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Reactive Mesothelial Cell and Mesothelioma of the Pleura

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Summary. The results of a light and electron microscopic study and enzyme histochemistry of reactive mesothelial cells and diffuse and localized (solitary) pleural mesotheliomas were compared, in order to establish a diagnosis and elucidate the cell of origin of the mesotheliomas. The reactive mesothelial cells were usually regular in appearance but could be cuboidal or columnar, or even peg-shaped with large nuclei and prominent nucleoli. Colloidal iron and alcian blue staining were positive in the plasma membrane, microvilli and in parts of the cytoplasm of mesothelial cells. These stains were mostly negative following testicular hyaluronidase treatment. The neoplastic cells of diffuse pleural mesothelioma had some epithelial characteristics such as microvilli, desmosomes, and a prominent basement membrane and were similar to reactive mesothelial cells with regard to cellular form and staining for acid mucopolysaccharides. In this study the colloidal iron and PAS stains appeared to be more intense in mesothelioma cells than in reactive mesothelial cells.

In enzyme histochemistry, naphthol AS-D acetate esterase and α-naphthyl acetate esterase activity were demonstrated strongly in neoplastic cells. The nuclear-cytoplasmic ratio, the numbers of microvilli and mitochondria and the amount of glycogen were greater in the neoplastic cells when studied by electron microscopy. Electron lucent intracytoplasmic fibrils appeared wider and more distinct in mesothelioma cells.

Localized mesotheliomas, thought to be an entirely different entity from reactive mesothelial cells, showed little characteristic morphology in light microscopic pictures, few cytoplasmic organelles were seen ultrastructurally.

Key words: Mesothelioma, benign and malignant – Pleural neoplasm – Lung neoplasm.

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Introduction

Morphological differentiation between reactive hyperplasia of the mesothelium and mesotheliomas is often difficult, especially on surgically resected and quantitatively limited material (Selikoff et al. 1965; Wang 1973). Reactive hyperplasia of the mesothelium is commonly seen in surgical pleural specimens from patients with spontaneous pneumothorax, but there have been few studies concerning reactive changes of the pleural and peritoneal mesothelium (Hourihane 1965; Rosai et al. 1975). In an effort to elucidate differential diagnostic characteristics and the histogenetic relationship between reactive hyperplastic mesothelial cells and mesotheliomas, surgically resected specimens of the pleura were studied by light microscopy, enzyme histochemistry, and electron microscopy.

Materials and Methods

At Keio University Hospital, Tokyo, surgically resected pleural specimens were obtained in 25 cases of spontaneous pneumothorax, 3 diffuse pleural mesothelioma cases, and 7 cases of localized pleural mesothelioma.

Light Microscopy. Tissue blocks were fixed in 10% formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin, colloidal iron, alcian blue including hyaluronidase digestion, and periodic acid Schiff (PAS) including diastase digestion.

Enzyme Histochemistry. Approximately 2 mm thick blocks were fixed in 10% formol-calcium (pH 7.1–7.2) for 24 h at 4° C, changed to cold gum sucrose and kept for 2 days in the refrigerator. Tissue sections cut 4–6 μm thick in a cryostat were processed for the following enzyme histochemical stainings: acid phosphatase (AcPase) (Barka et al. 1962), β-glucuronidase (β-Gase) (Hayashi et al. 1964), N-acetyl-β-glucosaminidase (N-Gase) (Hayashi et al. 1965), alkaline phosphatase (AlPase) (Burstone 1962), adenosin triphosphatase (ATPase) (Wachstein et al., 1957), α-naphthyl acetate esterase (α-N. acet est) and naphthol AS-D chloroacetate esterase (N.As-D chlor est) (Yam et al. 1971), and naphthol AS-D acetate esterase (N.AS-D acet est) (Burstone 1957).

Transmission Electron Microscopy. Pleural specimens were minced and fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.4, postfixed in 0.1 M phosphate buffered 1% osmium tetroxide for 2 h, dehydrated through graded alcohols and acetone, and embedded in epon 812. Thin sections were cut, doubly stained with uranyl acetate and lead citrate, and examined with a JEOL transmission electron microscope.

Scanning Electron Microscopy. Pleural portions of grossly normal appearing resection margins were fixed by immersion for several hours at room temperature in a mixture containing 2.5% glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.4, postfixed in phosphate buffered 1% osmium tetroxide for 2 h, dehydrated in graded alcohols, critical point dried using carbon dioxide, and examined with a scanning electron microscope.

