Survival of the Mouse Mammary Tumor Agent (MTA) in Frozen Tissue*

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It has been established that the mouse mammary tumor agent (MTA), with the properties of an infectious agent or virus, will withstand filtration, lyophilization, and desiccation (1, 2-4, 8-10, 15). Gye (11) and Mann (12-14) advanced the theory that the agent would be liberated in its active form following freezing and would induce mammary cancer within a short time, because of its "selective infectivity" for the mammary tubules. Although the MTA could be demonstrated in cell-free centrifugates of mammary tumors which had been frozen, the incidence and cancer ages were similar to those which were found when the agent was administered in extracts of unfrozen tumors (6-7), whether injected intraperitoneally or subcutaneously. When suspensions of the thawed tumor tissues were employed, after being frozen and/or dried according to the technic described by Gye (11), viable cells could be demonstrated (6-7, 15).

MATERIALS AND METHODS

Tissue from a transplanted mammary carcinoma, AZF; tumor No. 8415, was frozen 1/4/50

* Assisted by grants from the Citizens Aid Society of Minneapolis, the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, the Minnesota Division of the American Cancer Society, the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and the Graduate School Cancer Research Fund of the University of Minnesota.

Received for publication February 5, 1958.

TABLE 1

TUMOR-INDUCING ACTIVITY OF THAWED TISSUE OF A TRANSPLANTED MAMMARY CARCINOMA WHICH HAD BEEN FROZEN FOR APPROXIMATELY 4.5 YEARS (1/4/50–5/26/54)

<table>
<thead>
<tr>
<th>GM-SPECIFIC INJECTED</th>
<th>No.</th>
<th>Cancer (per cent)</th>
<th>Cancer Age in Days</th>
<th>Nonca.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 × 10^4</td>
<td>20</td>
<td>70.0</td>
<td>344</td>
<td>428</td>
</tr>
<tr>
<td>10^4</td>
<td>24</td>
<td>68.5</td>
<td>347</td>
<td>452</td>
</tr>
<tr>
<td>10^4</td>
<td>19</td>
<td>78.9</td>
<td>327</td>
<td>923</td>
</tr>
<tr>
<td>10^4</td>
<td>21</td>
<td>61.9</td>
<td>415</td>
<td>494</td>
</tr>
<tr>
<td>10^4</td>
<td>18</td>
<td>38.9</td>
<td>409</td>
<td>558</td>
</tr>
</tbody>
</table>

The observations obtained on the development of mammary cancer in ZBC females following the intraperitoneal administration of cell-free extracts of different concentrations of the thawed carcinoma are recorded in Table 1.

The mean incidence of mammary tumors for ZBC females which received from 2 × 10^-4 to (6, 7), according to the method used by others (11-14), and maintained at a temperature of −79°C. On 5/26/54 two vials, each containing 1 cc. of 5.3 per cent dextrose and 1 gm. of the original tumor mince, were placed in a water bath at 37°C until the tissue had thawed. Part of the thawed tumor-suspension was ground with sand in a mortar, and distilled water was added to make a 10 per cent suspension, which was centrifuged for 20 minutes at approximately 2500 r.p.m. The supernatant was diluted so that the administration of 0.5 cc., intraperitoneally, contained the MTA from the amount of tissue specified in the table. The test animals were 24-27-day-old agent-free ZBC females, and litter-mate controls were used in separating the animals to be injected with the different extracts. The experimental females were subjected to breeding, and only noncancerous mice which survived for at least 300 days have been included in the tabulations.

Two cc. of saline was added to 1 cc. of the thawed tumor mince, and 0.2 cc. of the resulting suspension was injected subcutaneously into ten AxZbF1 male mice, which were susceptible to transplants of the original tumor.

RESULTS AND DISCUSSION

After tissue of tumor No. 8415 had been frozen for 48 hours, it was shown that the injection, either subcutaneous or intraperitoneal, of a suspension of the thawed tumor would produce transplanted mammary tumors in male AxZbF1 hybrids (6, 7). In the present study, with tissue which had been frozen for longer than 4 years, no evidence of the survival of cells was obtained by inoculation into ten hybrids of the same generation, and no transplanted tumors were observed.

The observations obtained on the development of mammary cancer in ZBC females following the intraperitoneal administration of cell-free extracts of different concentrations of the thawed carcinoma are recorded in Table 1.

The mean incidence of mammary tumors for ZBC females which received from 2 × 10^-4 to
$10^{-4}$ gm.-equiv. of material was 68.3 per cent, with an average age of 366 days; this incidence was increased to 80.4 per cent when only the noncancerous females which lived to the average cancer age of each group are included. As may be seen from the data, a much lower incidence of tumors was obtained by the assay of the extract diluted 10$^{-4}$-fold.

The youngest mouse to develop mammary cancer following the injection of the extract containing $10^{-4}$ gm.-equiv. was 280 days, while the mean age for the others was 232 days; 215 days for the youngest cancerous animal receiving the extract diluted 50-fold and 218 days when the MTA from $10^{-4}$ gm.-equiv. of tissue was assayed. These ages for the development of tumors are similar to those observed when agent-containing extracts of unfrozen tissues of this and other spontaneous or transplanted mammary tumors were tested for their biological activity (5-7).

These observations demonstrate that the mammary tumor agent remained active in the frozen state for a period of nearly 4.5 years, and no significant decrease in the tumor-inducing activity was apparent until the extract being assayed contained less than $10^{-4}$ gm.-equiv. of material. No attempt will be made to compare the data for the frozen tissue with biological assays in which fresh and frozen tumors were used (5-7) because of the various factors known to influence the final incidences and cancer ages.

SUMMARY

The mouse mammary tumor agent (MTA) was demonstrated in extracts of thawed tumor mince which had remained frozen for approximately 4.5 years.

The agent present in $10^{-4}$ gm.-equivalents of tissue induced mammmary cancer in nearly 40 per cent of the test animals, while those which received the extract at dilutions of $2 \times 10^{-2}$ to $10^{-4}$ had a mean tumor incidence of 68 per cent.

There was no indication that the MTA had become "active" following freezing, as suggested by Gye and Mann, since the mean age for the youngest cancerous mice in the different groups was 242 days.

Viable tumor cells could not be demonstrated in the thawed suspension when tested on a small number of mice which were susceptible to grafts of the transplanted tumor.

REFERENCES

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