Both neoplastic and nonneoplastic cells are found in pleural and peritoneal effusions due to malignant neoplasms. Are these cells viable and capable of proliferation when they come to rest upon a suitable surface? More especially are neoplastic cells, floating in such fluids, still viable? If so, it would lend support to the idea that carcinomatosis of serous surfaces can result from implantation, independent of vascular and lymphatic dissemination. Also, can a colony be formed by the multiplication of a single neoplastic cell? This question is of considerable interest, as it bears upon the fundamental potentialities of neoplastic cells. These questions might be answered by studying the cellular constituents of pleural and peritoneal effusions in tissue culture.

MATERIAL AND METHODS

Pleural and peritoneal fluids were obtained through the cooperation of the staffs of the Hospital of the University of Pennsylvania and the Philadelphia General Hospital. In each instance the fluid was withdrawn aseptically, and about 10 to 15 cc. put into each of two test tubes. One of these was centrifuged and the plug of cells thus thrown down was fixed, embedded, sectioned, and stained. The other tube, destined to supply cells for culture, was centrifuged only if the suspension of cells was light. From the contents of this one, roller tube tissue cultures were made. If the cells had been packed by centrifuging, the plug of cells was removed and handled as has been previously described (1) for preparing roller tube cultures of biopsy material. If the cells had not been packed by centrifugation, several drops of the ascitic or pleural fluid were allowed to run down the inside of the culture tube and mix with chicken plasma. The plasma, when clotted, then held the scattered cells in position. A fluid medium consisting of human fetal serum and a physiological salt solution was added, and the tube stoppered and placed in a rotator (2) housed in an incubator. When more detailed cytological studies were desired than is possible through the wall of the pyrex culture tubes, subcultures were made in hanging drop preparations.

RESULTS

By these methods cells derived from the ascitic and pleural fluids of 23 patients with a variety of diseases have been studied in tissue culture (Table I). In some instances, several samples were obtained from the same patient.

<table>
<thead>
<tr>
<th>Source of fluid</th>
<th>Disease</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal cavity</td>
<td>Carcinoma of ovary</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Carcinoma of stomach</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cardiac failure</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis of liver</td>
<td>3</td>
</tr>
<tr>
<td>Pleural cavities</td>
<td>Fibrosarcoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Metastatic carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Leukemia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cardiac failure</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis of liver</td>
<td>4</td>
</tr>
</tbody>
</table>

Cells Derived from Peritoneal Effusions

It seems desirable to consider first those cells encountered whether or not a malignant tumor was present, since they can be expected to appear in all cases. The following types were found in peritoneal effusions from patients with cirrhosis of the liver or cardiac decompensation:

Macrophages.—These were the cells encountered most commonly. When cultures were examined immediately after preparation the macrophages appeared as scattered spherical cells, but within a few minutes after incubation they changed their shape, flattened out, formed pseudopodia, and exhibited sluggish ameboid movement. In most instances they tended to die out after a week or so of culture; less...
Like the polymorphonuclear leukocytes they persisted and even when the different samples of fluid were obtained from the same patient, but upon different days. Like the polymorphonuclear leukocytes they persisted for only a short time, usually no more than a week or 10 days.

Lymphocytes.—These cells were extremely numerous in some samples of fluid, almost absent in others, even when the different samples of fluid were obtained from the same patient, but upon different days. Unlike the polymorphonuclear leukocytes they persisted for only a short time, usually no more than a week or 10 days.

Macrophages, polymorphonuclear leukocytes, lymphocytes, mesothelial cells, and fibroblasts were found in about the same numbers as in fluid from the peritoneal cavity, and none of them showed any significant differences from those obtained from the ascitic fluids.

In addition, one culture yielded endothelial cells. These formed tubes resembling capillaries (Fig. 7), varying in caliber and in length and anastomosing to form a network as described by Lewis (4). A question arises as to the origin of these endothelial cells. It is conceivable that a few endothelial cells were carried away by the needle from vessels of the chest wall during the thoracentesis. No other plausible explanation of their origin has presented itself.

Neoplastic cells.—Cultures were made of cells in pleural fluids obtained from 2 patients with fibrosarcoma, from 1 with a metastatic carcinoma of the pleura, and from 1 with leukemia. The diagnosis in each instance was confirmed by biopsy.

From the cases of fibrosarcoma was obtained luxuriant proliferation of malignant fibroblasts (Fig. 8). These cells grew vigorously, surviving subculture in hanging drop preparations, and continued to grow profusely in the roller tubes. When the fluid itself was examined microscopically only an occasional cell was found with an appearance suggestive of malignancy, so that in these two cases it would have been well-nigh impossible to reach a diagnosis of fibrosarcoma by the usual histological methods of examining the fluid; yet in the cultures there could be no
growth has not been determined at this time, but the metastatic carcinoma showed the typical epithelial culture have been well described by Lewis (5).

