Acceleration in the Transplantation and Killing Time of Mammary Tumors in Mice Pretreated with a Heat-stable Tumor Tissue Preparation*

GEORGE MIROFF,† CARLOS MARTINEZ, AND JOHN J. BITTNER

(Department of Physiology, Division of Cancer Biology, University of Minnesota Minneapolis, Minn.)

In the course of experimentation it was noted that in mice treated with tumor tissue heated at 70° C. for 1 hour, prior to the administration of a viable tumor cell suspension, an acceleration in the transplantation and killing time of the tumors was produced in these animals when compared with that in untreated controls receiving the same tumor cell suspension. A search of the literature showed that the presence of a heat-stable accelerating material in tumors was first noted by Flexner and Jobling (8, 9). These authors heated the Flexner-Jobling sarcoma of the rat at 56° C. for 1 hour and injected this material into rats. After 10–50 days, the animals received an injection of viable tumor (Flexner-Jobling sarcoma). The tumors grew larger and more rapidly in treated animals than in the control rats. These same authors found no such growth-stimulating effect from emulsions of liver, spleen, kidney, muscle, and testicle (9). Jobling also found that the accelerant was present in rat carcinoma (11).

Chambers and Scott reported that when autolysed Jensen sarcoma, heated at 40° C. in toluene-saturated solution, was injected into rats the susceptibility of the rat to the Jensen sarcoma was increased (4, 6).

Berry and Dedrick described a heat-stable material prepared from myxoma and glycercinated or lyophilized fibroma. Administration of this preparation to rabbits resulted in the development of myxoma (1, 18). This agent was stable at temperatures between 56° C. and 85° C. and stable to alcohol treatment at these temperatures. This material was found in the supernatant of the tumor protein-precipitated solution. No reports dealing with a heat-stable accelerant have been found in the literature since the advent of inbred strains of mice. This report deals with experiments designed to show the presence of a heat-stable tumor accelerant in tumors and to demonstrate some of its chemical properties.

MATERIALS AND METHODS

PREPARATION OF ACCELERANT

a) Tumor.—The heat-stable accelerating material was prepared from Z mammary tumors repeatedly transplanted for 40 generations in ZBC animals. This tumor originally arose spontaneously in a Z strain breeding female.

The tumors used for the preparation of the accelerant were homogenised and diluted to a 5 per cent concentration by the addition of 0.9 per cent NaCl buffered at pH 7.4. This homogenate was placed in a large flask and submerged in a constant-temperature water bath at 70° C. for 1 hour. At the end of the heating period, the homogenate was cooled to room temperature, and the volume was adjusted to the original amount with distilled water. The heated suspension was dispersed uniformly by shaking. The test animals were injected subcutaneously with 0.2 cc of this suspension.

b) Other tissues.—Muscle, kidney, spleen, liver, lung, breast, and thymus tissues taken from ZBC mice were prepared as in (a) above.

c) Ether fractions.—The heat-treated tumor homogenate was frozen with dry ice and extracted 4 times with ethyl ether, using 10 times the volume of tissues for a 24-hour period. The ether solution was filtered and then dried in vacuo, and the dry material was suspended in a volume of 0.9 per cent NaCl equal to the volume of the original homogenate.

d) Ether residue.—The frozen residue from (c) was thawed and all excess ether removed in vacuo. The original volume was restored with distilled water.

e) Acetone fraction.—The heat-treated tumor homogenate was frozen with dry ice and extracted 4 times with acetone, and then dried in vacuo, and the dry material was suspended in a volume of 0.9 per cent NaCl equal to the volume of the original homogenate.

f) Acetone residue.—The excess acetone from (e) was drawn off in vacuo, and the original volume was restored with distilled water.
The control and experimental mice used in this experimental study were 6–8 weeks old at the time of the initial injection. ZBC mice, not possessing the mammary tumor agent, were injected subcutaneously with the heat-stable accelerant on the left side of the body. Twelve days later a viable tumor suspension prepared from spontaneous mammary tumors of the Z strain was injected on the right side of the body. The control group of animals received only the viable tumor suspension on the 12th day. The transplantation time, killing time, and the rate of growth of the tumors were recorded.

**Terminology**

*Transplantation time.*—The time required for 50 per cent of the animals of a given experimental group to develop tumors (minimum diameter of 0.8 cm.) which grew progressively.

