Mesothelioma: Profile of Keratin Proteins and Carcinoembryonic Antigen

An Immunoperoxidase Study of 20 Cases and Comparison With Pulmonary Adenocarcinomas

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The distribution of keratin proteins and carcinoembryonic antigen (CEA) in 20 diffuse pleural malignant mesotheliomas and 20 adenocarcinomas of the lung was determined with the use of an indirect immunoperoxidase method. Keratin proteins were identified in all of the mesotheliomas, with strong staining observed in 17 of the cases. Tumor cells of various histologic types (tubular, papillary, solid, and spindle) revealed staining for keratin proteins. A variety of staining patterns were observed, but the homogeneous pattern predominated, in either a diffuse (16 cases) or focal form (4 cases). CEA was usually absent (11 cases), but weak or equivocal staining was also observed (8 cases), and 1 case uniquely exhibited moderate staining for CEA. In contrast, adenocarcinomas of the lung usually stained weakly or negatively (18 cases) for keratin proteins and exhibited a predominantly peripheral staining pattern. All cases, however, stained strongly or moderately for CEA. The profile of strong keratin staining and weak or absent CEA staining appears characteristic of mesotheliomas and may be diagnostically useful in defining the epithelial element of these neoplasms and in distinguishing them from adenocarcinomas. (Am J Pathol 1982, 108:80–87)

MORPHOLOGICALLY, the diagnosis of mesothelioma is often difficult. Distinguishing many mesotheliomas from adenocarcinomas presents a particular problem. Histochemical methods have proven helpful, but in many cases they lack the sensitivity needed for a definitive pathologic diagnosis.[1-3] Recent immunohistochemical studies of keratin proteins and of carcinoembryonic antigens in tumors, however, have introduced a new approach to the diagnosis of mesotheliomas.

In a preliminary survey of the distribution of keratin proteins in a variety of tumors using an immunoperoxidase method, Schlegel et al[4] have described keratin proteins in a small group of mesotheliomas and a paucity or absence of these proteins in adenocarcinomas. Conversely, mesotheliomas reportedly appear devoid of carcinoembryonic antigen (CEA) by immunofluorescence,[5] whereas many adenocarcinomas of entodermal origin are rich in CEA.

This immunoperoxidase study defines the profile of keratin proteins and carcinoembryonic antigen in 20 cases of malignant pleural mesothelioma as compared with 20 cases of adenocarcinoma of the lung. The immunohistochemical staining patterns provide new criteria, which complement other established techniques, for accurate diagnosis of mesotheliomas.

Materials and Methods

Twenty diffuse malignant pleural mesotheliomas from the period 1977–1981 were obtained from the files of the Brigham and Women's Hospital. All cases were well or moderately differentiated and met the following criteria for inclusion: 1) characteristic epithelial (14 cases) or biphasic pattern (6 cases); 2) absence of periodic acid–Schiff–diastase-resistant mucosubstances[1]; 3) open biopsy and/or pleurec-

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tomy specimen; 4) thoracotomy with typical gross appearance and distribution of mesothelioma; and 5) careful clinical staging to exclude a primary carcinoma. Surgical specimens from 10 cases were examined by electron microscopy, and their ultrastructural features were typical of mesotheliomas.[6-9] Autopsies were performed in 7 cases and revealed a disease distribution typical of mesothelioma. Tissues were fixed in formalin. Most cases were also fixed in Zenker's–acetic acid solution, and some were fixed in Bouin's solution and B5[10] as well. We examined specimens of reactive mesothelial hyperplasia (1 of pleura overlying emphysematous blebs in a patient with a spontaneous pneumothorax and 2 of mesothelium from inguinal hernia sacs) to assess keratin protein and CEA localization in nonmalignant mesothelium.

For comparison, 20 adenocarcinomas of the lung (1 bronchoalveolar, 1 clear cell type, and 18 not otherwise specified) from the period 1971–1981 were also obtained from the files of the hospital. Cases were well or moderately differentiated adenocarcinoma in which pneumonectomy, lobectomy, or wedge biopsy had been performed and suitable formalin-fixed, paraffin-embedded tissue was available.

