

THE EXFOLIATIVE CYTOLOGY OF DIFFUSE MALIGNANT MESOTHELIOMA

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PLATES LXXXVI-XCI

ALTHOUGH excellent accounts have been published in recent years of the gross and histological pathology of neoplasms believed to be diffuse malignant mesotheliomas, there remains a scarcity of adequate descriptions of their exfoliative cytology. One purpose of this paper is to help correct this deficiency. Another is to present further evidence that diffuse malignant mesothelioma really exists, for undoubtedly there are still some who are not convinced that there is such an entity.

A search of the clinical and pathological literature on diffuse malignant mesothelioma revealed numerous references to the cytology of the accompanying serous fluids mostly, however, meagre and poorly illustrated. The earliest illustrated reference I have been able to find is that of Warthin (1897), who has a drawing of a stained wet film showing round and fusiform cells which are difficult to identify as mesothelial or malignant. No other illustrated report appeared until that of Coulter (1946), but the first indubitable illustrated example of exfoliated mesothelioma cells is that of van Assen (1953). The best illustrations and most informative descriptions are those of Papanicolaou (1956, 1960) and Klempman (1962). Papanicolaou's photographic illustrations are particularly noteworthy. They are in colour and the corresponding tissue sections are shown; nevertheless, this particular contribution has been received with some scepticism (Book review, this *Journal*, 1962).

This paper will present evidence that abnormal cells found in the effusions of 7 patients with cancer involving serous membranes were of mesothelial origin, that these cells were exfoliated from the neoplasms themselves, and that the neoplasms must, therefore, be malignant mesotheliomas.

MATERIAL AND METHOD

The material was received during a 4-yr period by the Department of Pathology of the University of Michigan. It consisted of smears of the sediment obtained by centrifugation of pleural or peritoneal effusions from 7 patients who had malignant neoplasms that diffusely involved one or more serous membranes. All smears were fixed while wet in 95 per cent. ethyl alcohol and stained by the Papanicolaou method. Adequate tissue was obtained in 6 cases and a histopathological diagnosis of malignant mesothelioma made.

CASE HISTORIES

Case 1. A man aged 47 had a pleural effusion which was detected by routine X-ray of the chest. He had no symptoms. Smears of the pleural fluid were reported as negative for tumour cells. At thoracotomy the visceral and parietal

pleura was studded with innumerable warty excrescences, diagnosed microscopically as malignant mesothelioma (fig. 1). The patient died 2 yr later; there was no necropsy.

This was the first mesothelioma encountered in the series. Cytologically it was the most differentiated, with the cells in the smears resembling greatly enlarged benign mesothelial cells. It was not until the diagnosis of mesothelioma had been made on the tissue that the possibility was considered that these apparently benign mesothelial cells were, in fact, cancerous.

Case 2. A man aged 65 had a pleural effusion, which was detected by routine X-ray of the chest. He had no symptoms. Smears of the pleural fluid were reported as malignant mesothelioma or extreme mesothelial hyperplasia, probably the former. The needle biopsy specimen of pleura was interpreted as highly suspicious for malignant neoplasm. A year later X-ray examination of the chest revealed numerous bilateral peripheral nodular masses. During the next 3 yr both lung fields became obscured by neoplasm. The patient died 4 yr after the discovery of the pleural effusion; there was no necropsy.

Though no satisfactory tissue specimen was ever obtained, there was no doubt, from the radiological findings and clinical course, that the patient had cancer involving the pleura. Significantly, the pleural fluid had the colour and consistence of honey, qualities that have been described in association with diffuse malignant mesothelioma (Meyer and Chaffee, 1939; Truedsson, 1951-52).

Case 3. A man aged 40 had abdominal pain and distension. Laparotomy revealed numerous neoplastic nodules studding the peritoneum; these nodules were interpreted microscopically as malignant mesothelioma or adenocarcinoma (fig. 2).

The patient died 6 mth later. A complete necropsy showed that the neoplasm, except for two minute pulmonary metastases, was confined to the peritoneum. The final diagnosis was diffuse malignant peritoneal mesothelioma. Smears were made from the peritoneal fluid obtained at necropsy. Three independent observers who were unfamiliar with the case interpreted these smears as malignant mesothelioma.

