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 intra-

<table>
<thead>
<tr>
<th>GM-EQUIV. INJECTED</th>
<th>No.</th>
<th>CANCER (per cent)</th>
<th>AV. AGE IN DAYS</th>
<th>NONC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 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>62.5</td>
<td>547</td>
<td>45</td>
</tr>
<tr>
<td>10^4</td>
<td>19</td>
<td>73.9</td>
<td>527</td>
<td>325</td>
</tr>
<tr>
<td>10^4</td>
<td>21</td>
<td>61.9</td>
<td>415</td>
<td>464</td>
</tr>
<tr>
<td>10^4</td>
<td>18</td>
<td>58.9</td>
<td>409</td>
<td>558</td>
</tr>
</tbody>
</table>

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 AxZbF1 male mice, which were susceptible to transplants of the original tumor.

MATERIALS AND METHODS

Tissue from a transplanted mammary carcinoma, AZF1 tumor No. 8415, was frozen 1/4/50

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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^-2-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 mammary cancer in nearly 40 per cent of the test animals, while those which received the extract at dilutions of 2 × 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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