Pathology of Human Malignant Mesothelioma

By Yasunosuke Suzuki

THE EXISTENCE of malignant mesothelioma was recorded before the end of the nineteenth century. The pleural lesion reported by Wagner[1] in 1870 is now undoubtedly considered to be a malignant mesothelioma.[2] In those days, terminology for mesothelioma was a matter of controversy, and the term "endothelioma" was frequently used for this tumor.[2,3] DuBray and Rosson[2] proposed the term primary mesothelioma of the pleura in 1920, having concluded that the tumors arose from the surface of the parietal pleura.[2] In 1931, Klemperer and Rabin[4] described two cases from Mount Sinai Hospital, and added three from the European literature. From these cases they developed many of what are now accepted as the modern concepts of the pathology of diffuse malignant pleural mesothelioma. They concluded that (1) the localized giant tumor connected to the visceral pleura was usually benign originating from the sub-mesothelial connective tissue; (2) the gross anatomy of malignant mesothelioma usually was diffuse originating from the mesothelial cells; and (3) malignant diffuse mesothelioma was highly variable histologically due to the probable multipotentiality of the mesothelial cell, which could differentiate into various cell lines. After this report, the term malignant mesothelioma became popular. The biologic behavior of the tumor, such as its ability to differentiate into distinct histologic types, remained of academic interest.[4-6]

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In 1953, Weiss[7] suggested that asbestos exposure was responsible for the induction of malignant mesothelioma in an asbestos worker. In 1960, Wagner et al.[5] reported on the strong association of asbestos exposure and malignant mesothelioma. They identified multiple cases of malignant pleural mesothelioma among residents in the Northwest Cape Province of South Africa, all but two of whom had had exposure to asbestos dust. Since then, others have confirmed and extended these observations both epidemiologically and in animal experiments.[9-12]

In the past, malignant mesothelioma was a rare tumor occurring in 0.1%–0.01% of autopsies.[13] In the past 20 yr, however, the incidence of the tumor has been rising.[5,9,12] Recent epidemiologic studies[9,12,14,15] indicate that there is a long latent period (20–40 yr) from asbestos exposure to the development of the tumor. These studies have also shown that malignant mesothelioma can occur in association with a wide range of asbestos exposures, including direct occupational, indirect occupational, and family contact exposures. Since submicroscopic asbestos fibrils can be seen by electron microscopy in the lungs of patients with mesothelioma who did not exhibit clinical evidence of asbestosis, it is possible that low level exposures to asbestos can induce malignant mesothelioma.

Generally, malignant mesothelioma is considered to be a malignant diffuse tumor arising from the mesothelial cell. Alternate explanations, however, come from some experimental studies. In these studies, both the mesothelial cell and the submesothelial mesenchymal cell[16,17] have been implicated as the precursors of various forms of malignant mesothelioma.

Regardless of the cell of origin, the surface linings of three serosal cavities, pleura, peritoneum, and pericardium, are known to be the major sites of malignant mesothelioma. The incidence of pericardial mesothelioma is considerably less frequent than pleural or peritoneal mesothelioma. In material submitted to our laboratory, the ratio of pleural:peritoneal:pericardial mesotheliomas has been 57.1%, 39.5%, 1%, respectively. The site of origin of the remaining 2.4% could

not be determined, since both pleura and peritoneum were extensively involved by the neoplastic process. In addition to these three major sites, rare mesotheliomas of the serosa of the genital tract and organs such as the tunica vaginalis, ovary, uterus, and fallopian tubes have been reported.[18 27] Both benign and malignant forms have been seen. These mesotheliomas have not as yet been associated with asbestos exposure, with the possible exception of one patient with malignant mesothelioma of the tunica vaginalis.[19]

Klemperer and Rabin[4] classified mesotheliomas as either benign, which was usually localized and histologically showed only a fibrous form, or malignant, which was usually diffuse, and three forms could be distinguished histologically: epithelial, biphasic (mixed), or mesenchymal or fibrous (sarcomatous). On the other hand, Stout[28] also divided mesothelioma into benign and malignant, but both were classified into diffuse and localized. He further divided the localized and diffuse into epithelial, mesenchymal, and biphasic forms on the basis of light microscopy. These diverse classifications were based on necropsy findings.

DIAGNOSTIC CRITERIA

A comprehensive approach using gross anatomical observations, histology, histochemistry, electron microscopy, and possibly biochemistry of the fluid and cytology have been attempted for diagnosis of malignant mesothelioma.

Gross Appearance

As shown in Fig. 1 (pleural), and Fig. 2 (peritoneal), malignant mesothelioma has a tendency to develop along the serosa, with invasion into surrounding tissues such as the submesothelial connective tissue, and the chest wall, abdomen, diaphragm, and the subserosal parenchyma of various organs such as lung, liver, and intestines. Both pleural and peritoneal mesotheliomas are frequently accompanied by hyaline plaques in the perineoplastic fibrotic tissue.[1,29,30] Pleural effusion and ascites are frequently observed and the fluid is usually bloody in nature. Peritoneal mesothelioma generally show a pattern of extensive intraabdominal spread even when first diagnosed. Occasionally malignant pleural mesothelioma may appear somewhat localized. The tumor tissue of mesothelioma is usually white and hard, but occasionally the tumor cannot be distinguished from the surrounding fibrotic tissue.

Fig. 1. Malignant pleural mesothelioma. Showing gross anatomical appearance of malignant pleural mesothelioma. Left pleura was totally infiltrated by neoplastic tissue. The lung parenchyma was compressed by diffuse, thick neoplastic tissue. (From Dr. I.J. Selikoff's slide collection.)

Metastases may be seen with malignant mesothelioma.[31,32] Regional lymph nodes, lungs, brain, adrenal glands, and liver are potential sites of metastases from pleural mesothelioma. Abdominal and pelvic lymph nodes, liver, lung, pleura, and pericardium are organs in which metastases from peritoneal mesothelioma may be detected. Both pleural and peritoneal mesothelioma can infiltrate through the diaphragm into the other body cavity. Histologic evaluation of the metastases sometimes demonstrates considerable variability from the original tumor. The presence of metastases can sometimes confuse the diagnosis grossly. However, diffuse process in the chest or abdomen should immediately bring to mind the possibility of mesothelioma.

Fig. 2. Malignant peritoneal mesothelioma. Abdominal surface of diaphragm diffusely infiltrated by fine neoplastic nodules. (From Dr. I.J. Selikoff's slide collection.)