Results

Reactive Mesothelial Cells

Histologically, reactive mesothelial hyperplasia was commonly found on the pleural surface adjacent to bullae, mixed with phagocytotic mononuclear and mast cells. Mesothelial cells were flat, cuboidal, columnar, or sometimes peg-shaped, with large nuclei and prominent nucleoli. The colloidal iron stain was

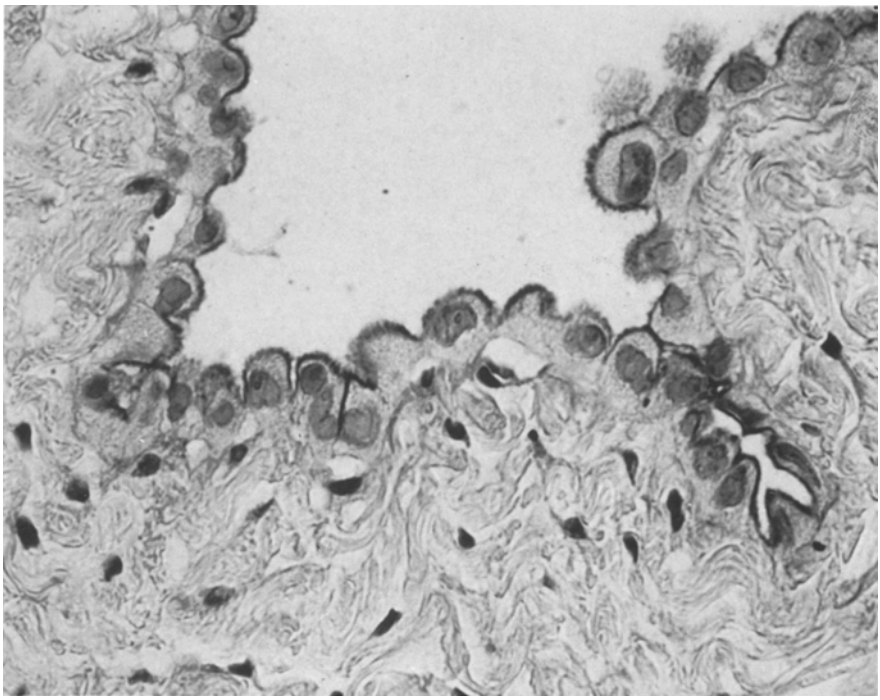

Fig. 1. Reactive mesothelial cells on the surface of the pleura. Colloidal iron stain-positive material in the cytoplasmic membrane and microvilli. Colloidal iron, ×600

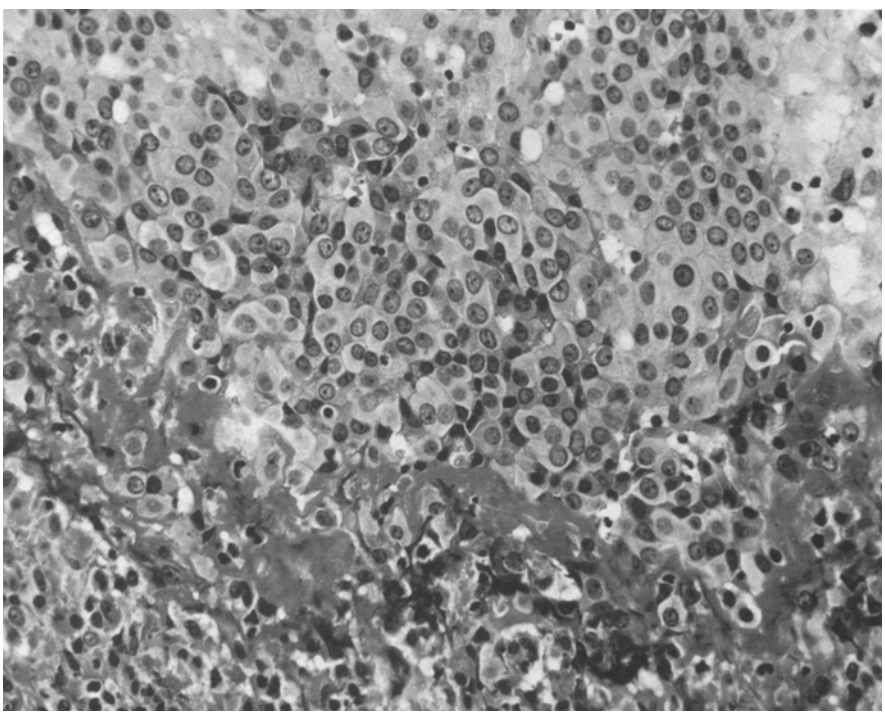

Fig. 2. Mesothelial hyperplasia were composed of sheets of mesothelial cells with fibrin and there was little or no supporting stroma. H&E ×300

