# Infiltrative potentiality of brain tumors in organ culture

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✔ The invasive potential of 15 brain tumors (seven meningiomas, and eight astrocytomas) as studied in cultures with Gelfoam used as a substrate. A three-stage system is introduced to classify *in vitro* invasiveness according to the depth of Gelfoam infiltration after 2 weeks' culture. This system proved to be simple and accurate. Meningiomas revealed greater activity in culture as compared to astrocytomas and infiltrated deeper into the Gelfoam. Dura mater explants were placed next to the culture of the tumors to study their interaction. Dura mater acted as a relatively strong barrier against tumor cell invasion in culture. In the majority of the explants, tumor cells revealed either contact arrest or spread underneath the dura and rarely spread over it. Tumor cells were seen infiltrating the dural explants in three cases, all of which were meningiomas.

KEY WORDS • **tissue culture** • **meningiomas** • **astrocytomas** • **brain tumors**

TISSUE culture has been used extensively to study the morphological features of brain tumors and to correlate them with their histopathological types and clinical behavior.[4,8-10,16,17] The majority of these studies were done on monolayer cultures on glass.

Although organ culture has been employed in the study of many nonneurological tumors,[7] it has rarely been used in investigating brain tumors.[4,9] This technique provided an ideal model for *in vitro* evaluation of the invasiveness of malignant tumors, and their interaction with other tissues.[18] Several methods of organ culture have been used for tumors, including plasma clot,[2,3] cellulose matrix,[6] rayon net,[13] perforated metal grid,[14,16] and chick mesonephros.[18] Recently we reported a new method using Gelfoam sponge.[11]

The purpose of this paper is to report some observations on the use of this new method in evaluating the infiltrative capacity of brain tumors into Gelfoam sponge. It also aims at determining the *in vitro* interaction between brain tumors and dura mater.

## Materials and Methods

The study included 15 patients, seven with meningioma and eight with astrocytoma. The tumors were classified histopathologically following Kernohan and Sayre's system.[5] During surgery biopsies were taken from the

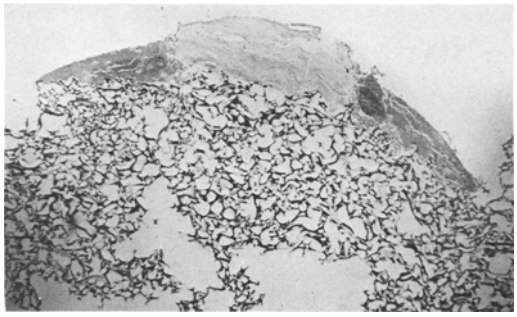


FIG. 1. Position of tumor explants on either side of the dural fragment. H & E, × 15.

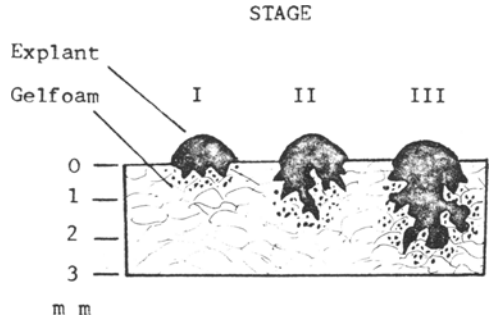



FIG. 2. Diagram showing the three stages of Gelfoam infiltration by tumor.

tumor, and from the dura mater at a site distant from the tumor; the tissue was then transferred aseptically to the tissue-culture laboratory.

Each biopsy was divided into two sections. One section was sent to the pathology laboratory for confirmation of the histopathological diagnosis and the viability of the tumor as a control. The other section was divided into numerous small explants of $2 \times 2 \times 2$ mm, and cultured on Gelfoam sponge rafts in special screwcapped bottles. A total of 234 tumor explants were used in this study. Of these about two-thirds were cultured alone; the other third were placed about ½ mm apart from dural explants, in such a way that each dural fragment was interposed between two tumor fragments taken from the same patient (Fig. 1).

