The minimum number of cells of a solid tumor which must be transplanted in order to produce a new tumor has been estimated by several investigators. Kahn and Furth (2) prepared a suspension of cells of a mouse sarcoma and injected counted numbers of cells into mice. By this technique they found that the sarcoma could be transmitted with 50 cells. Reinhard, Goltz, and Warner (3) ground young tumors of the Marsh-Simpson adenocarcinoma, filtered the mash through handkerchief linen, and counted the cells in a hemocytometer after treating the suspension with erythrosin to color dead cells. With this technique they obtained two tumors in 57 animals with an inoculum containing 80 cells. Over 5 million cells were needed to give 100 per cent takes. With mouse adenocarcinoma db & B, the smallest number of cells necessary to induce tumors was eighteen (three takes out of 57 mice). For 50 per cent takes, 500 cells were necessary; for 100 per cent takes, 2,590 cells were required (4). Hewitt (1), working with a cell suspension of a spindle-cell carcinoma in CSH mice, found that 80–90 per cent of the cells in such suspensions could be presumed nonviable as indicated by the diffusion of trypan blue into the nuclei. Such suspensions were diluted 24,300 times and were assumed to contain five cells/inoculum of 0.1 ml. This cell number gave four out of twelve takes. Twelve out of twelve takes were obtained with about 133 cells. With Sarcoma 37, however, about 2,000 viable cells were needed to give 100 per cent takes.

The method used by these workers, which involves grinding tumors and injecting diluted suspensions of ground material into animals, suffers from the following drawbacks: (a) the number of cells in the inoculum cannot be determined with accuracy, for the possibility cannot be ruled out that clumps containing many cells have been inoculated; (b) the nature of the cells inoculated cannot be determined; and (c) damage is likely to be inflicted on the cells by the process of grinding. To overcome some of these difficulties the method here described was developed, involving the use of small cell populations derived from tissue cultures in which the identity and number of the cells could be accurately determined.

MATERIALS AND METHODS

Two tumors were investigated: (a) the glioblastoma 8110 obtained from the University of Minnesota and originally induced in the brain of a stock mouse by injections of methylcholanthrene and (b) the Walker carcinoma grown in the Sherman rat. The tumors were removed aseptically from the animals and cut into small fragments (about 1 mm.²). These fragments were implanted in a thin plasma clot formed on the surface of the upper slide of the double slide culture tube shown in Chart 1. This consisted of a Pyrex glass test tube (18 × 150 mm.), an upper slide (18 × 75 mm.), and a lower slide (5 × 75 mm.). To the lower slide five square coverslip fragments (3 × 3 mm.) were cemented with 0.5 per cent crystalline egg albumin. The tubes and slides were autoclaved separately, the slides being placed in individual Petri dishes. The smallest possible amount of egg albumin was used to cement the coverslip fragments to the slide. Each tube re-
ceived 4 ml. of nutrient medium containing 40 per cent horse serum, 50 per cent basal salt solution (BSS, Earle's formula), 10 per cent embryo extract. The 13 x 75 mm. slide was prepared by being coated on its upper surface with a film of chick plasma (Difco) clotted with 1 drop of embryo extract. After the clot had formed, fragments of tumor tissue about 1 mm.³ were placed on the surface of the clot and left for 30 minutes. The slide was then placed in a tube containing the medium so that the surface bearing the tissue fragments faced downward. Coverslip fragments of the tumor were transplanted by trocar to fresh animals. A minimum of three transplant generations from each in vitro culture was studied in the rodents with respect to the size, rate of growth, and histology of the tumors.

EXPERIMENTAL

Glioblastoma 8110.—The appearance of the deciduous cells from this tumor is shown in Figure 1. The cells were relatively uniform in size and morphology. They appeared to be all of one type. Table 1 summarizes the results of 87 transfers of cells ranging in number from two to over 100. The number of cells in these preparations had surprisingly little effect on the number of takes. Tumor production was obtained with as few as two cells,

<table>
<thead>
<tr>
<th>Cell count</th>
<th>No. implantations</th>
<th>No. takes</th>
<th>Per cent takes</th>
<th>Av. no. days required for growth of implant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-10</td>
<td>15</td>
<td>7</td>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>11-20</td>
<td>19</td>
<td>11</td>
<td>58</td>
<td>16</td>
</tr>
<tr>
<td>21-30</td>
<td>16</td>
<td>8</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>31-40</td>
<td>10</td>
<td>6</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>41-60</td>
<td>11</td>
<td>7</td>
<td>63</td>
<td>12</td>
</tr>
<tr>
<td>61-80</td>
<td>11</td>
<td>4</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Over 80</td>
<td>7</td>
<td>6</td>
<td>86</td>
<td>11</td>
</tr>
</tbody>
</table>

but even when cell populations were over 80, the percentage of takes did not rise to 100, nor did the number of cells in the original inoculum affect, to a significant extent, the time required for growth of the tumor in vivo. The histological appearance of the tumor before tissue culture and after its re-establishment in the mouse is shown in Figures 3 and 4. There can be no doubt that it is the same tumor. There was no evidence to indicate that it had been altered in any way by the tissue culture procedure.

Walker rat carcinoma.—The appearance of the deciduous cells from this tumor is shown in Figure 2. Transfers of these cells to rats gave only five takes out of 165 transplants. The cell counts of these five were 5, 9, 59, 61, and 72. Histological examination indicated that they had arisen from the Walker rat carcinoma cells from the tissue cultures.

DISCUSSION

These results are in accord with those of other workers in showing that tumors vary greatly in their transplantability. The glioblastoma 8110 could be transplanted readily with fewer than twenty cells. The Walker rat carcinoma was only rarely transplanted successfully by this technic.
It appears that each tumor may have its own characteristic limits of transplantability which may in turn be an expression of the tumor's malignancy. Further work is required to establish this relationship.

SUMMARY

A method was developed for isolating small populations of tumor cells by means of the double slide culture tube technic.

Known numbers of cells derived from mouse glioblastoma 8110 and the Walker rat carcinoma were transplanted, attached to the plasma film on which they were growing, under the skin of the appropriate strain of rat or mouse.

Glioblastoma 8110 was successfully transplanted with as few as two cells. Fifty to 60 percent takes were obtained with cell numbers ranging from two to 80.

The Walker rat carcinoma was transplanted successfully in only five out of 165 attempts. The successful transplants had cell counts ranging from five to 72.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Miss S. Halliday in this work.

REFERENCES

The Transplantation of Small Numbers of Tumor Cells

R. S. de Ropp and Doris McKenzie


Updated version: Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/14/8/588

E-mail alerts: Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions: To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions: To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.