Immunoperoxidase studies for assessment of intracellular keratin proteins and carcinoembryonic antigen were performed on paraffin sections as previously described for other tissue antigens.[11,12] Rabbit anti-human keratin serum was kindly provided by Dr. Susan Banks-Schlegel. Preparation of antigen and characterization of antiserum have been previously described.[13] Rabbit anti-human CEA was obtained from Dako Laboratories (Copenhagen, Denmark, U.S. distributor, Accurate Chemical and Scientific Co., Westbury, NY).

To minimize background staining, sections were initially incubated for 30 minutes with a solution of 2.5% egg albumin in diluted (1:20) normal swine serum. Sections were then incubated with the primary antiserum for 30 minutes at room temperature. Various dilutions of antiserum were tested (1:20, 1:40, 1:100, 1:200, 1:400 for keratin and 1:50, 1:200, 1:500, and 1:1000 for CEA). For most specimens, optimal dilutions for keratin were 1:40 and 1:100 and for CEA, 1:50 and 1:200. Formalin-fixed sections for keratin staining were also incubated with trypsin (Type II, pancreatic No. T-8128; Sigma Chemical Co., St. Louis, Mo; 0.125 mg/ml) in a solution of 0.134 g/dl $CaCl_2$ (dihydrate) adjusted to pH 7.8 for 20 minutes at 37 C, prior to incubation with antikeratin antibody. Specificity of staining was verified by parallel studies substituting for the primary antiserum either normal rabbit serum, or anti-human keratin absorbed with keratin, or CEA antiserum that had been absorbed with polymerized normal spleen and colon and with CEA. Control sections of normal skin (for keratin studies) and of colon carcinoma (for CEA studies) were included in all experiments.

For comparison of mesotheliomas and adenocarcinomas the following were employed: 1) formalin fixed tissue, 2) antiserum to keratin proteins at dilutions of 1:40 and 1:100, and 3) antiserum to carcinoembryonic antigen at dilutions of 1:50 and 1:200.

Staining intensity was estimated using the following guide, based on the proportion of cells stained and the intensity of their staining.

Strong (+3, +4): Most cells (>50%) stain intensely (dark brown).

Moderate (+2): Many cells stain moderately (medium brown); there is intense staining in ≤50% cells.

Weak (+): Many cells stain weakly (pale brown); <10% of the cells are intensely/moderately stained.

Equivocal: Some cells stain weakly; <1% of cells are intensely/moderately stained.

Results

Hyperplastic mesothelium and mesotheliomas exhibited strong cytoplasmic staining for keratin proteins, as defined by the immunoperoxidase technique (Figures 1 and 2). Comparison studies of different fixatives revealed optimal staining after Zenker's fixation; however, satisfactory results were also obtained with fixation in 10% neutral buffered formalin, Bouin's, and B5 solutions. The staining of formalin-fixed specimens was considerably enhanced by preliminary trypsin digestion. Prompt fixation was also helpful in optimizing staining. Control studies using normal rabbit serum or antiserum absorbed with keratin resulted in complete loss of staining.

Of the 20 mesotheliomas stained for keratin proteins, strong or moderate staining was seen in all cases (Table 1). Cytoplasmic staining in epithelial types of mesotheliomas accentuated their tubular, cordlike, and papillary patterns (Figures 2 and 3). Tumor cells proliferating on the mesothelial surface as well as the invading mesothelial cells were stained strongly (Figure 2). In biphasic types of mesotheliomas, keratin staining delineated epithelial tumor elements, which were inapparent or ill-defined in routine hematoxylin and eosin sections. Cords of cohesive round cells and cuboidal cells rimming ill-defined clefts were identified as epithelial elements by their keratin staining. In the predominantly sar-