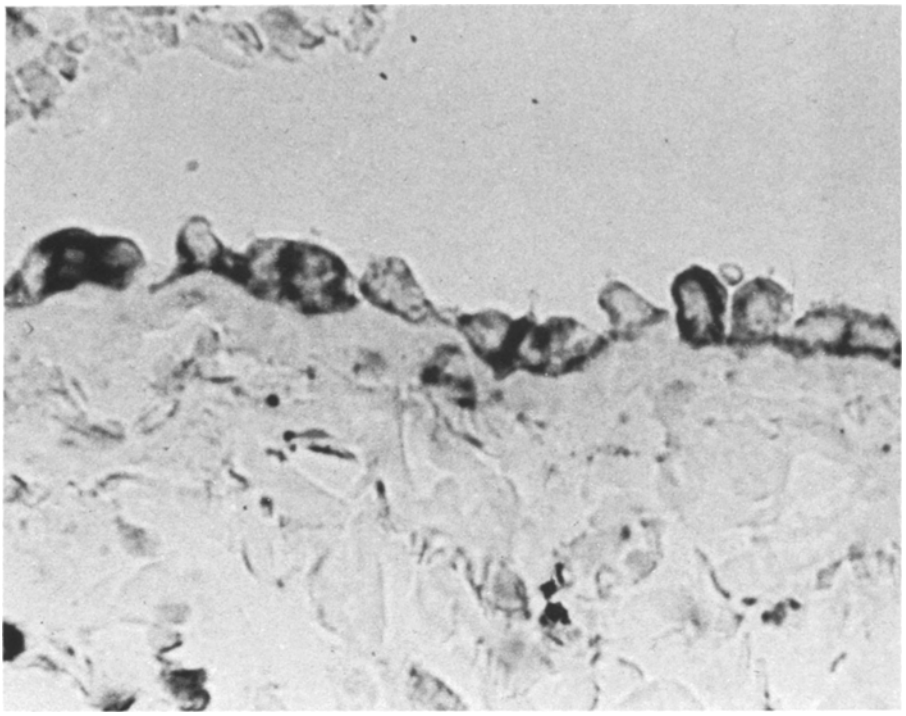

Fig. 3. Strong activity was demonstrated in the mesothelial cells. N. AS-D acet est reaction ×600

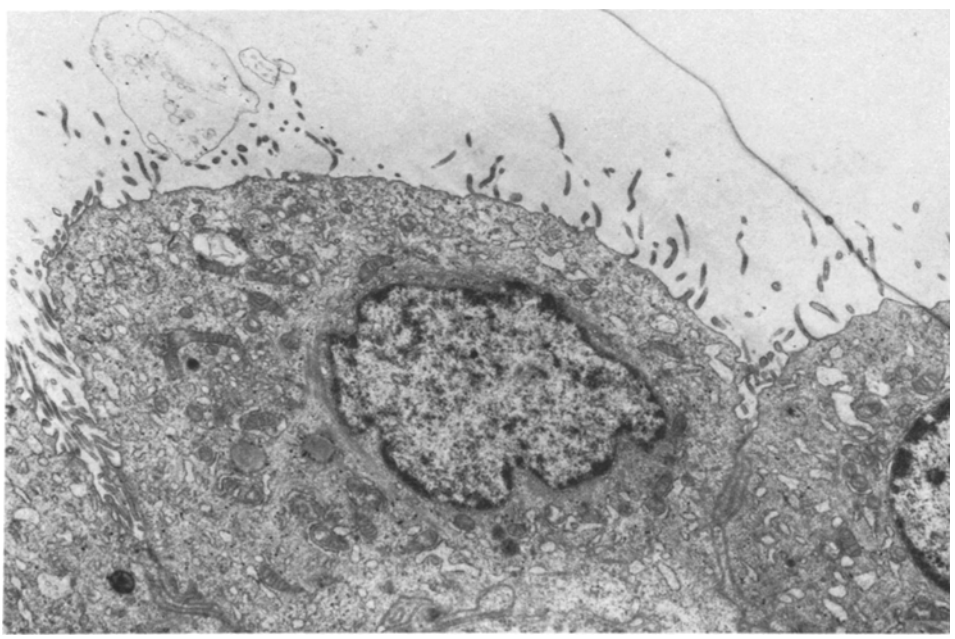

Fig. 4. Reactive mesothelial cells showed distended rough endoplasmic reticulum, mitochondria, Golgi apparati, prominent intracytoplasmic fibrils and several lysosomes. ×7,000 (orig. mag. ×4,800)

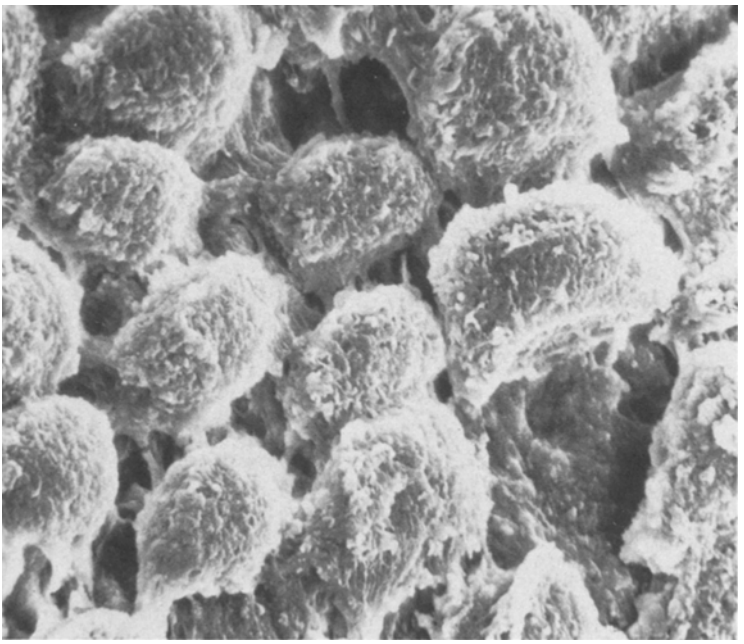

Fig. 5. Scanning electron microscopic features of reactive mesothelial cells. ×5,400 (orig. mag. ×3,000)

