TISSUE CULTURE OF INTRACRANIAL TUMORS

WITH A NOTE ON THE MENINGIOMAS

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It has been the purpose of this clinic for some time to utilize the tissue culture technique in the study of intracranial tumors. It was thought that observations on living tumor cells cultivated *in vitro* might profitably supplement the usual histological examination of fixed tissue and add not a little to our present knowledge of their histogenesis. This present paper records the results of some preliminary studies in this direction.

TECHNIQUE

The technique employed is the hanging-drop method of Lewis and Lewis.[1] A bit of healthy tumor tissue secured at operation is cut up in Locke or Tyrode solution into small pieces a millimeter or less in diameter. A piece is placed in the center of a clean sterile coverslip and a drop of heparinized human plasma is added. The coverslip is then inverted and sealed by means of a vaseline ring on a depression slide. In some cases a drop of tumor extract is added to promote growth. Aseptic precautions are observed throughout. The cultures are incubated at 37.5° C.

In successful cultures (Fig. 1) the cells migrate out within twenty-four hours on the under surface of the coverslip. One can then study under the highest powers of the microscope the morphology of the living cells as well as their reaction to vital dyes and to particulate matter.

TUMORS CULTURED

In order to ascertain what types of tumors would be favorable material for cultivation all tumors removed on the neurological service over a period of several weeks were cultured. A list of these

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together with the percentages of success with each is shown in the table below:

TABLE I

Tumors Cultured

	No. cultured	Satisfactory	Per cent
Meningioma	5	3	60
Metastatic carcinoma	2	1	50
Spongioblastoma multiforme	1	1	100
Pituitary adenoma	5	0	0
Astrocytoma	3	0	0
Acoustic neuroma	2	0	0
Cystic spinal tumor	2	0	0
Cysts	3	0	0
Unclassified malignant epithelial tumor	1	0	0
Total	24	5	21

The results were not satisfactory in many of the tumors. With the simple technique employed one could hardly expect the cells of slowly growing tumors like the astrocytomas to migrate actively in tissue culture. In a few of the pituitary adenomas a small number of epithelial cells wandered out from the explanted piece, forming a loose sheet; a growth pattern characteristic of glandular epithelium. In cultures of two cystic tumors of the spinal cord ciliated epithelial cells migrated out.

Satisfactory growth occurred in cultures of tumors of three types: meningioma, metastatic carcinoma, and spongioblastoma multiforme.* The success with the one specimen of spongioblastoma inspires the hope that the gliomas of relatively undifferentiated cell type may prove to be favorable material for study by tissue culture methods.

MENINGIOMAS

Active migration occurred in cultures of three out of five meningiomas. The cultures were kept alive for as long as three weeks by renewing the medium every three or four days and, in a few cases by subculturing the explanted piece. Attempts were made in our observations on these cultures to determine the precise nature of the outwandering cells.

The typical cells that grew out seemed to be of the mononuclear-

* Since Mr. Kredel made this preliminary study in the summer of 1927 we have had success with other types, notably with the acoustic neurinomas. H. C.

macrophage series rather than fibroblasts. Most of the cells were ameboid in shape. While some of them were pyriform and unipolar, the multipolar stellate form with many cell processes characteristic of fibroblasts was not in evidence during the first few days after explantation. Some cells showed a rosette of neutral red granules in the region of the centrosphere. In older cultures, however, the cells appeared gradually to assume a form more closely resembling that of fibroblasts.

To check these morphological observations with some physiological criterion the phagocytic power of the outwandering cells was tested by adding to the cultures a suspension of finely divided carmine in Locke solution. In cultures of a few days the cells ingested large numbers of carmine particles. Fig. 2 is from a seven-day culture of meningioma. This photograph illustrates both the characteristic morphology and the remarkable phagocytosis of carmine noted in the younger cultures.

After cultivation for a week or more the cells seemed to lose their phagocytic ability. Fig. 3 shows a group of cells in an eleven-day culture from the same tumor as the cells shown in Fig. 2. These latter cells did not phagocytize carmine to any noticeable extent. An anomalous result was obtained with a twenty-day culture, a field of which is shown in Fig. 4. The cells failed to ingest any carmine during the first half hour after the suspension was added. But at the end of four hours the cytoplasm had become tremendously swollen, distorted, and contained many carmine particles. We are inclined to believe that this last was an artifact due to the basicity of a poorly prepared carmine suspension, the reaction of which was found to be pH 7.9.

These observations, although suggestive, do not add much of critical importance to our present knowledge of the histogenesis of the meningiomas. If the view of Mallory [2] and Penfield [3] is correct that the type cell of these tumors is the fibroblast, the outwandering cells in our cultures must be clasmatocytes present in the stroma. This possibility must be considered, for Lewis and Gey [4] have shown that clasmatocytes migrate out abundantly in cultures of mouse sarcoma and have pointed out that the presence of large numbers of such phagocytes may well be overlooked in fixed sections. On the other hand, these observations conflict in no way with the view of Cushing [5, 6] that the histogenesis of the meningiomas is explained

on the basis of tumefaction of clusters of meningocytes that line the arachnoid villi and which possess phagocytic powers. The meningiomas when examined fresh by supravital technique are found to contain large numbers of these phagocytic cells and in his opinion they presumably represent a constituent part of the tumor.