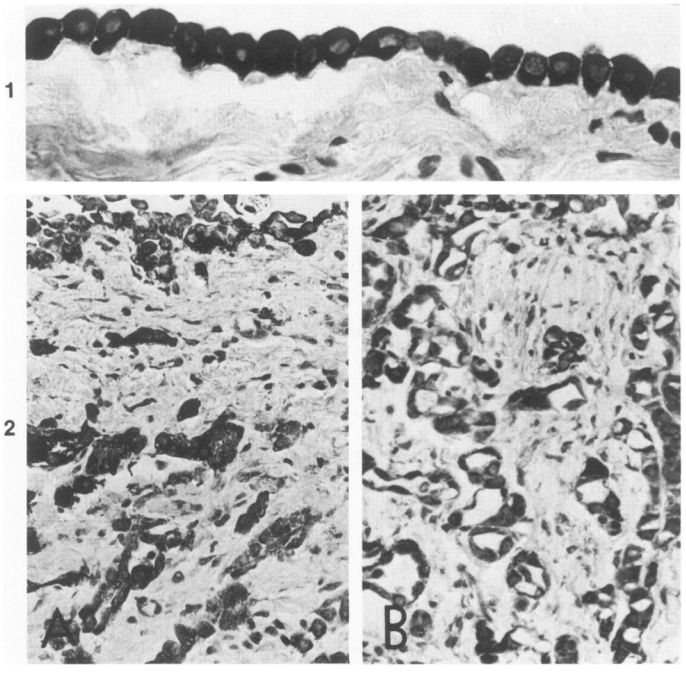

Figure 1—Hyperplastic mesothelium of parietal pleura from spontaneous pneumothorax case. Keratin proteins stain strongly (black) and homogeneously throughout the cytoplasm. (Keratin protein immunoperoxidase, hematoxylin counterstain, ×320) **Figure 2**—Epithelial type of mesothelioma stained by the immunoperoxidase method for keratin proteins. **A**—Tumor cells on the surface and within the fibrous stroma are stained strongly. **B**—Tumor forms tubules and cords. Keratin proteins of many of the cells stain strongly. (Hematoxylin counterstain, **A**, ×100; **B**, ×80)

comatous type of mixed mesotheliomas with large areas of spindle-shaped tumor cells, scattered spindle-shaped mesothelial tumor cells stained strongly for keratin (Figure 4).

A variety of cytologic keratin staining patterns were observed (Table 2), and several patterns were present in each mesothelioma. Homogeneous staining involved either all of the cell cytoplasm in the diffuse type or a portion of the cell cytoplasm in the focal type (Figures 3 and 5). Diffuse, homogeneous staining was the predominant pattern in most cases (Table 2). Striking ringlike staining was seen in all cases. The rings were perinuclear or peripheral (Figure 5) and were sometimes incomplete. Fine, linear filaments were also present in all cases (Figure 5A). These sometimes formed bundles, which occasionally were obliquely directed toward the cell surface, where they merged with peripheral rings. Vacuolar, punctate, and coalescent punctate staining were also seen but were usually observed in specimens that were not optimally fixed.

Staining for CEA differed markedly from the staining pattern for keratin proteins. CEA staining was much weaker and was absent in the reactive mesothelium and in most of the mesotheliomas by the immunoperoxidase technique. Optimal staining was obtained after fixation in 10% neutral buffered formalin. Satisfactory staining was also afforded by Zenker's-acetic acid and by Bouin's and B5 fixation. Prompt fixation was essential for optimal staining. Staining was obliterated by the substitution of antigen-absorbed anti-CEA antiserum or normal rabbit serum for the primary antiserum.

Only 9 of the 20 mesotheliomas stained for CEA.