Table 1. Three patients with malignant diffuse mesothelioma

Case	Occupation	Location	Surgery	Follow-up
1. 61 man	Policeman	Right	Pleural stripping	Alive 2yr.
2. 46 man	Decorator	Left	Pleural stripping	Died, no autopsy
3. 64 man	Plumber, Decorator	Left	Open Pleural biopsy, Thoracotomy	Died, autopsy No evidence of metastasis except lymph nodes of bronchus and cardia

positive in the plasma membrane (Fig. 1). The alcian blue stain was weakly positive but became negative after treatment with testicular hyaluronidase. Fine granules were deomonstrated by the periodic acid-Schiff (PAS) stain after diastase digestion. Sometimes diffuse hyperplasia of mesothelial cells was noted along visceral pleura producing tumor-like masses (Fig. 2). Phagocytic cells which were increased in the pleural cavity, also showed colloidal iron positive substance in the cytoplasm.

In enzyme histochemical methods, the activity of lysosomal enzymes such as β-Gase and AcPase was observed in most mesothelial cells. N-Gase was weakly active. Esterases such as N.AS-D chlor est, α-N.acet est and N.AS-D acet est were positive (Fig. 3), but activity of AlPase or ATPase was not demonstrated in the mesothelial cells of any case.

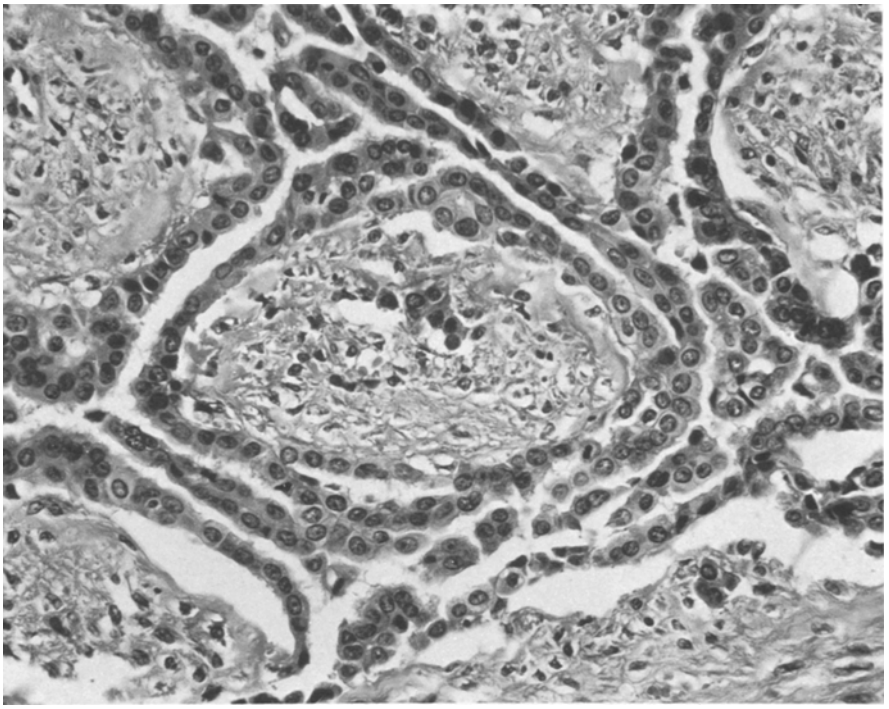

Fig. 6. Case 2: Epithelial form of malignant diffuse mesothelioma. Tubular and cord-like neoplastic cells were arranged in a single layer with fibromyxomatous stroma. The neoplastic cells were uniform in size and shape. H&E $\times 300$