The fluid obtained from the leukemic patient was of a slightly pink milky character and extremely rich in cells. The predominant type found upon examination of the hematoxylin and eosin preparations was the myeloblast. In culture there was seen a profusion of these myeloid elements for a few days only. Later there was a sharp decrease in their number and a notable increase in macrophages, which 2 weeks later had formed interlacing networks over the glass surface of the culture tube.

\textbf{DISCUSSION}

It has been generally thought that carcinomatosis of serous membranes could be produced by implantation though, as pointed out by Sampson (6), the evidence has never been more than circumstantial. The evidence is still not conclusive, but it has here been shown that neoplastic cells that have floated about in ascitic or pleural fluid remain viable and capable of proliferation when given a suitable surface for attachment and furnished with a nutrient medium. This strongly supports the contention that metastasis by implantation can give rise to carcinomatosis of the serous membranes.

Given the proper environment, a single neoplastic cell can multiply and produce a colony. This would seem to open up an interesting field for future exploration, for by studying such cells and their colonies in tissue culture it may be possible to answer several pertinent questions. For instance, do mutations occur? How closely do all the descendants of a single cell resemble each other and the original parent cell? Are there, in the same tumor, different strains of cells varying in their degree of anaplasia?

The culture of cells derived from pleural and ascitic fluids can, in some instances at least, be of diagnostic aid. But as determination of the ultimate value of this method would require considerable time and a large group of patients it could be employed only in an institution where the services of a tissue culture laboratory were readily available.

\textbf{SUMMARY AND CONCLUSIONS}

Cells derived from pleural and peritoneal effusions were grown in tissue culture by the roller tube method.

Macrophages, polymorphonuclear leukocytes, lymphocytes, mesothelial cells, and fibroblasts were cultured from all fluids. In one instance endothelial cells were found, and these produced structures resembling capillaries.

Cells from carcinomas and sarcomas grew vigorously, thereby indicating that such cells remain viable and capable of proliferation after floating in pleural or peritoneal fluids if given a satisfactory surface to which they may become attached. Thus support is given to the view that carcinomatosis of serous membranes can occur by implantation.

Small colonies were observed to develop from single neoplastic cells that had become isolated in the supporting plasma of the tissue culture. The significance of this observation is discussed as opening a field for further exploration.

\textbf{DESCRIPTION OF FIGURES 1 TO 8}

Fig. 1.—Macrophages in roller tube tissue culture showing formation of loose network after a week of existence \textit{in vitro}. These cells were obtained from ascitic fluid. When examined soon after the cultures were prepared the macrophages were spherical. Soon, however, they formed pseudopodia and were actively ameboid. Mag. X 110.

Fig. 2.—A single mesothelial cell in hanging drop subculture from roller tube. This cell was obtained from pleural fluid. Such cells sometimes formed loose sheets \textit{in vitro}; when single they sent out long processes. Most of the small dark dots within the cytoplasm are mitochondria. Multiple nucleoli are a common feature of mesothelial cells. Mag. X 1050.

Fig. 3.—Fibroblasts from ascitic fluid, in hanging drop subculture from roller tube. Fibroblasts are multipolar or elongated spindle cells with thin cytoplasm, few mitochondria, and variable amounts of fat. Mag. X 480.

Fig. 4.—Carcinoma cells forming an extremely loose and irregular sheet in roller tube culture. These cells were cultured from peritoneal fluid from a patient with carcinoma of the stomach. Carcinoma cells usually grew in delicate sheets one cell thick. They had a pronounced tendency to liquefy the supporting plasma. Mag. X 100.

Figs. 5 and 6.—Two stages in the formation of a small colony from isolated carcinoma cells in roller tube cultures. In Fig. 5 the 10 cells shown in Fig. 5 have increased by multiplication to 21 in 36 hours. Mag. X 400.

Fig. 7.—Endothelial cells in roller tube culture forming a branching capillary-like structure. These cells were cultured from ascitic fluid, but perhaps were derived from vessels of the chest wall dislocated by thoracentesis. Mag. X 110.

Fig. 8.—Fibrosarcoma cells in roller tube culture of pleural fluid. Malignant fibroblasts were frequently somewhat larger than normal fibroblasts, had a denser cytoplasm, and were often multinuclear. Commonly sarcoma cells showed larger nucleoli than normal cells and the nucleoli were often multiple. Mag. X 400.
It is suggested that the culture of cells from pleural and ascitic fluids can be of aid in diagnosis under favorable circumstances.

REFERENCES
Human Neoplasms in Tissue Culture. II. Observations upon Cells Derived from Peritoneal and Pleural Effusions

Dale Rex Coman