Cultures were fed twice weekly with a medium containing 50% autologous serum in Hank's balanced salt solution. Details of the tissue-culture technique have been previously reported.[11]

After 2 weeks, when tumors reached their maximal growth, cultures were fixed and embedded in paraffin, and serial sections were made for histopathological examination. Sections were examined for evaluation of tumor cell viability and extent of Gelfoam infiltration and dural invasion.

We classified the extent of Gelfoam infiltration into three stages according to the depth of infiltration into the Gelfoam matrix, with each millimeter depth representing one stage (Fig. 2). Interaction of the tumor cells with the dura mater was also assessed and it correlated with the histopathological patterns.

TABLE 1

*Histopathological diagnosis of tumors*

| Type | Subtype | No. of Cases |
|---|---|---|
| meningioma | meningothelial | 4 |
| | psammomatous | 2 |
| | fibrous | 1 |
| astrocytoma | Grade I | 4 |
| | Grade II | 2 |
| | Grade III & IV | 2 |
| total | | 15 |

TABLE 2

*Viability of tumors in culture*

| Tumor Type | Controls | | Explants | |
|---|---|---|---|---|
| | Infected | Grew | Degenerated | Viable |
| meningioma | 1 | 6 | 30 (28%) | 78 (72%) |
| glioma | 1 | 7 | 41 (33%) | 85 (67%) |
| total | 2 | 13 | 71 (30.4%) | 163 (69.6%) |

## Results

### *Tumor Viability*

The histopathological diagnosis of the tumors studied is presented in Table 1. Pathological study of the control sections revealed good viability of the tumor source. Explants derived from two tumors, a meningothelial meningioma, and a Grade II astrocytoma, became infected in culture and were omitted from the study. The overall viability of tumor cells after 2 weeks was 69.6% (163 out of 234). Meningioma cells, however, showed a slightly higher viability rate as compared to astrocytoma cells, namely, 72% as opposed to 67% (Table 2).

*Infiltration of the Gelfoam*

Table 3 shows the degree of infiltration of the Gelfoam by each tumor type. Most of the meningiomas infiltrated deeply into the Gelfoam, qualifying for Stages 2 and 3 of the proposed classification system (Fig. 3), while the majority of astrocytomas showed only mild infiltration of the Gelfoam matrix, and belonged mainly in Stage 1.

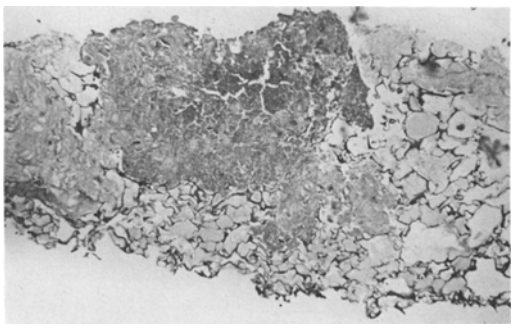
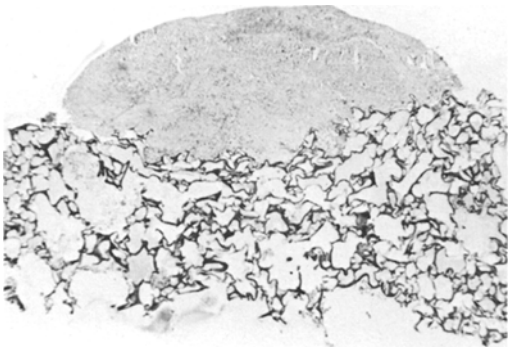