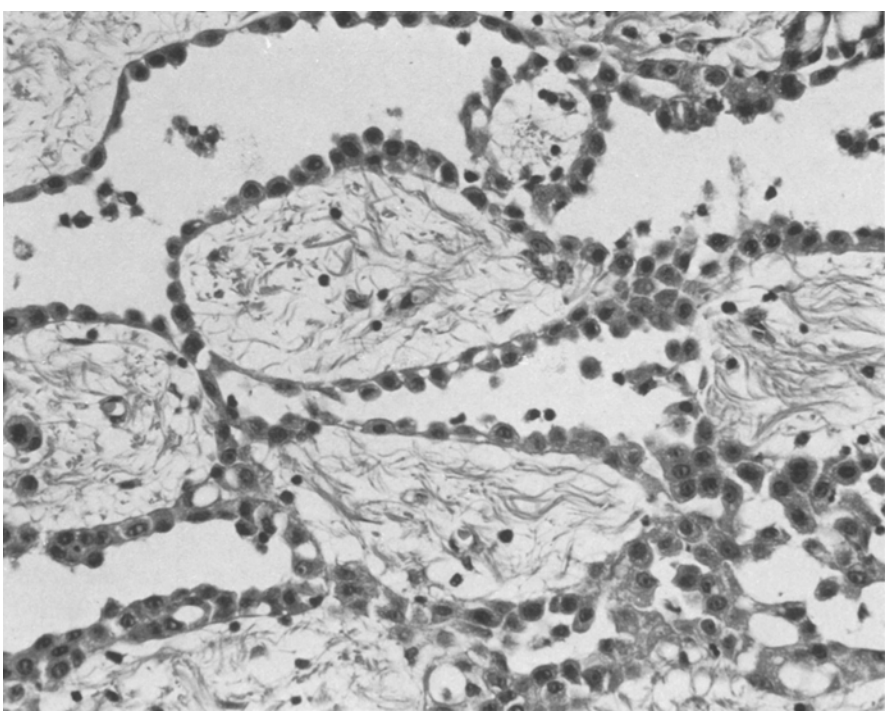

Fig. 7. Case 1: Cord-like pattern with fibrous stroma in epithelial form of malignant diffuse mesothelioma. H&E $\times 300$

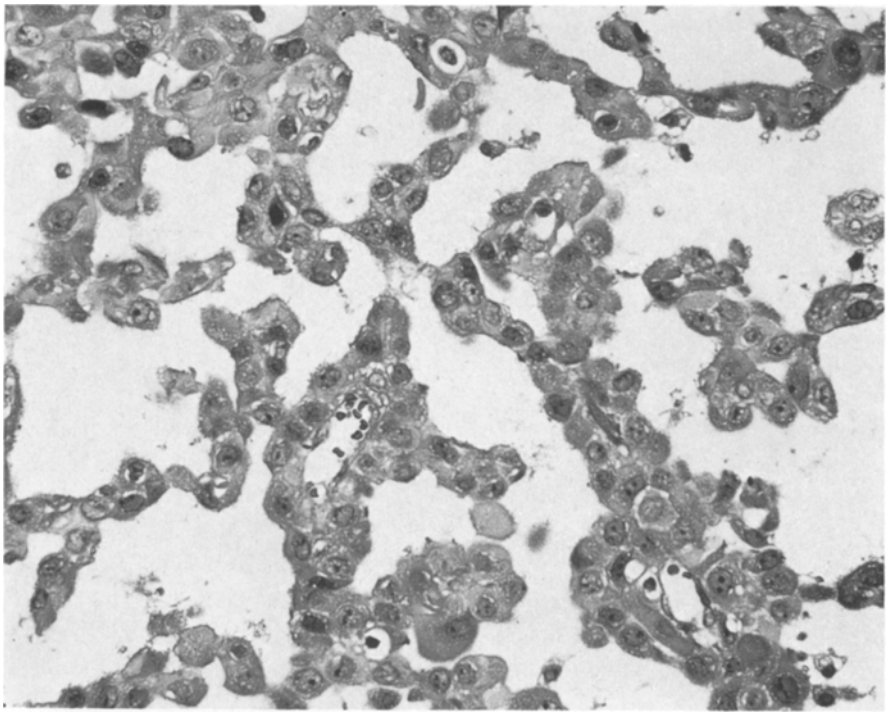

Fig. 8. Case 3: Papillary tumor cells were large and variable in size without fibrous stroma, pleomorphism and atypical figures were slightly seen. H&E ×300

In transmission electron microscopy, the mesothelial cells showed delicate microvilli and various intracytoplasmic organelles such as rough endoplasmic reticulum, mitochondria, Golgi apparati, perinuclear fibrils, lysosomes and sparse glycogen (Fig. 4). Linear structure recognized as basement membrane was present under these cells, and desmosomes were found between adjacent cells.

In scanning electron microscopy, mesothelial cells appeared uniform and rounded in a globular pattern. The plasma membranes of individual cells were closely connected to each other by cytoplasmic processes. The cell surface showed a fine meshwork of microvilli (Fig. 5).

Mesotheliomas

Histologically, 10 cases of mesotheliomas of the pleura were classified as diffuse or the localized (solitary) type (Ackerman 1954). Table 1 summarizes the clinical findings of the three patients with malignant diffuse mesothelioma. These cases were classified histologically into epithelial forms. These forms varied in histological patterns, papillary and tubular appearances were seen with a abundant fibrous stroma present in some areas. (Figs. 6, 7). In case 3, cellular growth consisted of pleomorphic cells with slightly eosinophilic cytoplasm resembling ground glass and large nuclei with prominent nucleoli were common. In larger