SUMMARY

Successful tissue cultures with the hanging-drop technique have been made from meningioma, metastatic carcinoma, and spongioblastoma multiforme.

Phagocytic cells migrated out in large numbers in meningioma cultures. The ability of these cells to ingest particulate carmine decreased progressively with the age of the cultures.

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3. Penfield, W. The encapsulated tumors of the nervous system. *Surg., Gynec. Obst.*, 1927, xlv, 178.
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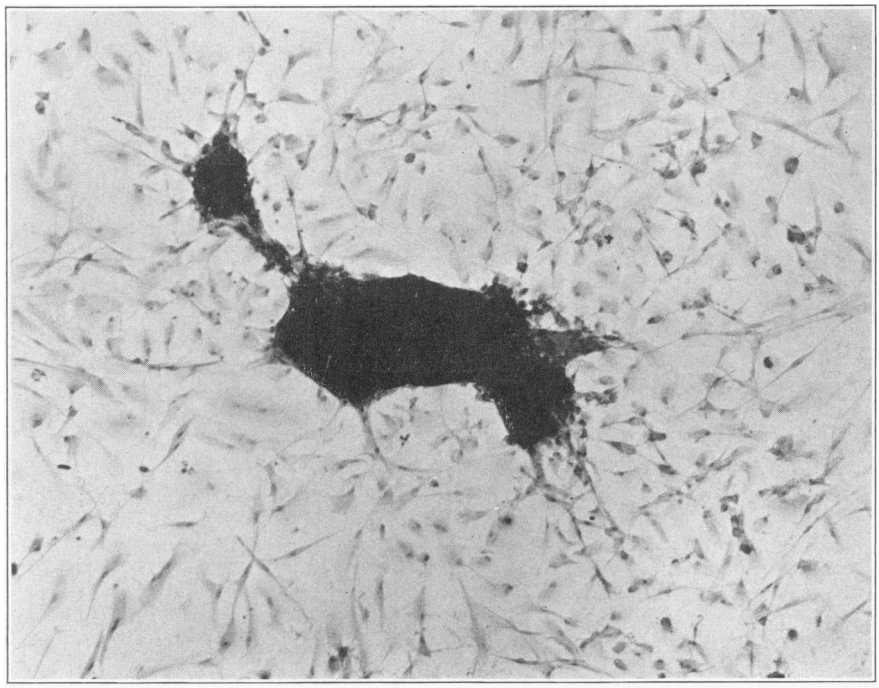
DESCRIPTION OF PLATES

PLATE 78

FIG. 1. An eight-day culture of a metastatic carcinoma (fixed in formalin, H. and E stain), showing an abundant outgrowth of multipolar and often multinuclear cells containing large granules not taking neutral red. × 100.

FIG. 2. A typical field of growing meningioma (seven-day culture, H and E stain). Most of the cells have ingested large numbers of carmine particles and are obviously phagocytic. × 600.



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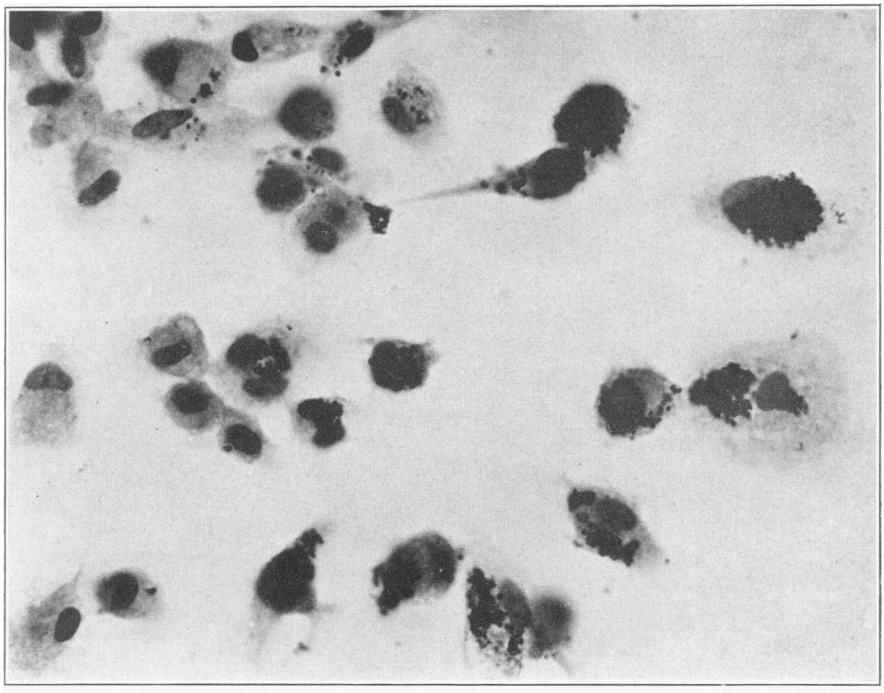