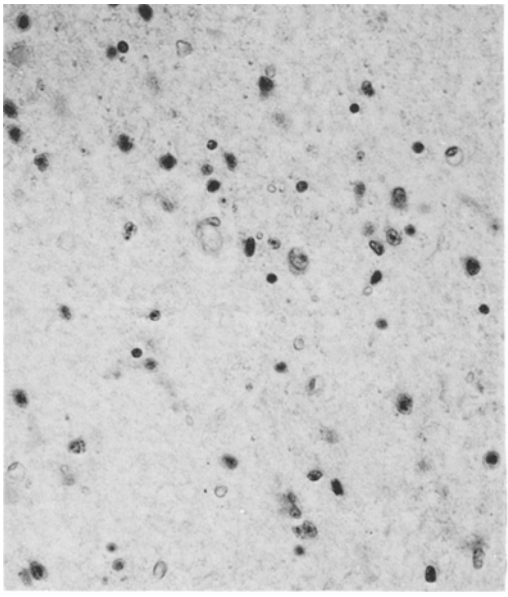
FIG. 3. Example of Stage 3 Gelfoam infiltration by a meningioma explant. H & E, × 15.

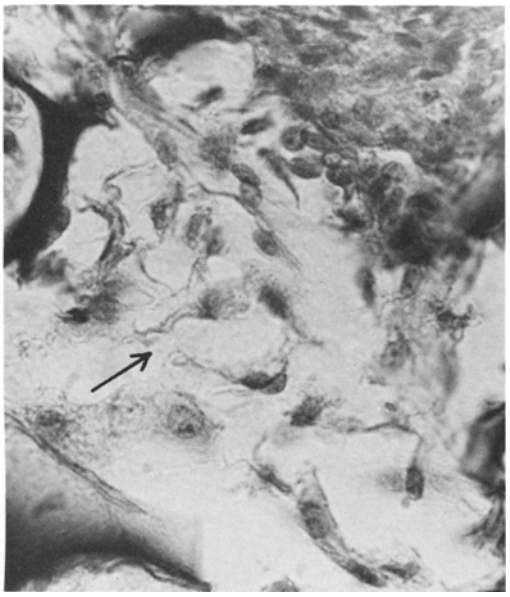
A correlation was made between the histopathological grade of astrocytomas *in vivo* and their infiltrative capacity in culture (Table 4). Deep invasion of Gelfoam, Stage 3, was only observed in explants derived from astrocytomas of Grades III and IV. Explants derived from Grades I and II astrocytomas behaved rather similarly *in vitro*, showing more limited invasion of Gelfoam to Stages 1 and 2. A correlation between the histopathological grades of astrocytomas with *in vitro* findings is shown in Figs. 4, 5 and 6.

Two distinct patterns of Gelfoam infiltration were consistently noticed in both varieties of the tumors. In one pattern densely packed masses of tumor cells invaded the

FIG. 4. Astrocytoma Grade I, H & E. *Lower Left:* Photomicrograph of control specimen. × 250. *Upper Right:* The same tumor showing Stage 1 Gelfoam infiltration. × 20. *Lower Right:* Higher power view *in vitro* showing uniform nuclei and fibrillary matrix (*arrow*). × 400.







Gelfoam matrix destroying and replacing its fibers and always preserving continuity with the original explant (Figs. 5 *right* and 7). The other pattern showed dispersed cells scattered inside the Gelfoam lacunae and lining their walls, with preservation of the Gelfoam architecture. These dispersed cells evidently lost their continuity with the original explants (Fig. 6 *right*).

TABLE 3

*Degree of Gelfoam infiltration*

| Tumor Type | No. of Explants | Stage 1 | Stage 2 | Stage 3 |
|---|---|---|---|---|
| meningioma | 78 | 19 (24.4%) | 38 (48.7%) | 21 (26.9%) |
| astrocytoma | 85 | 57 (67.1%) | 24 (28.2%) | 4 ( 4.7%) |

### *Interaction of Tumor Cells and Dura Mater*

Of the 36 explants that were cultured in contact with the dura mater, three infiltrated the latter (Fig. 8). In the remaining 33 explants there was no infiltration, and in these the tumor acted in one of three ways: it became arrested at the edge of the dura, spread underneath the dura, or spread above the dura (Fig. 9, Table 5).