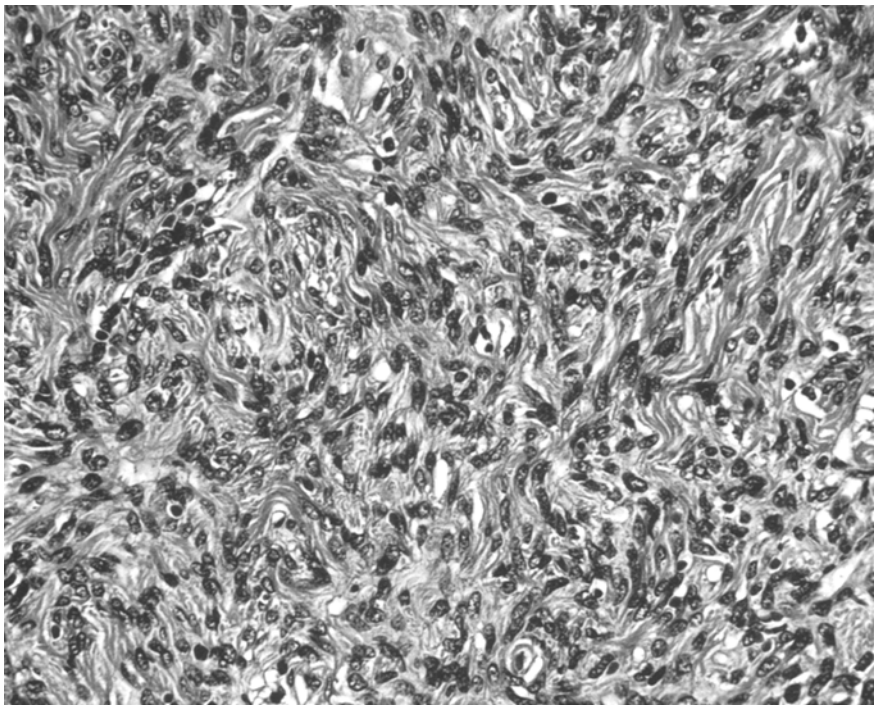

Fig. 9. Localized pleural mesothelioma with round tumor cells, fibroblasts and collagen fibers. H&E $\times 300$

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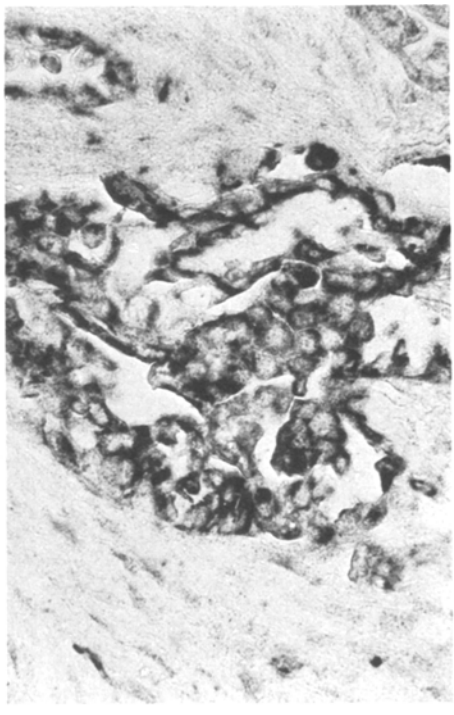

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Fig. 10. Strong diffuse activity was found in the neoplastic cells. α-N. acet est reaction $\times 300$

Fig. 11. Strong activity was demonstrated in the neoplastic cells. N. AS-D acet est reaction $\times 300$

Table 2. Enzyme histochemistry of malignant diffuse mesothelioma

Case	AcPase	β-Gase	N-Gase	AlPase	ATPase	Esterases		
						α-N. acet est	N. AS-D acet est	N. AS-D chlor est
1	++	±~+	−	−	−	±~+	+	±~+
2	+	+	+	−	−	++	++	+
3	±	±	+	−	−	++	+	+

± =weakly positive, + =positive, ++ =strongly positive

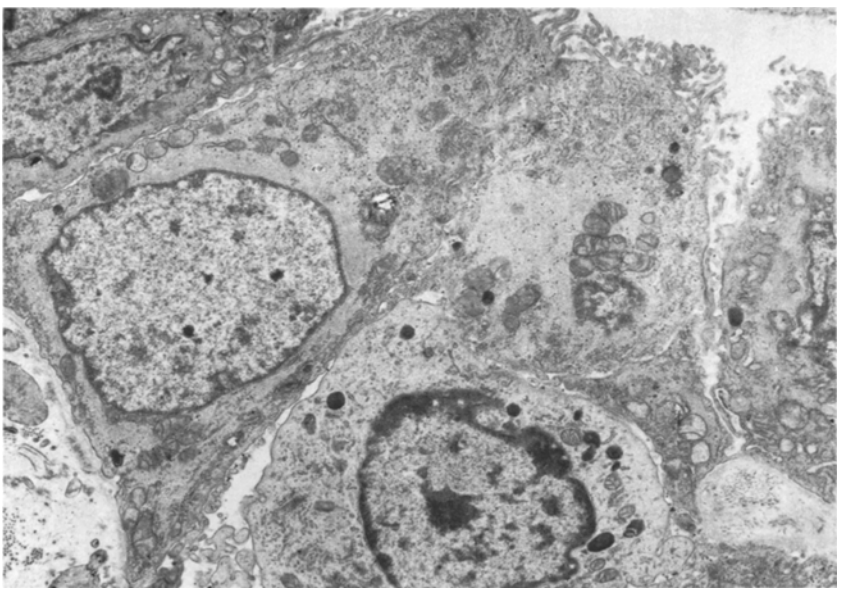


Fig. 12. Ultrastructure of diffuse pleural mesothelioma arranged in a layer having round or notched nuclei with prominent nucleoli. A basement membrane was present on the inner surface of tumor cells and abundant elongated, bush-shaped microvilli on the outer surface, ×5,700 (orig. mag. ×4,800)

specimens, however, a papillary arrangement of tumor cells could usually be found in some part of the tissue (Fig. 8). The plasma membranes of tumor cells were positive on colloidal iron and alcian blue stains, showing similarity with reactive mesothelial cells. Fine particles stainable by PAS which disappeared following diastase digestion, and were therefore interpreted as glycogen, were noted in the cytoplasm of tumor cells.