Table 1—Profile of Keratin Proteins and Carcinoembryonic Antigen in Malignant Mesothelioma and Adenocarcinoma of the Lung

		Staining intensity				
	No. of cases	Strong	Moderate	Weak	Equivocal	Negative
Keratin						
Mesothelioma	20	18	2	0	0	0
Adenocarcinoma	20	0	2	7	2	9
CEA						
Mesothelioma	20	0	1	2	6	11
Adenocarcinoma	20	16	4	0	0	0

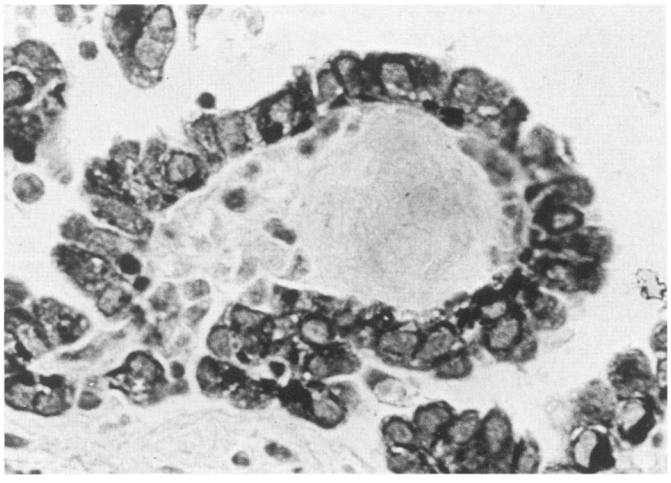

Figure 3—Epithelial type of mesothelioma forming papillary tuft. Cytoplasmic staining is diffuse in some cells and focal in others. (Immunoperoxidase for keratin proteins with hematoxylin counterstain, ×280)

Moderate cytoplasmic staining was noted in 1 case, while the remainder exhibited only weak or equivocal staining (Table 1). Several staining patterns were observed. The most common was a homogeneous pattern, which was diffuse or focal, without predilection for any particular areas of the cytoplasm (Figure 6). Punctate patterns were common, with solid round or ovoid granules about 2 μ in greatest diameter (Figure 6B). These sometimes formed coalescent, punctate patterns with irregular margins. Small vesicles, about the same size as the punctate foci, were also seen but were usually observed in suboptimally fixed specimens or in areas of intense lymphocytic infiltration of the tumor. Occasionally, membrane staining was seen that was limited to the periphery of the cell.

In marked contrast to the mesotheliomas, staining for keratin proteins in most of the 20 adenocarcinomas of the lung was absent, equivocal, or weak (Table 1). Staining of moderate intensity was seen in only 2 cases.

Staining for keratin proteins occurred predominantly at the periphery of the adenocarcinoma cells in or adjacent to the cell membrane (Figure 7) but was also noted as perinuclear rings and as diffuse, homogeneous staining of the cytoplasm.

Small foci of frank squamous differentiation were observed in 7 of the adenocarcinomas. They were characterized by spindle-shaped, triangular, and polygonal tumor cells that mimicked squamous cell carcinoma (Figure 8A). In these areas, staining was predominantly peripheral and often intercellular.

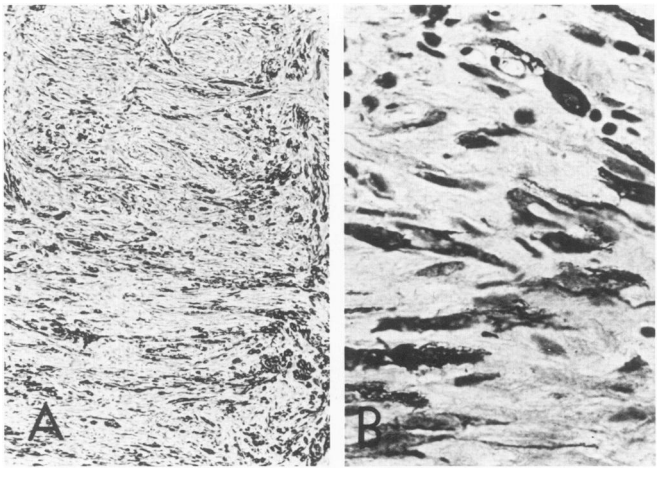

Figure 4—Sarcomatoid area of mixed type of mesothelioma. **A**—Many of the spindle-shaped cells in this area stain strongly for keratin. **B**—Higher power reveals strong cytoplasmic staining and includes staining of slender cytoplasmic extensions. (Keratin proteins, immunoperoxidase stain with hematoxylin counterstain, **A**, ×32; **B**, ×200)

Table 2—Keratin Staining Patterns in 20 Specimens of Malignant Mesothelioma

Pattern	No. of specimens
Homogeneous	
Diffuse	20*
Focal	20†
Ring	
Perinuclear	20
Peripheral	17
Filaments	20
Vacuolar	8
Bundles	5
Punctate	1

* Predominant pattern in 17.
† Predominant pattern in 3.