Differential Diagnosis

The absence of obvious primary tumor in any of the various organs such as lung, gastrointestinal tract, pancreas, or ovaries, coupled with a diffuse mesothelial tumor should alert one to the presence of a mesothelioma. Since other tumors can metastasize to the pleura or peritoneum, in the past many pathologists were unwilling to diagnose mesotheliomia except as a diagnosis of exclusion. There are now, however, specific histologic, histochemical and ultrastructural features that will allow for a more specific and antemortem diagnosis and these will be discussed in the respective sections.

Histology

Histologic evaluation is the most popular and important method to determine the diagnosis of malignant mesothelioma. In many series, the majority of tumors were diagnosed histologically from open or needle biopsies. Generally, the gross appearance is helpful and a greater quantity of tissue is preferable. Thus, open biopsy is probably the preferred method for obtaining diagnostic material. This histology of pleural, peritoneal, and pericardial mesotheliomas do not differ much. In each, the tumor can be classified into three forms: epithelial, biphasic (mixed), and fibrous (mesenchymal) varieties. This classification is based on the characteristics of the individual neoplastic cells. Our data from Mount Sinai Hospital in New York based on 210 consecutive malignant mesotheliomas, showed that 67% were epithelial, 26% were biphasic (mixed), and 7% were of the fibrous (mesenchymal) form. These mesotheliomas were classified as 177 definite (104 pleural, 66 peritoneal, 2 pericardial, and 5 both pleural and peritoneal) and 33 (16 pleural and 17 peritoneal) probable cases.

Epithelial Form

In this subtype of mesothelioma, the neoplastic cells may show various epithelial arrangements, such as papillary (Fig. 3a), tubular (Fig. 3b), tubulopapillary, cordlike (Fig. 3c) and sheetlike (Fig. 3d) patterns. Frequently, two or three patterns are mixed in a single case. The epithelial form, when present in sheetlike pattern, has occasionally been misjudged as poorly differentiated squamous cell carcinoma. However, neither intercellular bridging nor keratinization is found. The papillary and tubulopapillary forms must be differentiated from metastatic adenocarcinoma of lung, ovary, and gastrointestinal tract.

In general, the cytoplasm shows either eosinophilic or basophilic tones, which are related to ultrastructural features as discussed below. In the epithelial form, cells frequently produce intracellular (signet cell-like) and intercellular vacuoles. Generally, these vacuoles represent "empty spaces" on Hematoxylin–eosin stains. Using specific histochemical stains, hyaluronic acid may be demonstrated in some of the spaces. The nucleus of the cells is round or ovoid, and

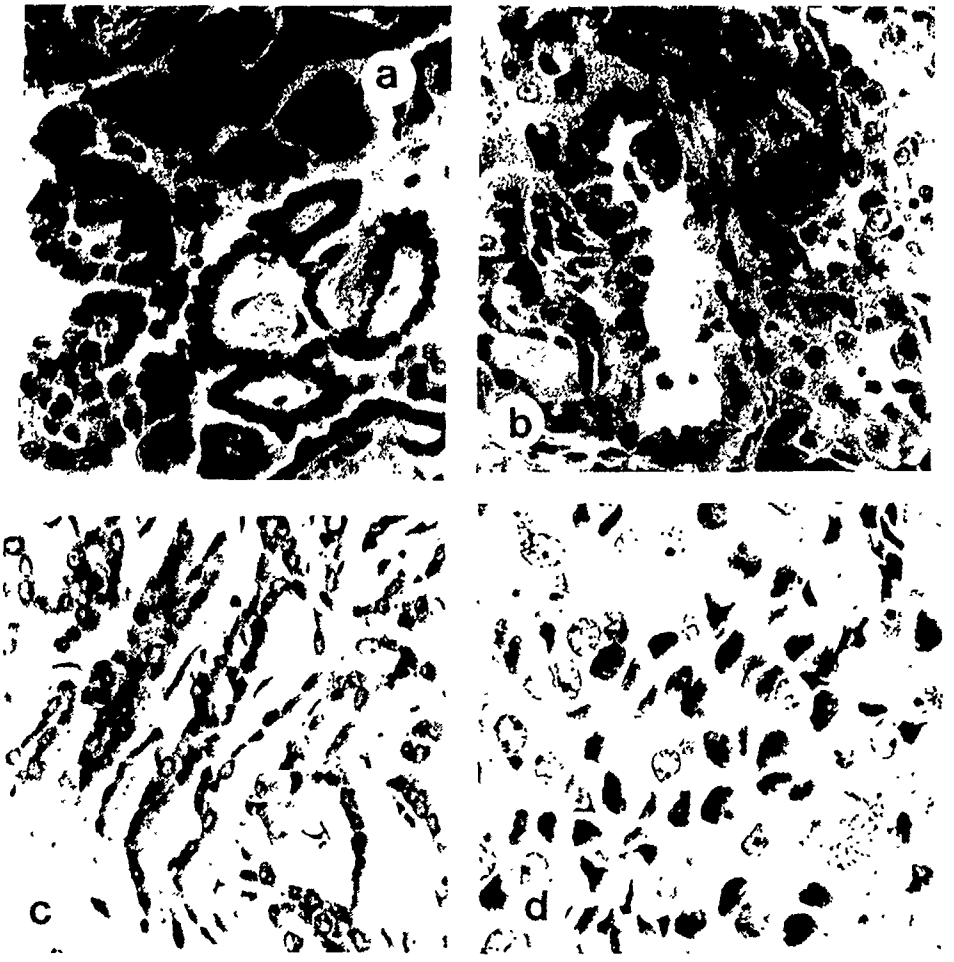


Fig. 3. Light micrographs showing histologic variability of the epithelial form. (a) Papillary pattern (H&E, ×350). (b) Tubular pattern (H&E, ×330). (c) Cord-like pattern (H&E, ×330) (d) Sheet-like pattern (H&E, ×530). ((a, b) adapted from *American Journal of Pathology*[74] with permission.)

the chromatin is generally fine. The nucleolus is usually well developed but fewer abnormal mitoses appear in mesothelioma than in other malignant tumors.

The stroma of the tumor varies. Frequently hyaline plaque (acellular hyaline degeneration of proliferated collagen) is seen in the neoplastic tissue, and sometimes, pseudomyxomatous degeneration of the stroma is observed.