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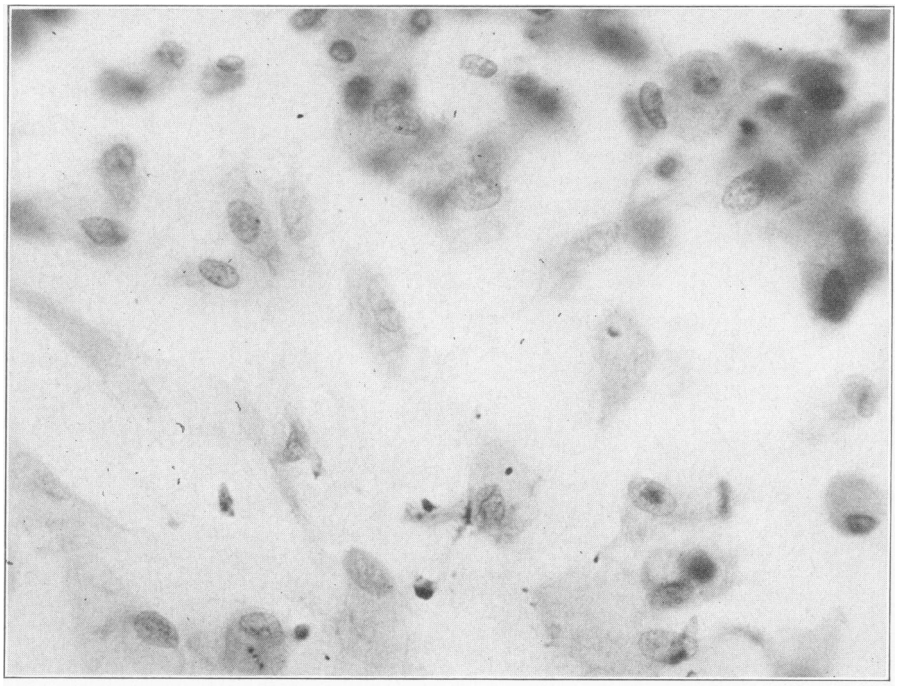
PLATE 79

FIG. 3. An eleven-day culture of same tumor as Fig. 2 (H and E stain), showing cells (fibroblasts or meningocytes?) which do not ingest carmine. × 600.

FIG. 4. Cells from same tumor as Fig. 2 after twenty-day growth (H and E stain), showing them richly laden with carmine particles. × 600.



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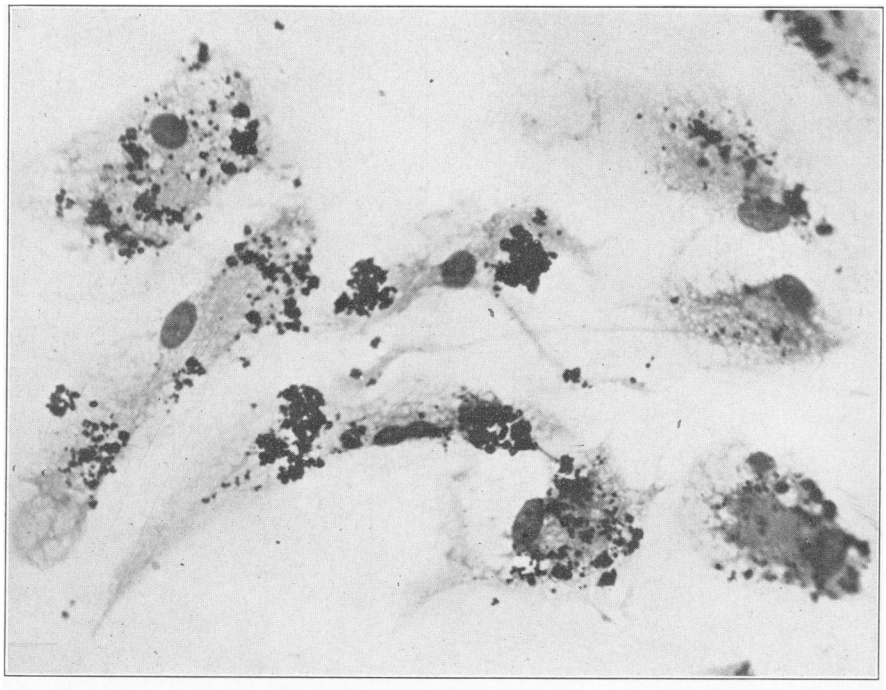

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