Long, intercellular, straight keratin spikes or slightly curved or irregular keratin bands were sometimes noted in the squamous areas extending the length of 2–4 cells (Figure 8B). In a few cases "micro" cysts about 20–50 μ in diameter were noted among the tumor cells and were highlighted by their lining of keratin-stained material. In a single case, keratin-stained cytoplasmic balls, 2–4 μ in diameter, were seen in the tumor cells. Residual normal glands containing keratin-staining cells were occasionally seen in the stroma of the adenocarcinomas and were distinguished from keratin-stained tumor cells by their morphologic features, including the absence of cytologic atypia characteristic of malignancy.

All of the 20 adenocarcinomas of the lung stained for CEA (Figures 9–11). In sharp contrast to the mesotheliomas, the staining was either strong or moderate in all cases (Table 1). It was diffuse and finely granular in the cytoplasm of tumor cells but was frequently accentuated at their luminal borders. Necrotic luminal contents usually stained strongly. Numerous fine cytoplasmic vacuoles were often present with staining of the periphery of the vesicles. Coarse cytoplasmic vesicles and large granules were seen occasionally.

Discussion

This immunohistochemical study defines a consistent staining profile for malignant mesotheliomas characterized by strong staining for keratin proteins and usually weak or absent staining for CEA. Our observations of strong cytoplasmic staining for keratin proteins in mesotheliomas confirm and extend the preliminary immunoperoxidase study of Schlegel et al.[4] The pattern of cytoplasmic localization of keratin proteins in our mesotheliomas reflects the distribution of intermediate filaments and tonofilaments noted in several electron-microscopic studies. Intermediate filaments, which include keratin proteins, have been noted in a perinuclear and in a random cytoplasmic distribution, as well as at the periphery of the cell as tonofilaments contributing to desmosomal complexes.[6–9]

Our studies of CEA reveal that most mesotheliomas are negative, though some may stain weakly or equivocally and a rare case stains with moderate intensity. Our findings differ from those of Wang et al, who observed no CEA staining in a group of 12

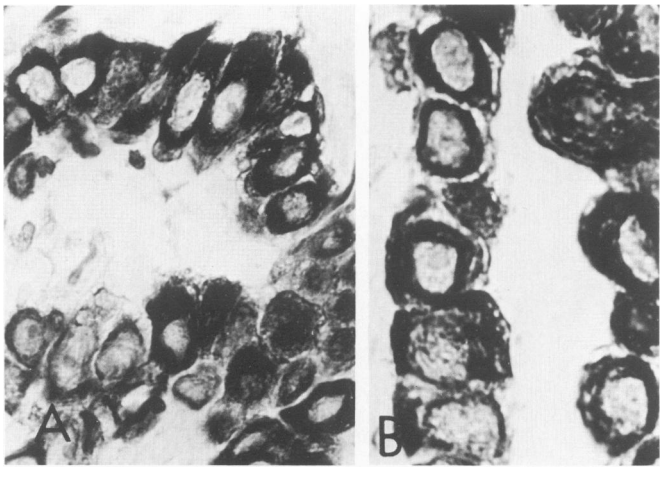

Figure 5—High magnification of epithelial type of mesotheliomas. **A**—The cytoplasm of many of the tumor cells stains diffusely and strongly for keratin proteins, and well-defined perinuclear rings are formed. In some cells, ill-defined longitudinal filaments can also be seen. **B**—Keratin proteins form characteristic strongly stained perinuclear rings. (Immunoperoxidase with hematoxylin counterstain, **A**, ×400; **B**, ×520)