Biphasic (Mixed) Form

The biphasic or mixed form is characterized by the presence of the two different cell types, epithelial and mesenchymal; frequently there are cells intermediate between the two cell types, as shown in Fig. 4b. When a tumor shows histologic characteristics of this subtype, one may confidently diagnose it as malignant mesothelioma. Figures 4a, b, and c illustrate the histology of the biphasic form. Epithelial cells showing a tubular pattern are seen in Fig. 4a and atypical (fusiform) epithelial cells are seen in Fig. 4b. Spindle shape fibrous cells are seen in Fig. 4c. These transitional cells will be characterized at the level of ultrastructure (see below). Small numbers of these intermediate cells are occasionally identified within tumors classified as either the epithelial or fibrous variety. The more one examines tissue sections of either form, the more likely one is to find these cells (Fig. 5).

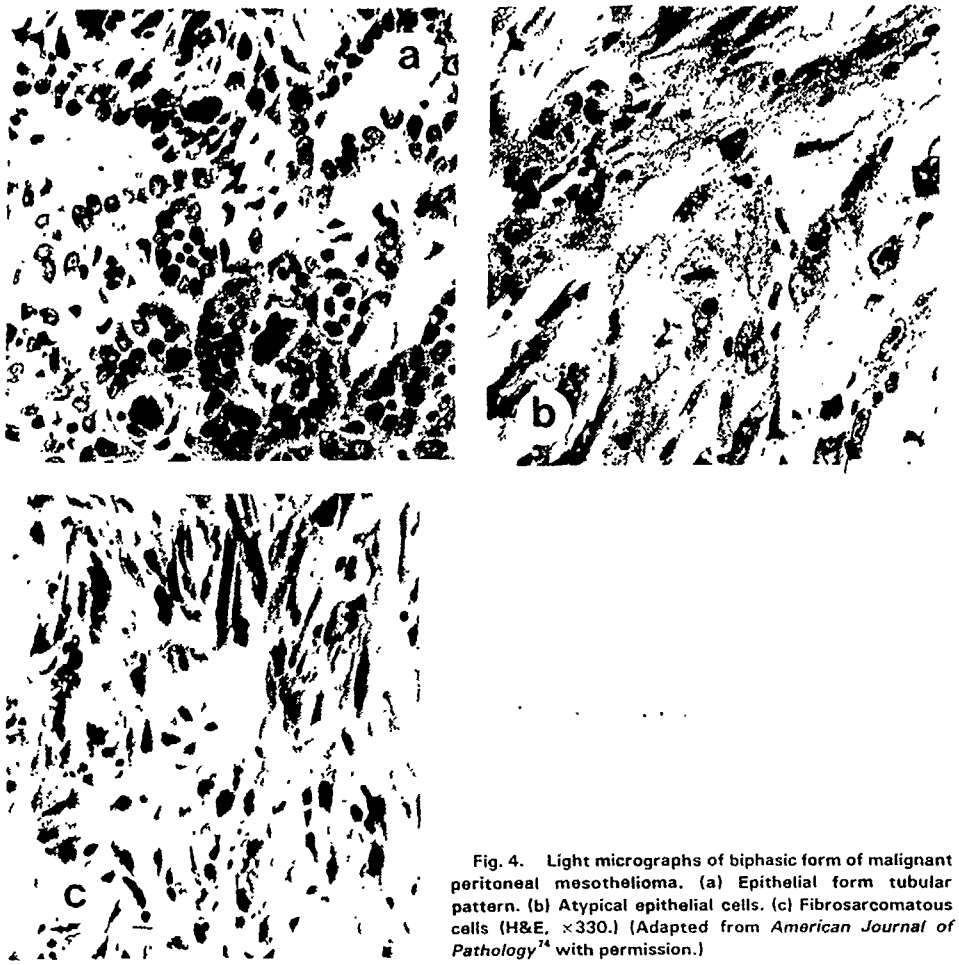


Fig. 4. Light micrographs of biphasic form of malignant peritoneal mesothelioma. (a) Epithelial form tubular pattern. (b) Atypical epithelial cells. (c) Fibrosarcomatous cells (H&E, ×330.) (Adapted from *American Journal of Pathology*[74] with permission.)

The identification of these intermediate "transitional" cells helps to establish the diagnosis of malignant mesothelioma.

Fibrous Form

The histology of the fibrous form is seen in Fig. 6. The cells in this form are spindle shaped and show a parallel arrangement and have an ovoid or elongated nucleus with well-developed nucleoli. The histologic aspects are similar to those of the fibrous portions of the biphasic (mixed) form, seen in Fig. 4c. Anaplastic features and pleomorphism are usually not striking.

Histochemistry

Malignant mesothelioma cells can produce hyaluronic acid. Accordingly, histochemical detection of this substance has been proposed for the diagnosis of the tumor. Meyer and Chaffee[33] first reported that the pleural effusion of a patient with mesothelioma contained a large amount of hyaluronic acid, a variety of acid mucopolysaccharide. Histochemically, colloidal iron or Alcian blue stains with or without hyaluronidase digestion have been used to study the presence of the material.[34,35] Figure 7a illustrates a section of mesothelioma stained with colloidal iron. The arrows in the illustration show the positively stained material in vacuoles. The positive material could be digested by hyalunidase (Fig. 7b) identifying it as hyaluronic acid. Hyaluronic acid can be found in both the cells and stroma of malignant mesothelioma. Hyaluronic acid content, particularly in the stromal tissue, can be detected in many other conditions,

Fig. 5. Spindle shaped neoplastic cells (arrows) are shown. Although the case was classified as an example of the epithelial form, it is an example of the presence of the intermediate cells in the epithelial form (H&E, ×350). (Adapted from *American Journal of Pathology*[74] with permission.)

such as bronchogenic carcinoma, mesenchymal tumors, inflammatory lesions, and young, active connective tissue. But our impression is that stromal hyaluronic acid seems to be greater in amount in mesothelioma than in the other conditions described above.

The exclusion of intracellular mucin (often produced in cells of adenocarcinoma of the lung or gastrointestinal tract) is also necessary for the diagnosis of mesothelioma, since mucin is not

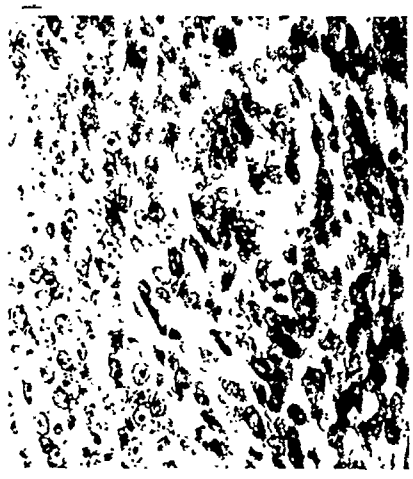


Fig. 6. Histology of fibrous form of malignant pleural mesothelioma. Cells are spindle shaped and in parallel arrangement. Also are reminescent of fibroblasts (H&E, ×350).

