rapid retrieval of data from the EM archives is necessary. It should be possible to rapidly identify past cases by patient name or pathology or hospital number, and locate diagnostic reports and stored micrographs and photographic negatives. Accessions of a particular tumor may be desired for retrospective studies, and interesting or unusual observations may be sought for presentation or publication. The laboratory information system used throughout the department for the storage and retrieval of routine light microscopy cases may serve the needs of the EM section, but many of the specimens accessioned for EM are not immediately sectioned and are simply stored, usually by EM number. In a large department with many EM specimens, it is more convenient and efficient to maintain a separate database for the EM specimens, and the diagnosis of record from the routine light microscopy and the report of the EM can be incorporated in the database. Information on the quality of the EM specimen and the numbers of representative tissue blocks are helpful when past specimens are retrieved for elective study, and a field can be used to record notable ultrastructural features.

The number of photographs taken on a particular tumor will vary depending on the complexity or level of interest of the neoplasm. Pathologists-in-training inevitably take more than an experienced pathologist. To keep the archives compact, redundant prints can be discarded when the case has been completed, particularly if the negatives are all being retained. In the old days when glass plates were used, storage problems included available space and sturdy cabinets. Now that sheet film is standard, these are no longer issues. The size of the data storage facility and speed of retrieval of particular cases or entities can be greatly facilitated by the use of electronic methods.

Quality assurance

Factors that influence the accuracy and efficiency of the work being performed in an EM diagnostic service should be assessed periodically to detect and correct problems. They include the level of preservation of the material being received, recurring technical problems in technical work such as chatter, low contrast, or stain or other contamination in thin sections, and the speed with which specimens are being studied and reported. Technical problems with the processing of tissue and the preparation of thin sections can be communicated verbally to the EM technicians, but it is worth also entering the information in
a log book maintained for the purpose. A well maintained log provides the head of the section a convenient way to monitor the quality of material and preparations over a period of time, and it will impress accreditation inspectors.

The degree of accuracy of the diagnostic work performed in the EM section is more difficult to assess, but it is necessary to develop a routine whereby this is achieved. Although it can be a time-consuming procedure for the assigned person, the diagnostic reports of the EM section can be correlated with the final diagnoses issued by the department. This responsibility will generally fall to the head of the EM section. It is easy to misinterpret a tumor specimen submitted for EM, and in a large department, more than one pathologist may share in the activities of the EM service. The responsibility for monitoring the quality of all the EM work that is performed rests with the head of the section. It is hardly feasible to restudy every biopsy and my policy has been to read the diagnostic reports and discuss any questions that might arise. When a pathologist is being trained to participate in the work of the section, the onus is on him or her to draw attention to any case in which there is a discrepancy between the EM and the routine light microscopy. On a controversial case, a second opinion can be sought from a suitably experienced pathologist in another hospital, making available light microscopy slides, clinical information, electron micrographs, and a representative EM tissue block.

Educational activities

Pathologists in training (residents or fellows – at the M.D. Anderson Cancer Center, residents in their final year of training are accepted and they are known as fellows) receive at least some instruction on tumor ultrastructure and its application in diagnosis. Hands-on involvement is also possible during an elective rotation of one month within the sections of EM and immunohistochemistry, but there are many subspecialties within the pathology department and the most important need in the final year of training is intense exposure to advanced light microscopy, so my view has been that a fellow should only select this elective if he or she anticipates an active role in diagnostic EM as a staff pathologist. Every fellow receives some exposure in two ways. A case submitted for diagnostic EM is returned with a written report and selected electron micrographs to the staff pathologist and fellow responsible for the case. In addition, a weekly conference devoted to EM in which I have conducted gives the opportunity to present and discuss past and current cases. As the publications included in this thesis show, fellows frequent coauthor papers based on findings from EM.

A fellow rotating through the EM service will understandably wish to perform the EM on current cases, but will not be sufficiently experienced to carry out the study rapidly or reach a reliable diagnosis. A compromise is for the staff pathologist to perform the EM study than pass the grids to the fellow without voicing his opinion on the case: once the fellow has performed his or her own study and reached a conclusion, the two can discuss the case together and review the electron micrographs once they have been printed. The fellow may be allowed under supervision to prepare the report of the EM study.

Dissemination of information on EM is facilitated by the publication of original findings in scientific journals. Clinical case reports that include light microscopy in addition to the ultrastructural observations are particularly useful to spread awareness of the value of EM in diagnosis. Much of the work from my section has been reported in the journal Ultrastructural Pathology where correlative studies are encouraged, but in order to reach a wider audience it is also necessary to utilize general pathology journals, and to periodically write reviews in which the diagnostic value of the technique is presented and correlated with routine light microscopy and immunohistochemistry. It is important in such articles to use
terminology and illustrations that can be comprehended by general pathologists with little or no past exposure to ultrastructural studies.

Current and future roles

I believe that the material in this thesis provides evidence that EM has a significant role in the diagnostic assessment and academic study of human tumors. Its contribution has been restricted by a number of factors including economic considerations, a lack of appreciation among many pathologists and most clinicians of the potential of the technique and the indications for its use, a constant shortage of competent pathologist-electron microscopists, and economic factors that have severely restricted the availability of electron microscopes and drastically limited their use in diagnostic pathology. The technique has been underutilized in the past, and with the current extensive use of immunoperoxidase methods, the situation in the United States is that there are now relatively few active diagnostic EM services. Units that continue to function in small hospitals depend for their survival on a constant supply of renal biopsies. Those in larger medical centers often owe their existence to the use of the facility by researchers.

Considerable pessimism is the logical response to the current situation and the future for diagnostic electron microscopy would appear to be bleak. It remains the case, however, that many tumors can not be confidently classified by routine light microscopy even when it is complemented by available immunohistochemical methods, and in many of these cases, ultrastructural study would be informative. In addition, the potential of EM in the retrospective study of interesting or controversial tumors is underutilized. For the technique to remain viable, it is necessary that electron microscopy units continue to receive specimens from their own institutions and from other pathologists in consultation. The service should be provided at a cost that is not so prohibitive that specimens will no longer be sent, and the diagnostic procedure must be performed efficiently and reported rapidly. To promote use of the service, and to further our knowledge of the ultrastructure of human tumors, there is a continuing need to keep before the attention of pathologists the value of electron microscopy, through incorporation of ultrastructural observations in pathology papers and production of readable review articles, and by presentations at pathology meetings. Given the opportunity, transmission electron microscopy can continue to provide important information on the structure, histogenesis and classification of tumors.
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