TABLE 4

*Correlation between grade of astrocytoma and stage of Gelfoam infiltration*

| Grade | No. of Cases | No. of Explants | Stage 1 | Stage 2 | Stage 3 |
|---|---|---|---|---|---|
| I | 4 | 48 | 37 | 11 | — |
| II | 1 | 13 | 10 | 3 | — |
| III & IV | 2 | 24 | 11 | 9 | 4 |
| total | 7 | 85 | 57 | 24 | 4 |

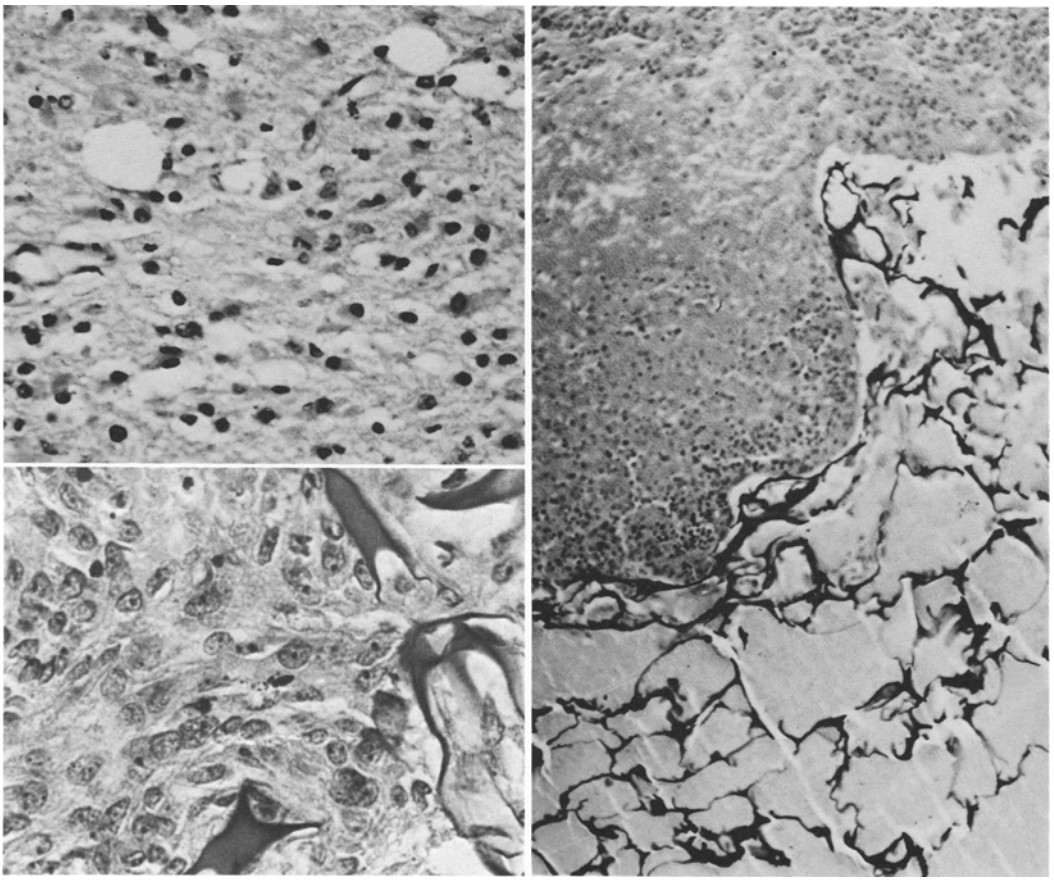


FIG. 5. Grade II astrocytoma, H & E. *Upper Left:* Photomicrograph of control specimen. × 250. *Right:* The same tumor showing Stage 2 Gelfoam infiltration. × 100. *Lower Left:* Higher power view *in vitro* showing moderate cellularity and pleomorphism. × 265.

*Infiltration of Dura Mater*

Dural invasion was seen in only three of the 26 meningioma explants. The tumor cells were seen infiltrating for a short distance (about 60 to 80 $\mu$) through the collagen bundles of the dura mater (Fig. 8). Two of these three explants came from one meningothelial meningioma, while the third came from a psammomatous meningioma. None of the astrocytoma explants, however, was found to invade the dura mater during the period of observation.