Tumor cells of the localized type appeared to be elongated and intermingled with abundant collagen in an interlacing pattern. Well-differentiated mesothelial cells were not seen in the tumor parenchyma, but only in invaginated pleural surface (Fig. 9). The tumor cells were negative for the alcian blue, colloidal iron and PAS stains.

Enzyme histochemically, the neoplastic cells of the diffuse type were strongly positive in the activities of α-N.acet est (Fig. 10) and N.AS-D acet est

Table 3. Comparative features of reactive mesothelial cells and mesothelioms

	Reactive mesothelial cells	Diffuse mesothelioma			Localized meso-thelioma
		Case 1	Case 2	Case 3	
Light microscopy					
PAS	weak	+	+	+	−
Coll. iron	+	++	+	+	weak
Alcian blue	+	+	weak	+	−
Electron microscopy					
Base. membrane	distinct	distinct	distinct	distinct	focal
Cell Junc.	desmosome	desmosome	desmosome	desmosome	junctional apparat.
Microvilli	variable	many	many	many	none
Rough E.R.	many (diffuse)	many	many (peripheral)	variable	variable
Golgi apparat.	variable	variable	variable	many	scarce
Intracyto. fibrils	variable-many (perinuclear)	many (fine)	many	many (fine)	scarce
Mitochondria	variable	many	many	many	scarce
Glycogen	scarce	variable	many	variable	none
Lysosome	variable	scarce	variable	variable	none

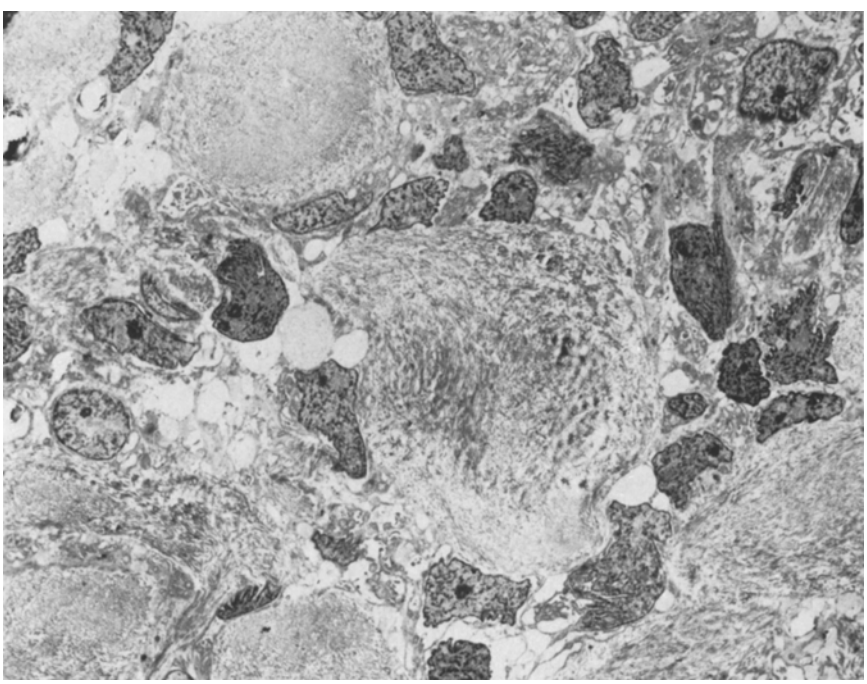


Fig. 13. Ultrastructure of localized pleural mesothelioma with scattered tumor cells within the fibrocollagenous stroma. ×2,200 (orig. mag. ×800)

(Fig. 11), but otherwise showed similar enzymatic reactions to those of the reactive mesothelial cells (Table 2).

In electron microscopy, tumor cells of the diffuse type showed abundant bushy microvilli on the cell surface, distinct increase in the nuclear-cytoplasmic ratio, and electron lucent fine fibrillary structure in perinuclear areas of the cytoplasm (Fig. 12). The cytoplasm also showed numerous mitochondria of variable size and finely dispersed glycogen. Tumor cells were bound to each other by desmosomes, and a linear basement membrane was present on the inner surface (Table 3).

Localized type tumor cells appeared among abundant collagen (Fig. 13). In contrast to the diffuse type, localized mesothelioma was characterized by the lack of microvilli and glycogen. Mitochondria were scarce, and the intracytoplasmic fibrillary structure indistinct.