Case 4. A man aged 48 complained of pain in the chest; a pleural effusion was detected by X-ray. Smears of the pleural fluid were reported as malignant mesothelioma. At thoracotomy the visceral and parietal pleura was studded with neoplastic nodules, diagnosed microscopically as malignant mesothelioma (fig. 3). The patient died 1 yr later; there was no necropsy.

Case 5. A man aged 70 complained of breathlessness and chest pain; a pleural effusion was detected by X-ray. Smears of the pleural fluid were reported as strongly suspicious for malignant mesothelioma. The needle biopsy specimen of pleura was interpreted as probably mesothelioma, possibly metastatic carcinoma. The patient developed ascites and died 9 mth later. A complete necropsy revealed diffuse pleural, peritoneal and pericardial neoplasm, diagnosed microscopically as malignant mesothelioma (fig. 4). There were a few small metastases in the liver, one kidney, and a peribronchial lymph-gland.

Case 6. A man aged 47 complained of breathlessness; a pleural effusion was detected by X-ray. Smears of the pleural fluid were reported as malignant mesothelioma. At thoracotomy the visceral and parietal pleura was studded with neoplastic nodules, diagnosed microscopically as malignant mesothelioma (fig. 5). He died 8 mth later; there was no necropsy.

Case 7. A man aged 61 complained of pleuritic pain; a pleural effusion was detected by X-ray. Smears of the pleural fluid were reported as malignant mesothelioma or adenocarcinoma, probably the former. At thoracotomy the visceral and parietal pleura was studded with neoplastic nodules, diagnosed microscopically as malignant mesothelioma (fig. 6). The patient died 6 mth later. A complete necropsy showed that the neoplasm was confined to pleura on one side. The diagnosis remained malignant mesothelioma.

OBSERVATIONS

The serous fluids of these patients are some of the most cellular we have ever encountered; nearly all smears contain enormous numbers of cells (fig. 7). One immediately gains the impression that they are atypical and of mesothelial origin. In 5 cases most of these cells were considerably larger than benign mesothelial cells, with some attaining gigantic proportions (figs. 8 and 9); in cases 5 and 6 they are, on the whole, only slightly larger than normal. Many are discrete, but often they form large clusters (figs. 7, 10 and 11); in the smears from cases 2 and 5 discrete cells are almost entirely absent. Sometimes the clusters have fairly smooth and round outlines, but most are knobby, the knobs being formed by single jutting-out cells (figs. 10 and 12).

Types of intercellular articulation can be seen that are common in normal mesothelial cell clusters and hardly ever occur in any other normal cell type. One type shows a cell embraced by pincer-like prongs of cytoplasm of a larger cell (figs. 13 and 14). In other instances a few cells are loosely connected and flattened at apposing surfaces (figs. 15-17), and in a third several cells fit together like a mosaic to form a flat sheet (fig. 18).

The Papanicolaou staining and density of the cytoplasm are quite distinctive. In all specimens the cytoplasm takes on the varied hues of grey-green, orange, brown, pink and mauve so characteristic of mesothelial cells. This variety is not encountered with such constancy in histiocytes or carcinoma cells recovered from serous fluids. The cytoplasm, except where vacuolated, also possesses great optical density, often contrasting with a foamy, lighter stained peripheral rim (figs. 8-10, 12, 14-17); these features also characterise the benign mesothelial cell. An interesting property of some of the cells is their strikingly abundant cytoplasm (figs. 19 and 20).

In all 7 cases a few of the atypical cells contain vacuoles: some large and dominant, occupying most of the cytoplasm (figs. 21 and 22); others small and few in number (figs. 8 and 23); occasionally numerous, imparting a finely bubbled effect to the cytoplasm (fig. 24). Not infrequently one cell seems to be ingested into a vacuole of another (fig. 25).

In 3 of the cases some nuclei are large and hyperchromatic and possess the irregularity of contour and the coarse uneven pattern of chromatin that suggest malignancy. But except for size these nuclear abnormalities are not as pronounced as those found in exfoliated cells of squamous-cell carcinoma or adenocarcinoma. This is so even in case 7, which has the most malignant-appearing nuclei (figs. 23 and 26). Most of the nuclei even in these three cases, and all the nuclei in the other four, are of a type that cannot be readily recognised as malignant, for they are not disproportionately large, their borders are not especially heavy, and their outlines are smoothly round or oval (figs. 14, 17-20). However, they show a slight but definite coarseness and irregularity

of chromatin pattern that is more readily appreciated by comparison with mesothelial cells known to be benign (fig. 27).