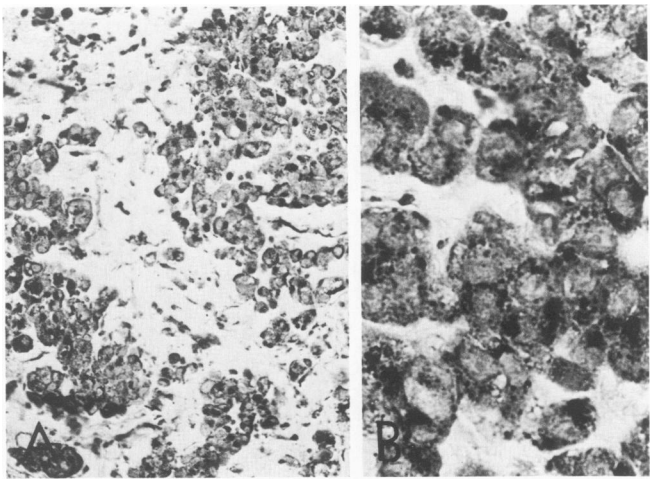

Figure 6—Epithelial type of mesothelioma stained by the immunoperoxidase method for CEA. In this unique case, dark staining of the cytoplasm of many of the tumor cells is seen. **A**—Under low power, cytoplasmic staining is seen to be diffuse in some cells, but focal cytoplasmic staining predominates. **B**—Under higher power, punctate cytoplasmic staining is seen. Occasional cells with focal, homogeneous cytoplasmic staining are also present. Many of the stained granules are small, round to ovoid, and discrete, but coalescent clusters are also seen. (Immunoperoxidase with hematoxylin counterstain, **A**, ×80; **B**, ×320)

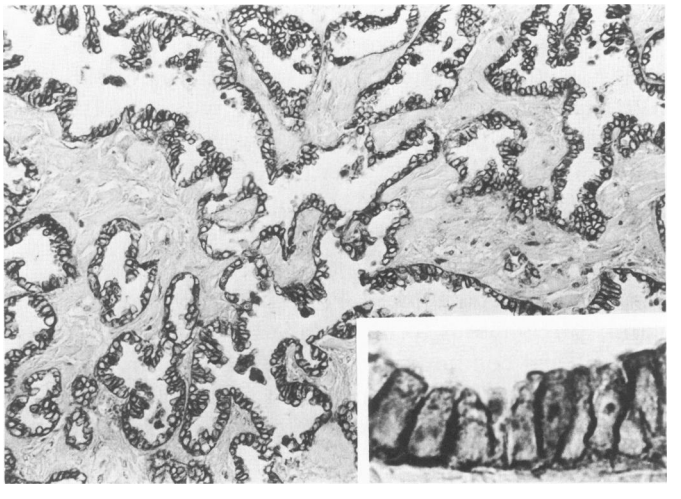

Figure 7—Adenocarcinoma of lung, bronchoalveolar type. The pattern of diffuse, moderate staining for keratin proteins is unusual. **Inset**—Well-defined, peripheral staining pattern. (Keratin protein immunoperoxidase with hematoxylin counterstain, ×80; **inset**, ×520)