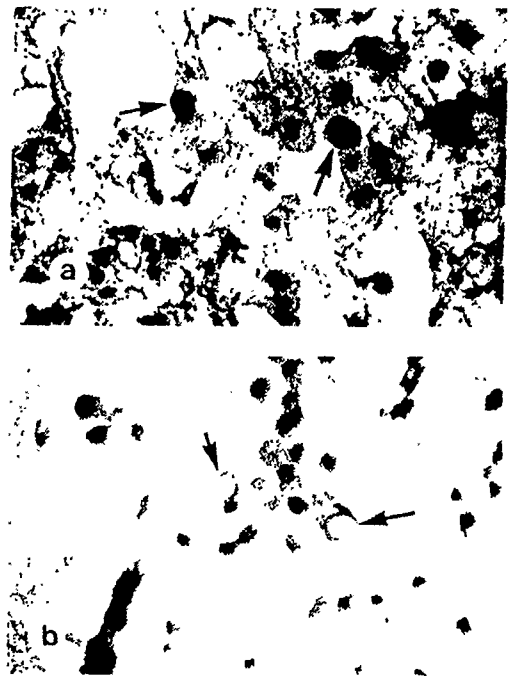


Fig. 7. A systematic histochemical study of a case of malignant pleural mesothelioma. (a) Mowry's colloidal iron. Intracellular vacuoles (arrows) are filled with colloidal iron positive materials. Mowry's colloidal iron stain, ×574. (b) Mowry's colloidal iron stain after hyaluronidase digestion. The material is digested by the enzyme before the staining. Accordingly, the intracellular vacuoles do not show the positive material by subsequent staining with colloidal iron. Digested material is therefore probably hyaluronic acid. Mowry's colloidal iron stain with hyaluronidase digestion, ×574. (Adapted from *American Journal of Pathology*[74] with permission.)

usually produced in cells of mesothelioma. The presence of mucin will exclude mesothelioma. Periodic-acid-Schiff stain (PAS) with or without diastase digestion or mucicarmine stains are frequently used for the histochemical identification of mucin. Cells of mesothelioma may contain glycogen, but this, unlike mucin, is removed by diastase digestion. For histochemical evaluation of possible mesothelioma several stains are therefore useful: periodic-acid-Schiff stain (PAS) with or without diastase digestion (or mucicarmine stain), colloidal iron stains (or Alcian blue stains) with or without hyaluronidase digestion.

Biochemical Detection of Hyaluronic Acid

Biochemical detection of hyaluronic acid in effusions has been proposed as a diagnostic crite-

ria of malignant mesothelioma.[33,35–39] Effusions caused by other diseases, such as metastatic cancers and inflammatory diseases, are also known to be rich in hyaluronic acid.[38–41] Therefore the identification of hyaluronic acid in the pleura is not diagnostic of mesothelioma. When present there is, however, generally greater amounts of hyaluronic acid found in malignant mesothelioma.[39] A quantitative relationship between other diseases and mesothelioma has been demonstrated with dried pleural tissues of mesothelioma containing over 0.10 mg of hyaluronic acid per gram tissue while pleural tissues from carcinoma or fibrous pleura contained 0.02 to 0.03 mg/g of tissue.[37] Thus a pleural or peritoneal effusion with a large quantity of hyaluronic acid has a reasonable likelihood of being derived from mesothelioma.

Cytology

Since effusions are commonly seen in malignant mesothelioma and since malignant cells may exfoliate into the effusions, cytological diagnosis of the tumor has been attempted using light and electron microscopy of the cell blocks.[42–47] Although many cytopathologists favor the use of cytopathology for diagnosis of malignant mesothelioma,[42,46,47] some still remain critical because of the high false positive and negative rate.[43–45] The well-differentiated floating epithelial cell of mesothelioma is usually difficult to separate from reactive mesothelial cells. Naylor[44] has, therefore, recommended using combined cytologic and chemical analyses of effusions to strengthen the diagnosis of malignant mesothelioma. However, the use of electron microscopy combined with specific cytochemical staining may make cytologic preparation sufficient for diagnosis. Ultrastructural characterization of floating mesothelioma cells has been proposed.[48,49] All three cell types have been identified by electron microscopy in effusions[49] and will be discussed below.

Immunopathologic Diagnosis

Recently, immunopathologic diagnosis of malignant mesothelioma has been attempted.[50,51] Indirect immunofluorescence staining for carcinoembryonic antigen (CEA)-like material was negative in malignant pleural mesothelioma, but detected in bronchioloalveolar cell carcinoma and adenocarcinoma, suggesting that the test may be useful in excluding these tumors from the differential diagnosis of malignant mesothelioma.[50] Antimesothelial cell serum was tested as a diagnostic reagent for malignant mesothelioma,[51] and mesothelioma cells were stained with the serum, suggesting that this technique may prove useful for antemortem diagnosis. These immunopathologic approaches have yet to be attempted in comparison with routine methods and judged for accuracy. It is likely, however, that these and similar techniques may become useful, particularly as the technology improves.

Electron Microscopy

Echevarria and Areans[52] first reported on the ultrastructure of malignant pleural mesothelioma. Subsequently, electron microscopy of malignant mesothelioma has been gaining acceptance for diagnostic purposes.[18,24–27,48,49,50,52–57] Transmission electron microscopy of ultrathin sections is the most commonly used method, but scanning electron microscopy has also been utilized.[58,59] There are certain ultrastructural features of malignant mesothelioma that are unique and will support the diagnosis. These will be discussed according to the various cell types.