Multiple nuclei are observed about as frequently as in benign mesothelial cells; usually there are 2 or 3 nuclei but occasionally many more (fig. 28). Nucleoli are fairly prominent in 6 of the 7 cases; generally they are solitary and neatly round, but occasionally multiple or elongated. A mitotic figure is rarely seen with even the most cellular specimen; the average count is less than one per smear. Only one of those seen is atypical.

In each case the atypical exfoliated cells are morphologically similar to those seen in the sections of the corresponding pleural or peritoneal neoplasm. Compare figs. 14, 15 and 18 with fig. 29.

The remaining components of the smears are similar to those of most serious effusions. There are what appear to be benign mesothelial cells, which may in reality be neoplastic. Also present are lymphocytes, histiocytes and a few neutrophil and eosinophil leucocytes. In case 2 the background of the smear has an unusual flocculent density (fig. 11), which may be related to the honey-like consistence and high hyaluronic acid content of the pleural fluid. The high hyaluronic acid content of the pleural fluid of this case, the only one in which there was no histopathological diagnosis, is strong evidence to support the diagnosis of mesothelioma (Meyer and Chaffee, 1939; Blix, 1951-52; Dvoskin, 1954; Wagner, Munday and Harington, 1962). Unfortunately, hyaluronic acid estimations were not performed on the fluids of the other 6 cases.

DISCUSSION

The first conclusion to be drawn is that the unusual cells encountered in these smears are of mesothelial origin. The knobby clumps; the dense cytoplasm, with its distinctive tinctorial properties, peripheral foaminess, and single or many fine vacuoles; the central smoothly round or oval nuclei; and the types of cellular articulation are all characteristic of the mesothelial cell, and of no other cell encountered in serous fluids.

Are they neoplastic or do they represent merely a combination of mesothelial hyperplasia and hypertrophy? It will be recalled that in 6 of the 7 cases there was histological evidence of neoplasm involving serous membranes; in case 2 the evidence was clinical and radiological only. It is very unlikely that serous membranes so heavily involved by neoplasm would fail to shed their cells into the fluid bathing them and that such cells would not be demonstrated by standard cytological methods. Yet none of the serous fluids showed cells resembling those of adenocarcinoma, squamous carcinoma, or undifferentiated carcinoma, but in their place numerous abnormal cells of mesothelial origin, morphologically very similar to those seen in the sections of the neoplasm. Since the abnormal cells were of mesothelial

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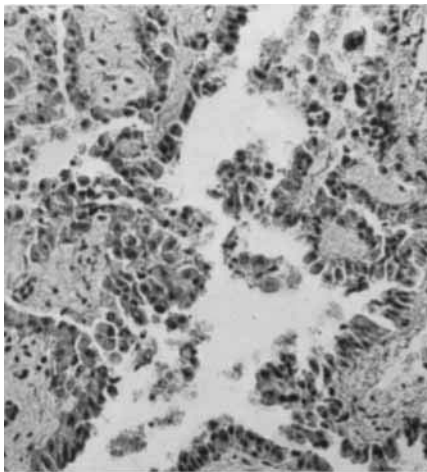


FIG. 1.—Case 1. Papillary malignant mesothelioma of pleura. Haematoxylin and eosin. $\times 150$.

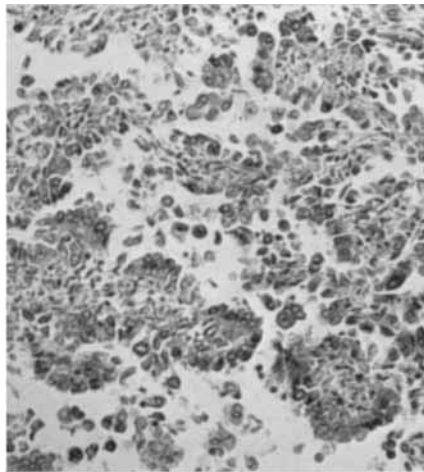


FIG. 2.—Case 3. Papillary malignant mesothelioma of peritoneum. HE. $\times 150$.