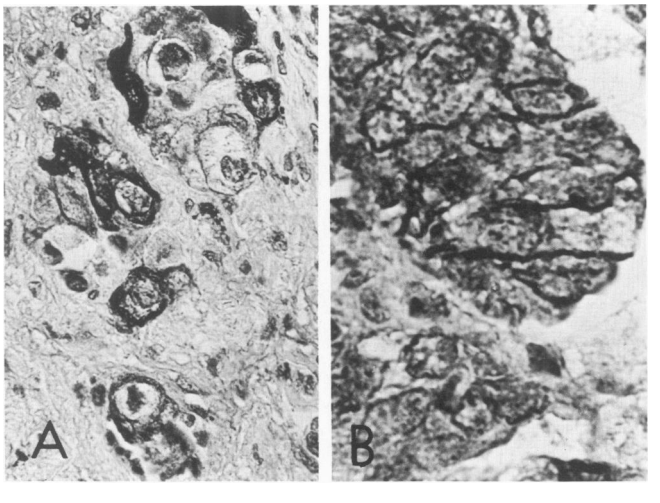

Figure 8—Adenocarcinoma of the lung with foci of squamous differentiation stained for keratin proteins. **A**—Frankly squamous area. Staining is confined to the periphery of some tumor cells, but homogeneous staining of the cytoplasm is also seen. **B**—Peripheral staining pattern and long bands of keratin extending between cells. (Immunoperoxidase with hematoxylin counterstain, **A**, ×280; **B**, ×520)

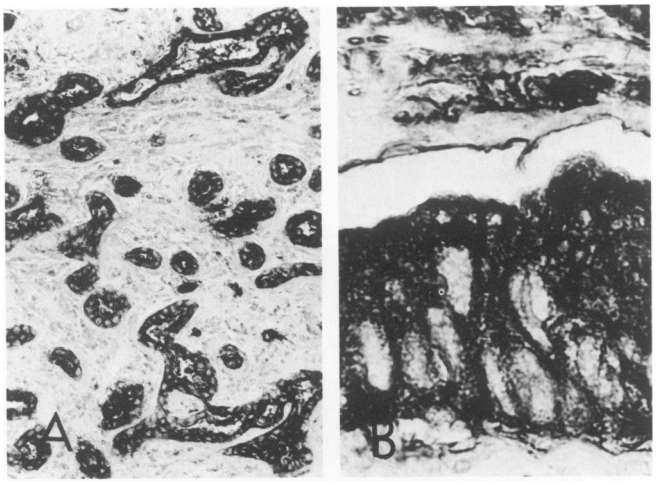

Figure 9—Adenocarcinoma of the lung stained for CEA. **A**—Intense, diffuse staining of the cytoplasm of infiltrating tumor tubules is seen. **B**—Higher power reveals intense, cytoplasmic staining of adenocarcinoma cells. Fine vacuoles and faint granularity are apparent in the otherwise homogeneous staining. The upper half of the figure is tumor gland lumen with stained necrotic cellular detritus. (Immunoperoxidase with hematoxylin counterstain, **A**, ×80; **B**, ×520)

mesotheliomas, using an immunofluorescent antibody method applied to tissue sections.[5] This difference may reflect increased sensitivity of CEA detection when the indirect immunoperoxidase technique is used, particularly since most positive cases revealed weak or equivocal staining.

The results of our immunoperoxidase study may provide useful diagnostic criteria for accurate identification of mesotheliomas. For example, in predominantly sarcomatous forms of mixed mesotheliomas, the epithelial component may be ill-defined and the diagnosis in doubt. Recognition of a clearly defined epithelial component would help to establish the presence of a biphasic pattern and to confirm the diagnosis of mesothelioma of mixed sarcomatous and epithelial types. The epithelial component of such cases may be precisely defined by the keratin staining of cords and tubules or as the staining of keratin filaments within spindle cells (Figure 4). The keratin staining represents the immunohistochemical correlate of keratin tonofilaments and desmosomes observed in electron micrographs of spindle cells of mesotheliomas. The latter ultrastructural features have been emphasized as useful clues in the diagnosis of mesotheliomas.[8]