Epithelial Form

The cells of the tubulopapillary form are quite similar in ultrastructure to hyperplastic mesothelium with polarity and presence of well-developed microvilli and a continuous basement membrane. Cells of the epithelial form (Fig. 8a) show microvilli formation and a basement membrane that are less well differentiated or discontinuous respectively when compared to hyperplastic mesothelium. The epithelial cell shown in Fig. 8c is a well-differentiated cell and contains intracellular vacuoles (large arrow). Again in this cell microvilli are present. Glycogen granules can be observed in the epithelial neoplastic cells. Microvilli may be covered by a fuzzy material (Fig. 8d); only occasionally the epithelial cells lack microvilli. Tonofilaments are frequently seen in the form of bundle structures (Figs. 8b and e), and they may be connected to the junctional structure (Fig. 8e). Cilia and mucinous secretory granules are not found in the mesothelial cells and their identification eliminates mesothelioma. Unlike secretory granules containing mucin, the contents of the vacuoles in mesothelioma lack electron opacity. Mytochon-

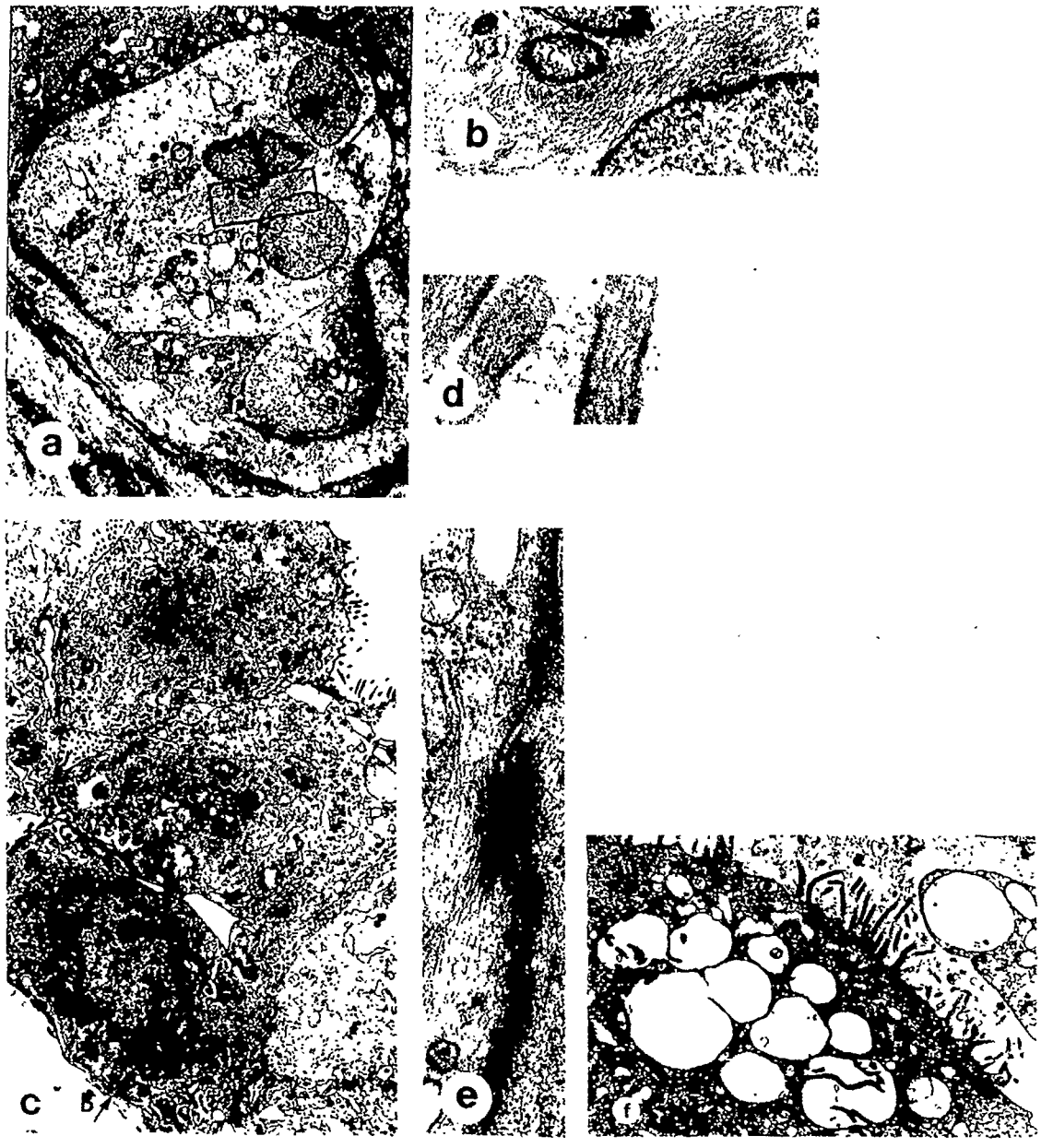
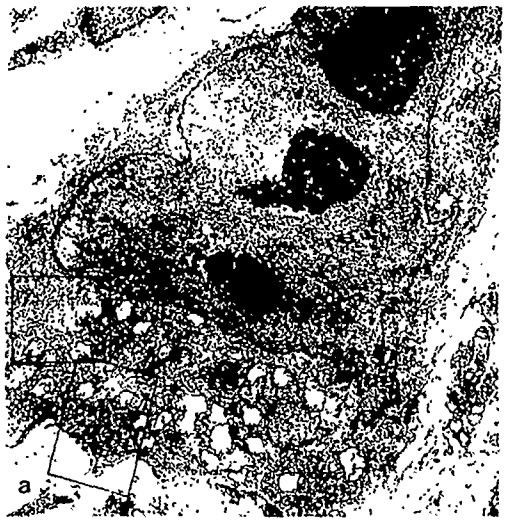


Fig. 8. (a) Ultrastructure of less differentiated epithelial cells. Dark (D_1–D_3) and clear (C) cells are easily seen. An arrow indicates a desmosome. The basement membrane is discontinuous and disappears beneath one of the neoplastic cells (D_2). ×2900. (b) Higher magnification of the rectangle in (a) illustrating tonofilaments. ×11,600. (c) Well differentiated epithelial form with intercellular gaps in the malignant pleural mesothelioma. Basement membrane is indicated at B by arrow. ×3500. (d) Shows microvilli covered by ill-defined material. ×65,000. (e) Shows a desmosome connected by tonofilaments. ×50,000. (f) Demonstrates intracytoplasmic vacuoles containing microvilli. ×4160. (Figures 8a–f adapted from *American Journal of Pathology*[74] with permission.)