FIG. 3.—Case 4. Malignant mesothelioma of pleura. Thickened subpleural fibrous connective tissue at the top. HE. $\times 150$.

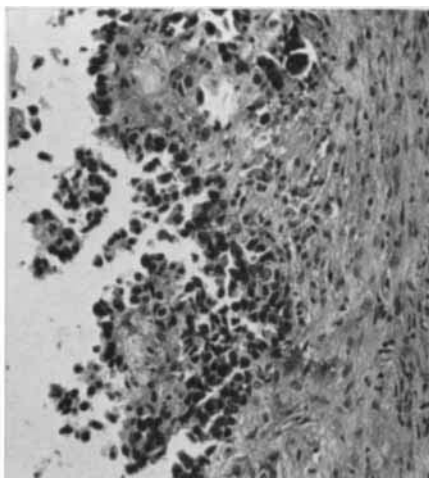


FIG. 4.—Case 5. Malignant mesothelioma in the peritoneum. Smooth muscle of small intestine on the right. HE. $\times 150$.

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FIG. 5.—Case 6. Malignant mesothelioma of pleura. Subpleural fibrous connective tissue at the top. HE. $\times 150$.

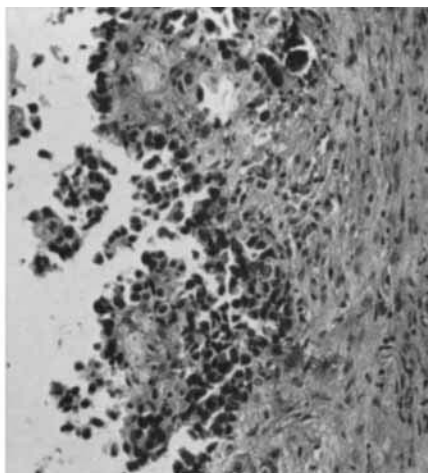


FIG. 6.—Case 7. Papillary malignant mesothelioma. Thickened subpleural fibrous connective tissue on the right. HE. $\times 150$.

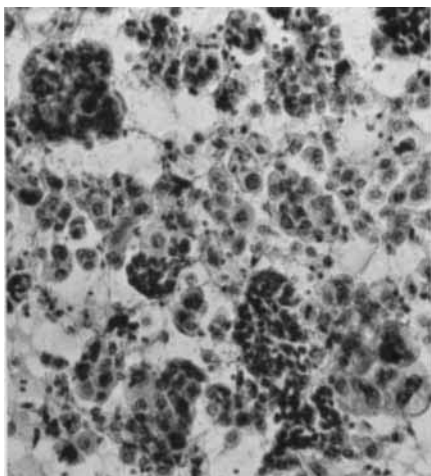


FIG. 7.—Case 4. Numerous clusters of mesothelioma cells. Figs. 7-28 are from pleural fluid. Papanicolaou (Pap.) stain. $\times 150$.

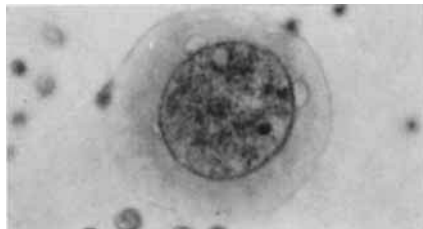


FIG. 8.—Case 7. Giant mesothelioma cell. The cytoplasm is quite dense except where it is vacuolated or faded at the periphery. The nucleus is hyperchromatic with rather coarse chromatin. Pap. $\times 600$.

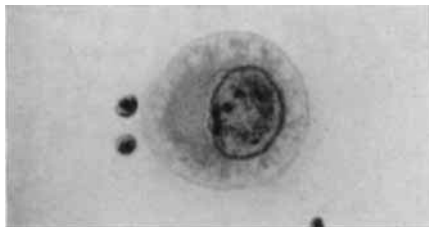


FIG. 9.—Case 7. Giant mesothelioma cell. The cytoplasm shows pronounced peripheral foaming. The nucleus is hyperchromatic and slightly irregular in contour. Pap. $\times 600$.

Figs. 7-9 from pleural fluid.