TABLE 5

*Interaction of tumor cells with dura mater*

| Tumor Type | No. of Explants | Infiltration | No Infiltration | | |
|---|---|---|---|---|---|
| | | | Under Spread | Contact Arrest | Over Spread |
| meningioma | 26 | 3 | 16 | 5 | 2 |
| astrocytoma | 28 | 0 | 20 | 7 | 1 |
| total | 54 | 3 | 36 | 12 | 3 |

## Discussion

There are very few reports in the literature on organ culture of brain tumors. Holstrom, *et al.*,[4] using plasma clot, compared the invasiveness of benign and malignant brain tumors. They measured cell proliferation by autoradiography, and graded tumor invasiveness by the depth of fibrin clot infiltration. They noticed that the growth pattern of benign and malignant tumors was somewhat different, insofar as the former showed less or no infiltration. The plasma clot technique, however, is rather tedious due to the considerable frequency of clot liquefaction.

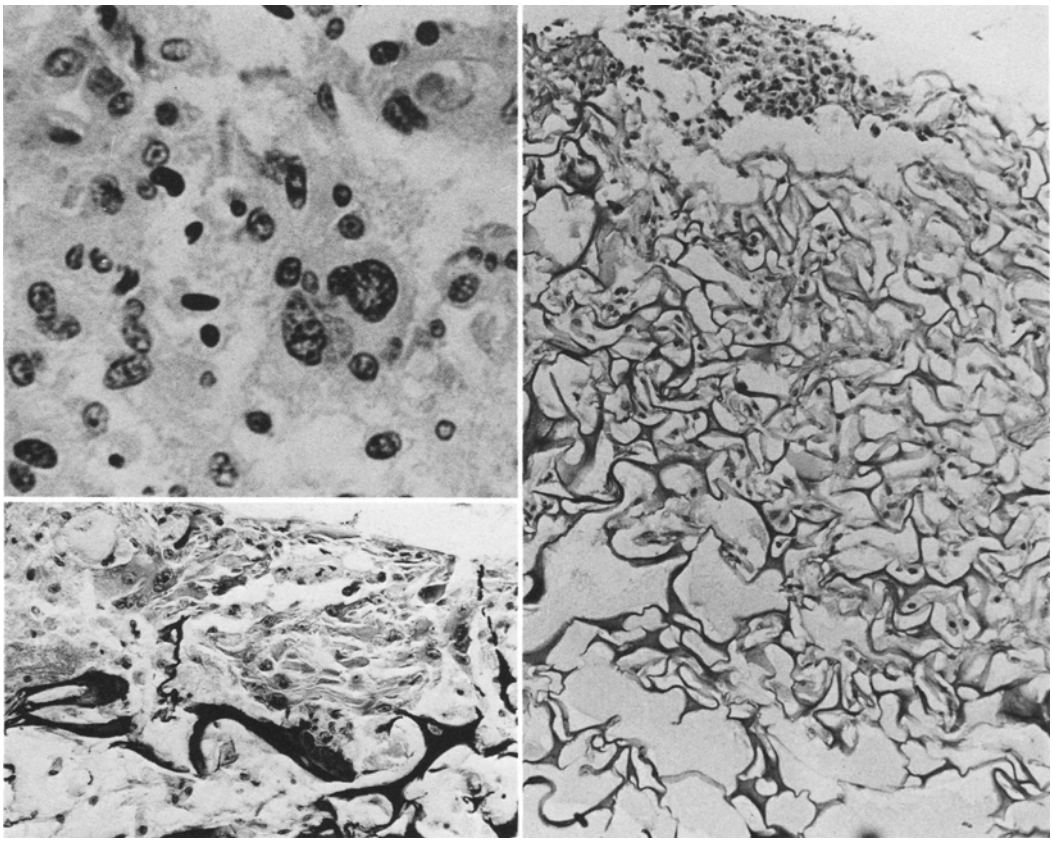


FIG. 6. Grades III and IV astrocytoma (glioblastoma multiforme), H & E. *Upper Left:* Photomicrograph of control specimen. × 250. *Right:* Same tumor on Gelfoam with widespread invasion of matrix by dispersed growth in the lacunae; Stage 3 infiltration. × 100. *Lower Left:* Higher power view *in vitro* showing pleomorphism, giant gemistocytic cell. × 150.