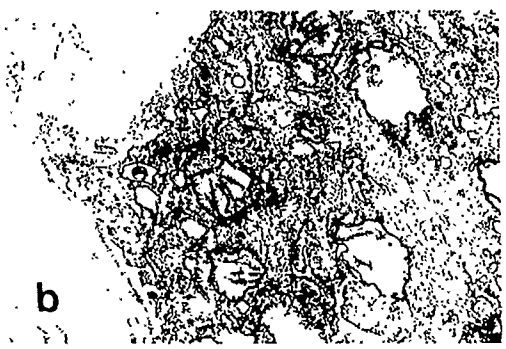


Fig. 9. (a) Ultrastructure of an atypical epithelial or intermediate cell seen in biphasic form of malignant peritoneal mesothelioma, for which light micrographs were shown in Fig. 4. (b, c) Higher magnification of rectangles 1 and 2 in (a): a basement membrane (b) and the remnants of microvilli (c). (a) ×1995; (b) ×13, 968; (c) ×3500. (Figures 9a–c adapted from *American Journal of Pathology*[74] with permission.)

dria are relatively large in size and the matrix is frequently pale. Lysosomal granules and osmiophilic lamellar structures can be rarely observed; these organelles are not characteristic of mesothelioma. The nucleus of the cells, regardless of cell type, show certain common features: the nucleus is filled with fine chromatin and a nucleolus is almost always well developed (Figs. 8a, 9a, and 12a). Microvilli are sometimes seen in the intracytoplasmic vacuole (Fig. 8f). Thus, for the differentiated epithelial form typical ultrastructural features include basement membrane, microvilli, tonofilaments, rather large mitochondria, cell junctions, cytoplasmic glycogen, and occasionally microvilli within the intracytoplasmic vacuoles. Less differentiated epithelial cells lack one or more of these epithelial features.

Biphasic or Mixed Form

The epithelial variety of the biphasic form is the same in ultrastructure as the epithelial form. Electron microscopy of an atypical epithelial cell of the biphasic form is shown in Figs. 9a–c. In the atypical epithelial cell, epithelial characteristics are not clearly seen with this low magnification (Fig. 9a) but enlargement shows a discontinuous basement membrane (Fig. 9b) and remnants of microvilli (Fig. 9c). This cell was thus classified as an intermediate cell. The ultrastructure of a fibrous or mesenchymal cell is seen in Fig. 10. Like a fibroblast, the fibrous cell is

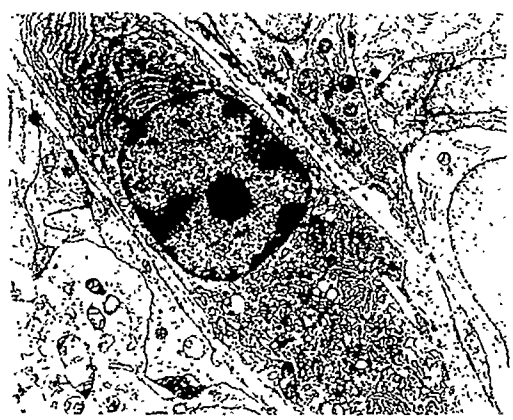


Fig. 10. Ultrastructure of biphasic form of malignant pleural mesothelioma. A mesenchymal cell type. With an arrow indicated direct contact of the cell with another neoplastic cell. ×3288. (Adapted from *American Journal of Pathology*[74] with permission.)

Fig. 11. Ultrastructure of biphasic mixed form. Collagen fibrils can be seen in the intercellular space of the mesenchymal neoplastic cells. ×22,800. (Adapted from *American Journal of Pathology*[74] with permission.)

rich in the rough-surfaced endoplasmic reticulum. There is, however, direct contact of two adjacent cell membranes (arrow). The interstitial spaces may be filled with collagen fibrils (Fig. 11). Another mesenchymal cell is shown in Fig. 12a; this cell highly resembles a fibroblast, since there is no cell to cell contact. Figure 12b illustrates a cell intermediate in appearance between an epithelial form cell (Fig. 9a) and a fibrous cell (Fig. 12a). Direct contact of two adjacent cells, occasional appearance of tight junction (arrows) and lack of other epithelial appearances, such as microvilli, a basement membrane, and tonofilaments, are indications of the intermediate cell type. Electron microscopically, three cell types, the epithelial, intermediate, and mesenchymal, are identified in the biphasic or mixed form of mesothelioma.

Fibrous Form

The ultrastructural features of a fibrous form cell (seen on light microscopy in Fig. 6) are shown in Fig. 13. The ultrastructure of this fibrous form cell is quite similar to that of the mesenchymal variety illustrated in the biphasic form (Figs. 10 and 12a). Kay et al.[54] have stated that epithelial cells can be observed among the cells of the fibrous form. It is possible that occasional atypical epithelial (intermediate)

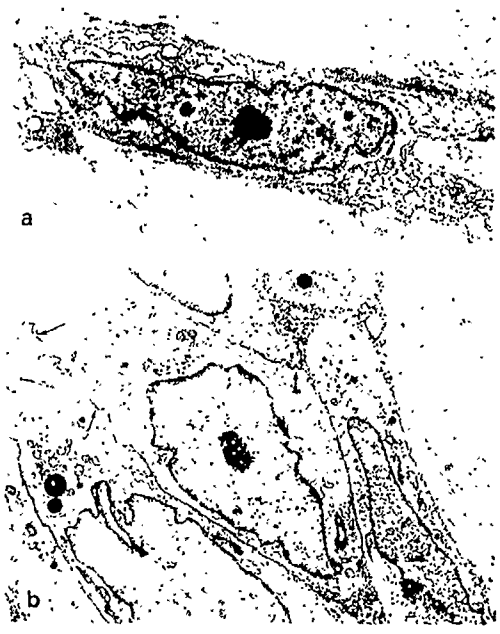


Fig. 12. Electron micrographs of another biphasic form of malignant pleural mesothelioma. Showing both fibrous (a) and intermediate (b) cell types. Arrows in (b) show tight functions. (a, ×3127; b, ×3024.)

cells may be found in the fibrous form with the electron microscopic if a sufficiently large number of neoplastic cells were screened. Thus, the fibrous form is characterized by cells lacking the characteristics of the epithelial form cells

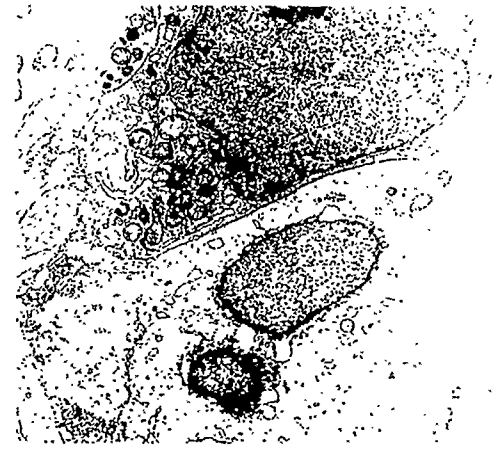


Fig. 13. Electron micrograph showing fibrous form of malignant pleural mesothelioma, for which the light micrograph is shown in Fig. 6. Ultrastructural aspects of neoplastic cells are similar to those of fibrous or intermediate cells in the biphasic form. ×5400.

such as microvilli, basement membrane, and tonofilaments. They have the appearance of fibroblasts but frequently exhibit direct cell to cell contact. Only occasionally may intermediate cells be identified.