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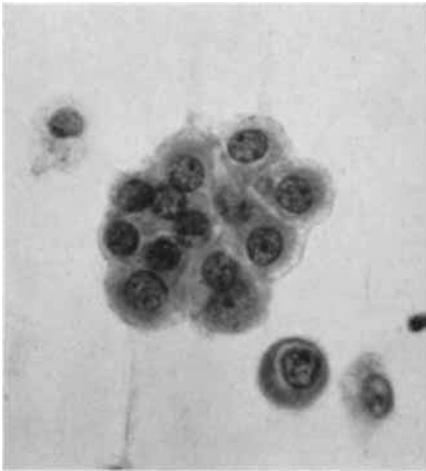


FIG. 10.—Case 1. A cluster of mesothelioma cells. The knobby outline of the cluster as a whole is characteristic. The cytoplasm is particularly dense and shows peripheral foaminess. Pap. $\times 600$.

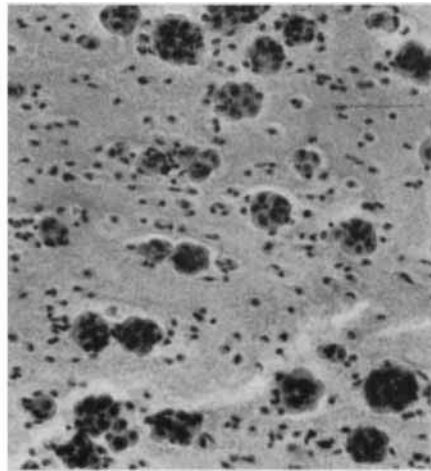


FIG. 11.—Case 2. Many large clusters of mesothelioma cells. The discrete cells are histiocytes. There is an unusual dense flocculent background. Pap. $\times 150$.

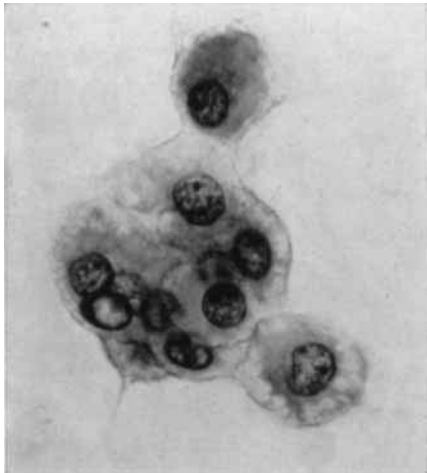


FIG. 12.—Case 1. A cluster of mesothelioma cells showing a "knobby" contour. The cytoplasm is dense except where it shows pronounced peripheral foaminess. Pap. $\times 600$.



FIG. 13.—Case 1. A pair of mesothelioma cells. One cell seems to be grasped by pincer-like prongs of cytoplasm of the other cell. Pap. $\times 600$.

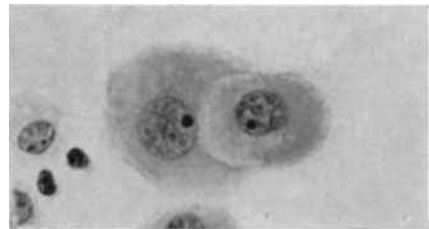


FIG. 14.—Case 1. A pair of mesothelioma cells. The articulation is similar to that in fig. 13, though the "embrace" is not as full. The cytoplasm is dense and shows peripheral fading. The smooth contour, light border and fine chromatin of the nuclei do not suggest malignancy. Pap. $\times 600$.

All figs. from pleural fluid.

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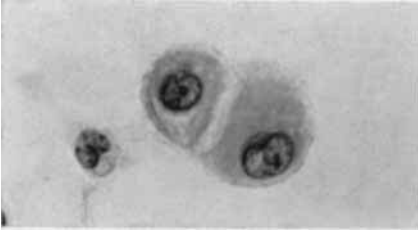


FIG. 15.—Case 1. A pair of mesothelioma cells. Note flattened apposing surfaces. The cytoplasm is dense and shows peripheral foaming. The appearance of the nuclei does not suggest malignancy. Pap. $\times 600$.