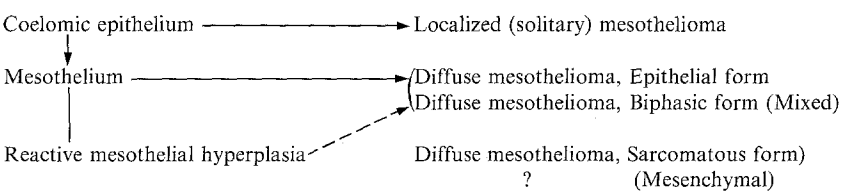
Discussion

A search of the literature revealed few previous studies on the enzyme histochemistry of human pleural mesothelium, although light and electron microscopy has been performed (Kawai et al. 1978; Klima et al. 1977; Ratzer et al. 1967; Suzuki et al. 1972). Wang (1974) observed rare ultrastructural alterations in human pleural mesothelial cells. In a variety of inflammatory and circulatory diseases the mesothelial cells of the pleura, pericardium and peritoneum become hyperplastic or even pleomorphic and may present diagnostic problems of differentiation from neoplasms when surgical specimens are small. Hyperplastic pleural mesothelial cells resulting from inflammatory diseases of the lungs may frequently appear in papillary and tubular patterns, resembling an epithelial form of mesotheliomas, a sheet-like arrangement and squamous metaplasia are also occasionally seen. Spindle cell foci of mesothelial cells are not a feature of mesothelial hyperplasia (McAllister et al. 1978). This hyperplasia is frequently accompanied by proliferation of macrophages and distinction between the former and the latter cell type is extremely difficult in hematoxylin-eosin stained specimens. This distinction may be unimportant as it has been reported that freefloating mononuclear cells settle on the denuded surface of the lung following which they spread out and develop features typical of mature mesothelial cells (Ryan et al. 1973). Nevertheless, moderate to strong PAS and colloidal iron activity observed within the cytoplasm, strong lysosomal activity and nonspecific esterase activity distinguished macrophages from mesothelial cells.

On routine light microscopic preparations the usual histopathologic criteria for a neoplasm, such as pleomorphism and mitosis of the tumor cells and the degree of stromal proliferation may aid in establishing the diagnosis, but no histological stains provides a definitive differential diagnosis between reactive mesothelial cells and neoplastic cells (Churg et al. 1965; Hourihane 1965; Kannerstein et al. 1978; Mostofi et al. 1973). In this study, the colloidal iron and PAS stains appeared to be more intense in mesothelioma cells than in reactive mesothelial cells. In enzyme histochemistry, the activities of most of the enzymes tested in the mesothelial cells in reactive hyperplasia were indistinguishable from those of mesothelioma cells only in naphthol AS-D acetate esterase and α-naphthyl acetate esterase the neoplastic cells showed stronger activities.

Our study and the reports of others (Davis 1974; Kawai et al. 1978; Kay

Table 4.



et al. 1971; Klima et al. 1977; Suzuki et al. 1972; Wang 1973) indicate that electron microscopy allows recognition of some morphological features characteristic of mesotheliomas. When reactive mesothelial cells are compared with the epithelial forms of mesothelioma, the nuclear-cytoplasmic ratio, the numbers of microvilli and mitochondria and the amount of glycogen were greater in the neoplastic cells. Electron-lucent intracytoplasmic fibrils were recognizable in reactive mesothelial cells but appeared wider and more distinct in mesothelioma cells (Table 3).

Malignant diffuse mesothelioma can be classified into epithelial, mixed or biphasic, and sarcomatoid or mesenchymal forms (Churg et al. 1965; Suzuki et al. 1976). We believe that both the epithelial and mixed or biphasic forms of diffuse mesothelioma have some epithelial characteristics, and are surely derived from mesothelial cells. However, it has not been confirmed that these mesotheliomas develop from mesothelial cells through reactive mesothelial hyperplasia (Table 4). Klima et al. (1977) emphasized that cases with prominent mesothelial cell lesions in biopsies, especially those with crowed mesothelial cells, should be followed up closely to exclude the possible existence of, or to detect at as early stage as possible the development of malignant mesothelioma.

Experimentally induced peritoneal neoplasms in mice by appear to be the so-called sarcomatous or mesenchymal forms of diffuse mesothelioma. Our light and electron microscopic studies suggested that these tumors were probably myosarcoma or fibrosarcoma (see Kawai 1979). If these forms are devoid of mesothelial cell characteristics as described above, they should not be categorized as a sarcomatous form of diffuse mesothelioma and require further discussion. However, some of the authors have suggested that mesothelial cells have the multipotence to assume a mesenchymal form accounting for the variation in histological pattern (Brandenburg 1953; Bolen et al. 1980).

The neoplastic cells of localized (solitary) pleural mesothelioma were not similar to reactive mesothelial cells or diffuse mesothelioma on light microscopic study. Ultrastructurally the cytoplasmic organelles were generally scanty (Fernandez 1979). We attempted to classify the different pleural lesions in relation to the development of mesothelioma. Because of the fibrous mesenchymal nature of the tumor pattern, mesothelioma of the localized type which had been called "solitary" by Stout (Ackerman 1954) presented no problems of differentiation from reactive hyperplasia of the mesothelium.

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