ELECTRON MICROSCOPY OF CELL BLOCKS FROM CYTOLOGY PREPARATIONS

Although routine Papanicolaou stained preparations from effusions have been somewhat disappointing in their diagnostic accuracy for malignant mesothelioma, it is possible that cell blocks examined by electron microscopy may lead to a better diagnostic rate for this tumor. Ultrastructure of the cells obtained from a mesothelioma effusion is shown in Figs. 16a, b, and c. Microvilli, tonofilaments, nucleoli, and junctional structure typical of epithelial mesothelioma are observed. The ultrastructural details in this case led to the diagnosis of malignant mesothelioma. Attention, however, still must be focused on the differentiation of mesothelioma cells from reactive mesothelial cells. Electron microscopy of the cell block is effective in identifying whether the cells are of mesothelial origin. However, differentiation of reactive mesothelial cells from well-differentiated epithelial mesothelioma cells is not yet possible. For this reason, prior to electron microscopic observation, the malignancy should be confirmed with the cell block sections using light microscopy. Further study is needed to develop specific criteria which will allow for the identification of mesothelioma from cell blocks obtained from effusions.

ELECTRON MICROSCOPIC DIFFERENTIAL DIAGNOSIS OF MALIGNANT MESOTHELIOMA FROM METASTATIC TUMORS

Occasionally there are pleural or peritoneal tumors which are very difficult to differentiate mesothelioma from metastatic disease. Figure 14a illustrates the light microscopy of such a pleural tumor. By light microscopy the diagnosis in this case was controversial (malignant mesothelioma; epithelial form vs. metastatic adenocarcinoma). Figures 14b and c show the ultrastructural details of this tumor. Unlike malignant mesothelioma cells, the tubular lumen is filled with electron opaque material, and the cytoplasm is rich in lysosomal granules (Fig.

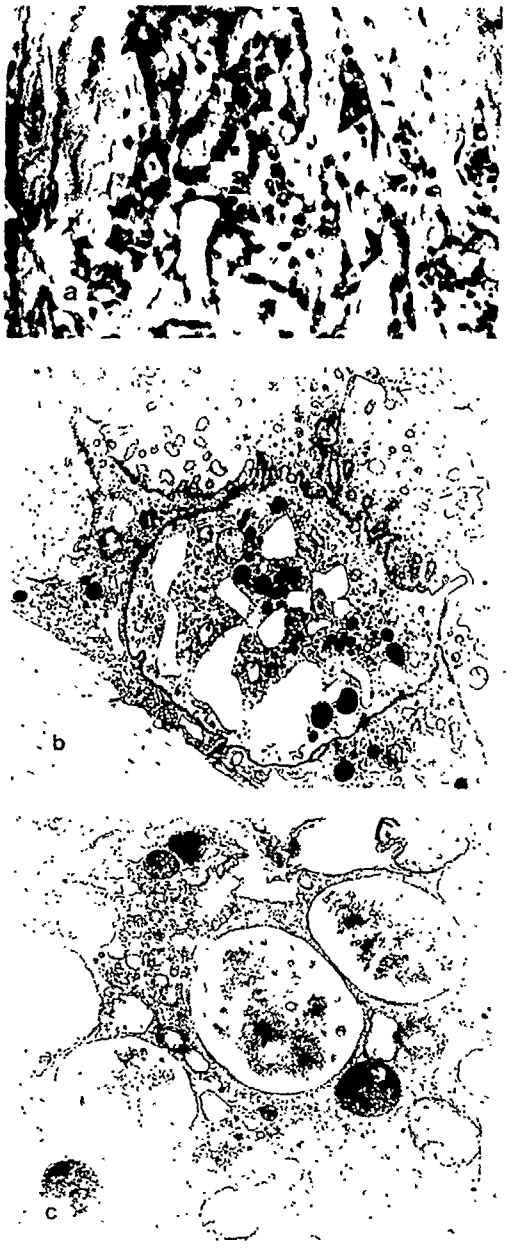


Fig. 14. (a) Light micrograph taken from a pleural tumor in which the histologic diagnosis was unclear between metastatic adenocarcinoma and malignant mesothelioma (H&E, ×254). Electron micrographs of neoplastic cells are quite revealing. However, (b) shows a tubular luminar filled with electron dense substance (mucoprotein?) and a cell cytoplasm rich in lysosomal granules, ×7384. (c) Some intracytoplasmic vacuoles are also filled with electron dense substance, ×14,840. These ultrastructural details strongly suggest adenocarcinoma. (Adapted from *American Journal of Pathology*[74] with permission.)

14b). Intracytoplasmic vacuoles are also seen with electron dense material (Fig. 14c). The electron opaque material may represent mucin or a mucoprotein, which are frequently produced in adenocarcinoma. Histochemically, the material was stained with PAS and was not digested by diastase. Both histochemical stains and the electron microscopic analyses lead to the conclusion that this pleural tumor was not a malignant mesothelioma. Figure 15a illustrates the light microscopy of a pleural tumor that was originally diagnosed as malignant mesothelioma; fibrous form. However, with electron microscopy (Fig. 15b) erythrophagocytosis, phagosomes, and poorly developed cell organoids are seen. These findings are not consistant with malignant mesothelioma but suggest malignant fibrous histiocytoma. These cases illustrate the value of performing these special techniques to distinguish between primary mesothelial tumors and metastatic deposits.

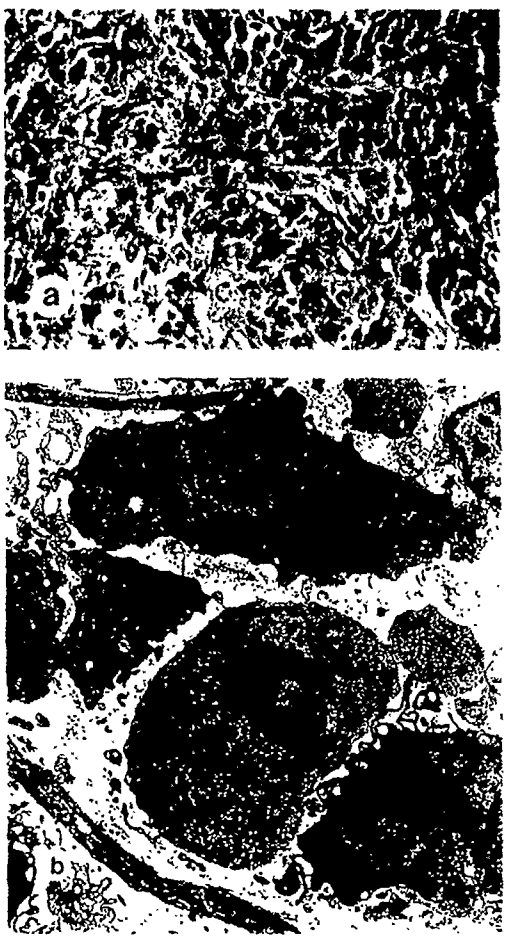


Fig. 15. (a) Light microscopy of a pleural tumor first diagnosed to be malignant mesothelioma (fibrous form), H&E, ×395. Electron micrograph of the pleural tumor, however, shows distinct features in (b). Ultrastructure is different from that of fibrous mesothelioma. There is a lack of cell contact and the presence of both erythrophagocytosis and phagosomes, ×3819. This leads to the suggestion that this tumor is a malignant fibrous histiocytoma.