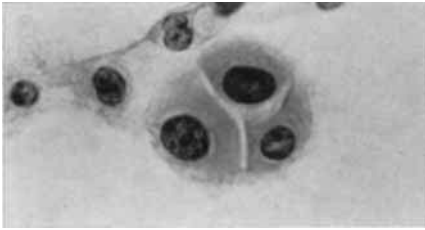


FIG. 16.—Case 1. A triad of mesothelioma cells. Note flattened apposing surfaces. The cytoplasm is dense. Pap. $\times 600$.



FIG. 17.—Case 1. A tetrad of mesothelioma cells. Note flattened apposing surfaces. The cytoplasm is dense. The appearance of the nuclei does not suggest malignancy. Pap. $\times 600$.

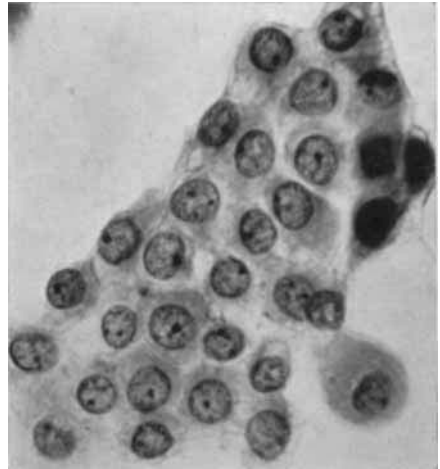


FIG. 18.—Case 1. Mesothelioma cells fitting together like a mosaic to form a flat sheet. The appearance of the nuclei does not suggest malignancy. Pap. $\times 600$.

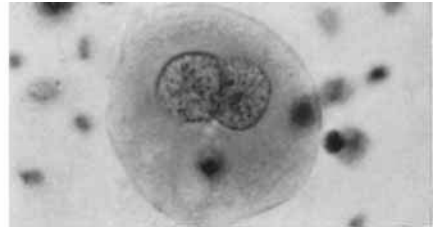


FIG. 19.—Case 7. A giant binucleate mesothelioma cell. The cytoplasm is abundant. The nuclear contours are smooth and the chromatin is fairly fine and evenly distributed. Pap. $\times 600$.

All figs. from pleural fluid.

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FIG. 20.—Case 1. A mesothelioma cell with a very low nucleocytoplasmic ratio. Pap. $\times 600$.

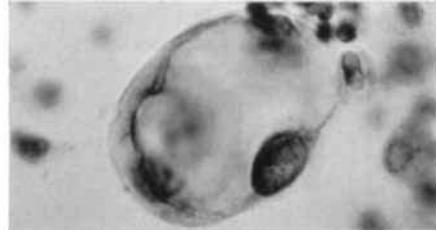


FIG. 21.—Case 7. A mesothelioma cell dominated by one large vacuole. Pap. $\times 600$.

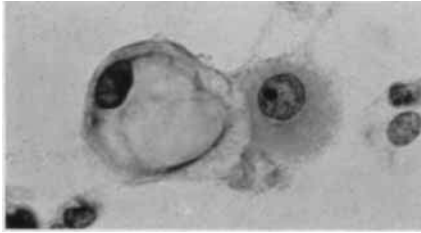


FIG. 22.—Case 1. A pair of mesothelioma cells. One is dominated by a large vacuole; the other, to which it is joined, has typical mesothelial characteristics. Pap. $\times 600$.



FIG. 23.—Case 7. Giant mesothelioma cell with a few small cytoplasmic vacuoles. The nucleus shows characteristics of malignancy. Pap. $\times 600$.

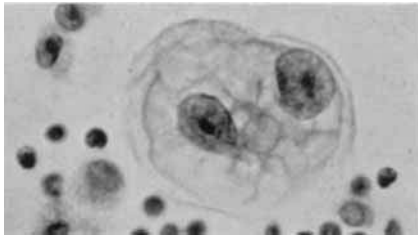


FIG. 24.—Case 7. A giant binucleate mesothelioma cell with numerous small vacuoles which give a finely bubbled effect to the cytoplasm. Pap. $\times 600$.

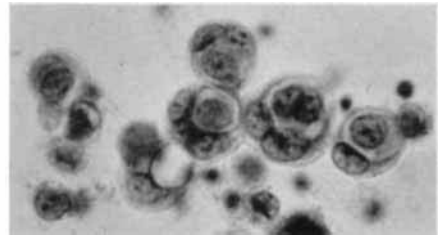


FIG. 25.—Case 6. Mesothelioma cells, some of which seem to contain an ingested mesothelioma cell. Pap. $\times 600$.