Lumsden[9] used the Wolf method for evaluating invasiveness of a glioblastoma into the chick embryo mesonephros. He reported that the tumor retained its cytological characteristics and invasiveness in culture. No further details were mentioned.

The present technique for organ culture of brain tumors with Gelfoam presents several advantages. Apart from its simplicity and economy, it allows observation of living tumor cells by phase-contrast microscopy. Moreover, serial sections can easily be cut

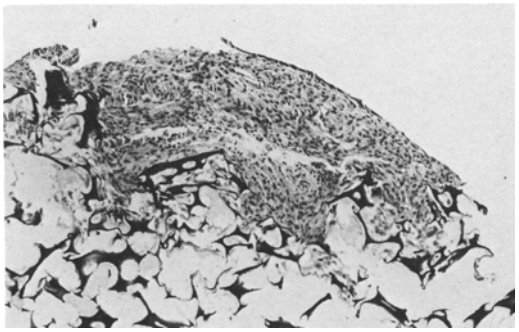


FIG. 7. Photomicrograph showing Gelfoam infiltration by densely packed cells of a meningioma. H & E, × 150.

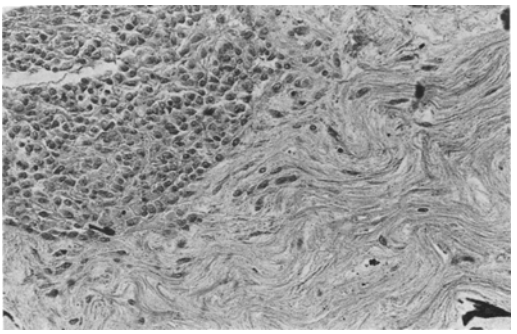


FIG. 8. Photomicrograph showing the cells of a meningioma infiltrating the dura mater. H & E, × 125.