SCANNING ELECTRON MICROSCOPY

In addition to conventional electron microscopy, scanning electron microscopy has been applied to the diagnosis of malignant mesothelioma.[58-59] There is a report that the microvilli of the mesothelioma cells are widely distributed and show variation in appearance, irregular overlapping and the presence of fused and branched forms.[58] It is quite possible, however, that under a scanning electron microscope the appearance of the microvilli may vary in structure and development between the various cell types, since a transmission electron microscopy has revealed that the mesenchymal cell lacks the microvilli, and the intermediate cell is rarely equipped with the microvilli.[74] For this reason, further careful studies are still needed to obtain detailed information on the scanning electron microscope appearances in the various cell types. Hopefully such studies will increase the diagnostic utility of this analytical method.

PATHOGENESIS OF MALIGNANT MESOTHELIOMA

Etiological Factors

Since Wagner et al.[8] reported the relation of asbestos exposure to malignant pleural mesothelioma, their conclusion has been widely confirmed.[9-13,60,64] Recently, Baris et al.[75] have reported that fibrous erionite, a type of zeolite mineral, was responsible for an endemic occurrence of pleural mesothelioma in Turkey. Animal studies have also indicated that various agents other than asbestos can induce malignant mesothelioma. Fibrous glass, brucite, and fibrous erionite are known to induce experimental mesotheliomas.[63,66,75] Stanton et al.[61] have suggested that the carcinogenicity of asbestos is related primarily to the structural characteristics represented by long (over 8 μ), thin (less than 1 μ) fibrous shapes, rather than its physicochemical properties. In addition to fibrous inor-

ganic agents, some organic compounds, including diethylstibestrol benzo(o)pyrene, and radioactive substances such as $^{239}PuO_2$ have been reported as carcinogenic agents in producing mesothelioma and other tumors.[67-70] From such observations, one might suspect that agents other than asbestos can be implicated alone or as cocarcinogens in the evolution of mesothelioma. Further studies are obviously needed to define the various components in the etiologic development of this tumor.

Histogenesis of Malignant Mesothelioma

Klemperer and Rabin provided an important basic concept concerning the histogenesis of mesothelioma. They explained in part that histocytologic variability of the tumor was due to the unique multipotential of the mesothelial cell, which could differentiate into various cell lines.[6] Later, other investigators reported that cultured mesothelioma cells could transform into different cell types.[71,72] One might question the applicability of tissue culture study for human mesothelioma. Tissue culture studies at Mt. Sinai Hospital of New York have revealed that cultured cells change strikingly from a cell containing hyaluronic acid (well differentiated epithelial form) to the nonhyaluronic acid containing cell of the poorly differentiated epithelial form, with appearance of many lipid granules. Such alterations may have resulted from artificial conditions in the medium. Nevertheless, the unique potential can be supported by light and electron microscopic evidence that the intermediate cells between the epithelial and mesenchymal cells are frequently identified in mesothelioma. Unlike cultured cells, mesothelioma cells transplanted into nude mice have kept their original cellular characteristics over 1 yr, with repetitive transplantation[73] suggesting that there may be great differences between cells maintained in vitro and in vivo. The transplanted mesothelioma may be a good model for further study of pathobiologic mechanisms. They may also be a model for testing antineoplastic therapies.

Unfortunately we still lack data concerning the evolution from the normal mesothelium to malignant neoplastic mesothelium, and the role of the hyaline plaque in the development of malignant mesothelioma. We also lack information about similarities and differences in histogenesis of the tumors induced by different agents. Answers to these and similar questions will contribute to development of a better understanding of the biology of mesothelioma which in turn might lead to prevention, earlier diagnosis, or better applications of treatment.

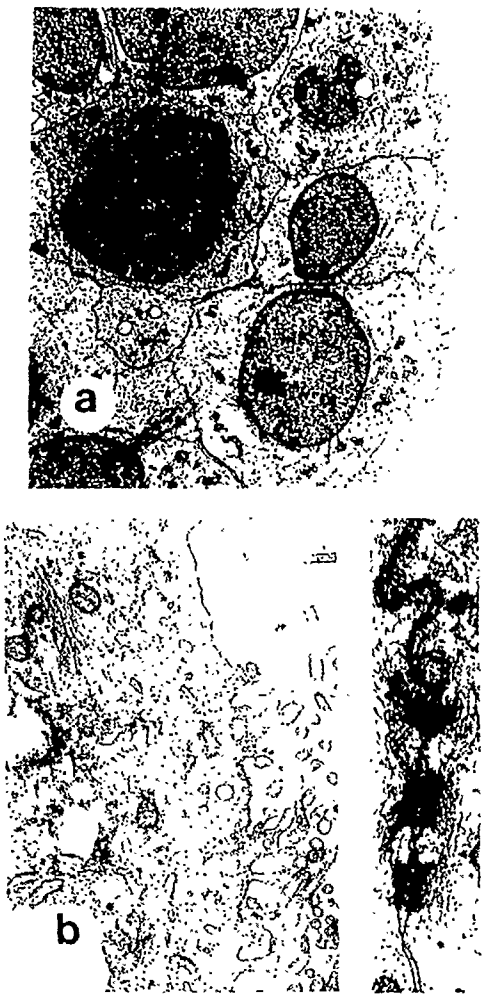


Fig. 16. (a) Electron microscopy of a suspected neoplastic cell obtained from a peritoneal effusion in a patient with mesothelioma, ×2000. (b) Higher magnifications of portions of (a) show well-developed microvilli and tonofilaments. Desmosomes are seen in (c). (b, ×28,000; c, ×54,600.) These findings, however, may still be confused with reactive mesothelial cells—see text. (Adapted from *American Journal of Pathology*[74] with permission.)

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