All figs. from pleural fluid.

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FIG. 26.—Case 7. A pair of mesothelioma cells: the nuclei show characteristics typical of malignancy. Pap. $\times 600$.

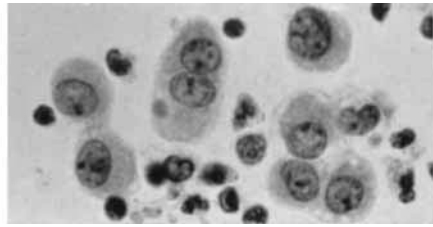


FIG. 27.—Mesothelial cells from the pleural fluid of a patient who did not have any neoplasm. Their grouping, size and other morphological features are typical of mesothelial cells. Pap. $\times 600$.

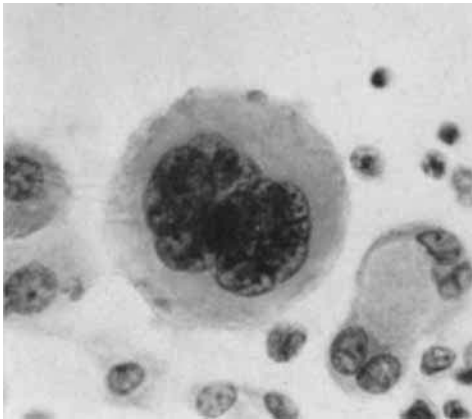


FIG. 28.—Case 1. A giant mesothelioma cell containing approximately 20 nuclei. Pap. $\times 600$.

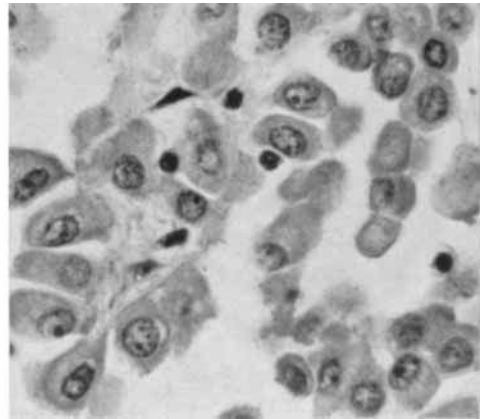


FIG. 29.—Case 1. A section of pleural neoplasm. Notice that the cells composing it are similar to those in figs. 14, 15 and 18, which are from the pleural fluid of the same case. Pap. $\times 600$.

Figs. 26-28 from pleural fluid.

type, and also were exfoliated from the neoplasm, one cannot help but conclude that the neoplasms were malignant mesotheliomas.

The diagnosis of malignant mesothelioma by exfoliative cytology requires a thorough familiarity with the benign mesothelial cell, which may show considerable variation in diameter, nuclear size and density, and cytoplasmic staining reaction. Mesothelial hyperplasia and hypertrophy can easily be mistaken for malignant mesothelioma and vice versa. It may be imprudent to do more than suggest malignant mesothelioma if there is not good supporting clinical or radiological evidence. The exfoliated malignant mesothelioma cell must also be distinguished from the adenocarcinoma cell, by far the commonest malignant cell recovered from serous fluids, which is well described in standard texts of exfoliative cytology.

The distinction may be of more than academic interest. Practically all patients with metastatic carcinoma involving a serous membrane die within a year. But, as this small series shows and as Le Roux (1962) has emphasised, some patients with diffuse malignant mesothelioma will survive for several years. The longer life expectancy of these patients may allow the development of more effective treatment.

SUMMARY

In six cases of pleural and one of peritoneal mesothelioma it is shown that the abnormal cells of the effusions are of mesothelial type, and are exfoliated tumour cells. These observations afford further proof that diffuse malignant mesothelioma is an entity.

I wish to acknowledge with thanks the aid given to me for this investigation, while I was at the Papanicolaou Cancer Research Institute, Miami, Florida, by grant T-28E from the American Cancer Society, New York. Also I am most grateful to Mr Constantine Railey for taking the photographs, and to him and Miss Emma L. Bridges for their valued criticism of the manuscript.

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