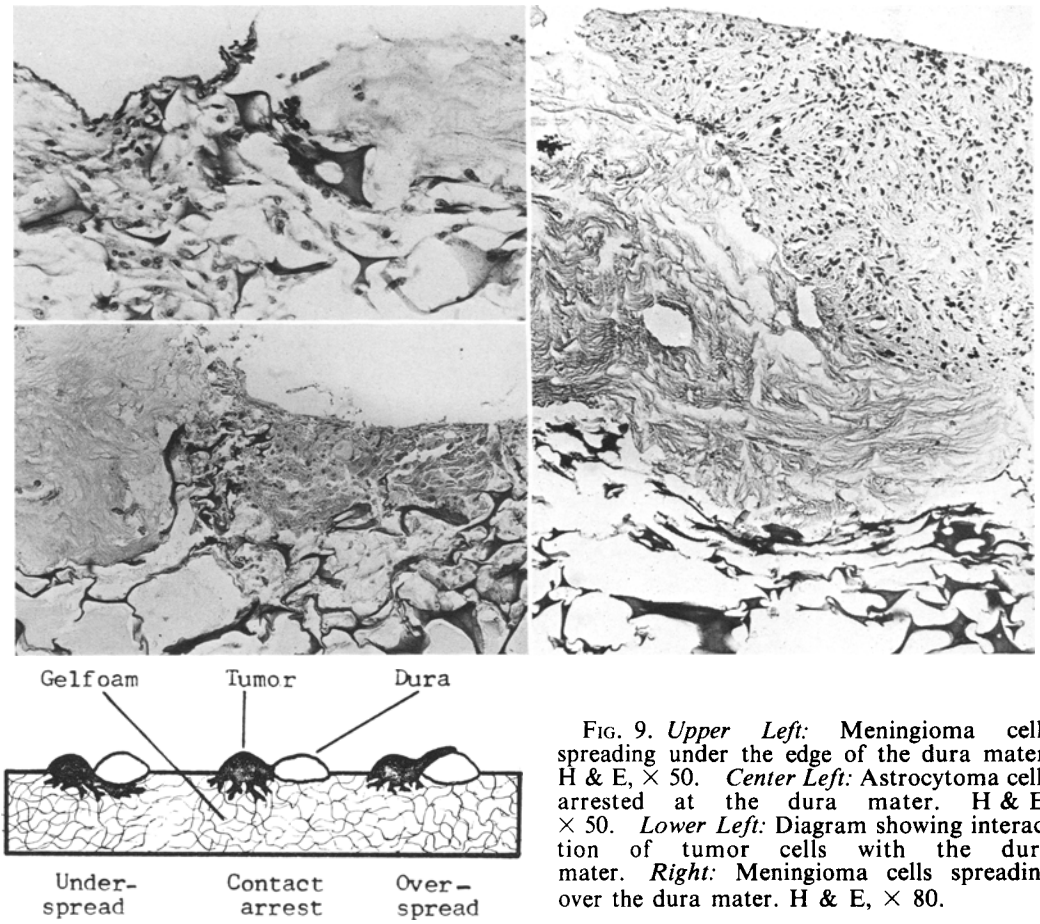



FIG. 9. *Upper Left:* Meningioma cells spreading under the edge of the dura mater. H & E, × 50. *Center Left:* Astrocytoma cells arrested at the dura mater. H & E, × 50. *Lower Left:* Diagram showing interaction of tumor cells with the dura mater. *Right:* Meningioma cells spreading over the dura mater. H & E, × 80.

after fixation of the cultures to study their organized structures, and to evaluate the infiltrative potentiality of the tumor and its interaction with other tissues. By this technique, we achieved a longer tumor viability in culture than workers using other methods.[1,12]

Gelfoam as a substrate proved to be quite favorable for studying invasion *in vitro* as compared to plasma clot, rayon net, metal grid, or chick mesonephros. Gelfoam, being inert, does not release any biological substance that could affect the rate of cell growth, thus causing a bias in the evaluation of the invasive capacity. Moreover, it offers minimal resistance to the advancing tumor cells compared to other synthetic fabrics like rayon. It also does not undergo liquefaction in culture like fibrin clot.

The system of measurement in millimeter stages introduced here provides a simple yet accurate method for quantitative estimation of tumor invasion in culture. It can be applied to tumors from any source. In this study meningiomas were found to have a greater activity in culture compared to astrocytomas, and invaded the Gelfoam matrix much more deeply. This is in line with observations of other investigators who used monolayer cultures.[4,8,9] The greater growth potential of meningiomas is possibly due to their mesoblastic origin as compared to astrocytomas which are neuroectodermal.

No active cell growth occurred from the dural explants, probably because of the fibrous nature of the dura and the scanty, well differentiated cellular elements it contains. It is a well-known fact that dura mater stands as a strong barrier *in vivo* against infiltration of gliomas, while it is not uncommonly infiltrated by meningiomas. We tested this observation *in vitro* by placing explants of tumors next to explants of dura mater. In most cases the dura mater resisted infiltration by the tumor cells; the tough collagen bundles act as a barrier against tumor cells. Once the cells reach this barrier they are arrested for some time, then they spread either underneath or above the dura. The greater frequency of underspread compared to overspread is probably due to the better nutritive condition and the more natural (three dimensional) environment offered by the Gelfoam matrix.

On three occasions, actual invasion of the dura mater by tumor cells was demonstrated; these were all cases of meningioma. We believe that this is the first time invasion of the dura mater by meningioma cells has been demonstrated *in vitro*, and has thus confirmed the pathological observation. The reason for the ability of meningioma cells to infiltrate the dura mater is not certain. It may be presumed that meningiomas are of mesoblastic origin, and hence have a greater growth potential and greater power of invasion than neuroectodermal tumors.

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