In well-differentiated pleural mesotheliomas, histo-

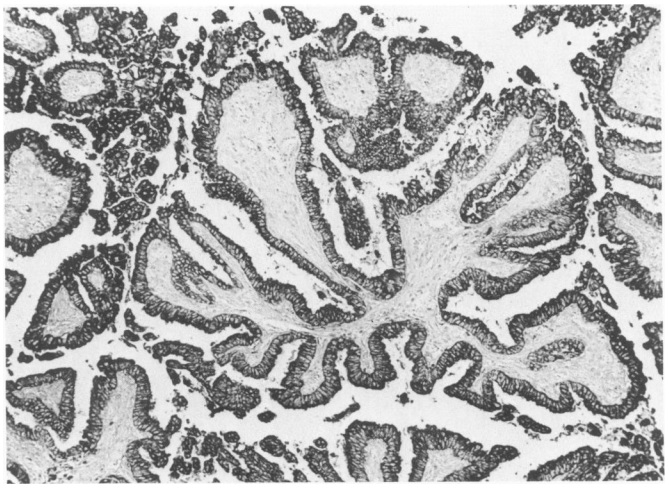

Figure 10—Adenocarcinoma of the lung; papillary clear cell type stained for CEA. Diffuse, strong staining of tumor cells is present. (Immunoperoxidase with hematoxylin counterstain, ×52)

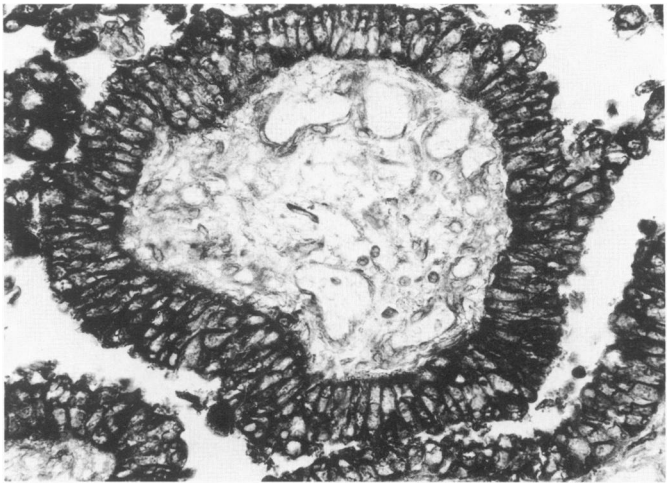

Figure 11 — Higher magnification of Figure 10. Staining is diffuse and strong, with peripheral accentuation of cytoplasmic staining. (CEA immunoperoxidase with hematoxylin counterstain, x 200)

logically, the major diagnostic consideration is usually adenocarcinoma of the lung. In contrast to mesotheliomas, adenocarcinomas of the lung were characterized by weak or absent staining for keratin proteins and strong staining for CEA. The pattern of weak keratin staining we observed in the adenocarcinomas of the lung was similar to that reported by Schlegel et al.[4] It is to be stressed, however, that our observations were made on fixed, paraffin-embedded tissues. Keratin proteins have been identified in glandular epithelia, and reasonably strong staining has been reported in a variety of adenocarcinomas when frozen sections were used.[14,15] The fixation and paraffin embedding as used in our study diminishes keratin staining in adenocarcinomas sufficiently to make the method particularly useful in differentiating mesotheliomas from adenocarcinomas. The staining for CEA in 20 of 20 of the adenocarcinomas in our series compares with the results of Pascal et al,[16] who observed positive staining in 10 of 14 adenocarcinomas of the lung and 6 of 8 bronchoalveolar carcinomas, and of Wang et al, who reported positive staining in 4 of 4 adenocarcinomas of the lung and in 8 of 8 bronchioloalveolar carcinomas,[5] but contrasts with the positive staining of only 5 of 16 adenocarcinomas of the lung reported by Goldenberg et al.[17] Differences in antiserums and staining methods may account for the variability in the percentage of tumors in which staining was positive among the different series.

Qualitative differences in keratin localization were also observed and may also prove to be of diagnostic utility. Although peripheral keratin protein localization was often present in the mesotheliomas, the predominant pattern was homogeneous cytoplasmic staining, which was usually diffuse but was often associated with perinuclear staining appearing as prominent rings. In the adenocarcinomas of the lung, on the other hand, the predominent pattern was peripheral. Long intercellular bands and keratin-lined microcysts, which were not seen in the mesotheliomas, were present and reflected the presence of squamous differentiation within the adenocarcinomas. These distinctions provide additional criteria for the evaluation of mesotheliomas, and complement histochemical methods[1-3] and more recently, electron-microscopic techniques,[6-9] for accurate diagnosis of these neoplasms.

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