*Death time.*—The time required for 50 per cent of the animals with tumors of a given experimental group to die.

### Table 1

**Accelerating Effect Produced by Heat-denatured Tumor Tissue**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exp. A</th>
<th>Exp. B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Spleen</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Kidney</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Muscle</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Thymus</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Breast</td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

**Per cent acceleration of transplantation time.**—The per cent acceleration of the transplantation time was expressed as:

\[
\frac{C - T}{C} \times 100
\]

where \(T\) is the transplantation time of the treated animals and \(C\) the transplantation time of the controls.

**Per cent acceleration of death** (16,17),

\[
\frac{d - t}{d} \times 100
\]

where \(d\) is the time required for 50 per cent of the control group of animals to die, and \(t\) is the time required for 50 per cent of the treated group to die. Calculations were based upon the number of mice developing tumors.

In mice treated with tumor tissue that had been heated to 70°C for 1 hour, the growth of subsequently inoculated spontaneous mammary tumors was accelerated (Exp. A, Table 1). The transplantation time of the treated and control groups was 14 and 24 days, respectively. This represented an acceleration of 41.5 per cent. The pretreatment with heat-denatured material likewise accelerated the death time of mice receiving the spontaneous mammary tumor suspension. The death time for the treated and control groups was 33 and 56 days, respectively. This represented an acceleration of 41.2 per cent (Table 1).

In a second experiment (Exp. B, Table 2), the heat-denatured material produced an acceleration in the transplantation time of 63.5 per cent and an acceleration in the death time of 56 per cent with another mammary tumor.

The transplantation time of tumors was not altered by tissue preparations made from spleen, liver, and lung. Muscle, thymus, and breast tissue preparations showed a small amount of acceleration. Preparations from the kidney retarded the transplantation time of tumors (Table 2). A slight acceleration in the death time was produced by preparations from liver, lung, and muscle, but spleen, kidney, thymus, and breast produced retardation (Table 2).

The tumor accelerant was extractable from heat-treated tumor tissues with ethyl ether or acetone (Table 3). The residue from these extractions contained little, if any, of the accelerant material.

The transplantation times of the tumors in mice pretreated with the ether and acetone fractions were 29 and 35 days, respectively, and 45 days for the controls. This represented an acceleration of 35.5 per cent and 22.3 per cent for the ether and acetone fractions (Table 3). The death time was accelerated by the ether and acetone fractions. The 50 per cent death time produced by pretreatment with the ether fraction was 76 days, as compared with 63 days for the acetone-treated ani-
mals and 145 days for the control animals. The acceleration of death time produced in mice receiving the ether fraction was 47.5 per cent and that by the acetone fraction was 56 per cent (Table 3). The presence of a small amount of accelerant activity in the ether residue (Table 3) demonstrated that the ether extraction was not so effective as acetone in extracting the accelerant material.

The results of transplanting tumors of the same size from the accelerated and control groups into untreated ZBC female hosts are shown in Table 4. The transplantation time in the initial accelerant experiment was 14 days for the treated animals and 24 days for the control group. When tumors of the same size from both groups were retransplanted into untreated hosts, the transplantation times were 10 and 16 days, respectively. Tumors of the same size taken from the treated and control groups after one passage through untreated ZBC hosts were retransplanted for a second time into untreated ZBC hosts. The transplantation time of the treated and control tumors in this second retransplantation was 11 and 14 days, respectively. This gave an acceleration of 21.4 per cent.

The accelerated death times of treated tumors remained accelerated when these tumors were retransplanted into untreated ZBC hosts. In the original acceleration study, the death time for the treated and control tumors was 83 and 56 days, respectively (Table 4). This represented an acceleration of 41.5 per cent. In the first retransplantation into untreated ZBC hosts, the death time for the treated and control tumors was 30 and 37 days, respectively. This represents an acceleration of 23.2 per cent. In the second retransplantation into untreated ZBC hosts, the treated tumors showed a death time of 35 days as compared with 39 days for the control tumors. This represented an acceleration of 10.5 per cent.

The mean per cent increase per day of the tumor diameters (Exp. A) for a given tumor size was greater in the treated mice than in the control animals (Table 5). This difference was significant for tumors with diameters greater than 1.2 cm. The error in measurement of the tumor diameters might, in part, account for the lack of significance of the differences in the mean tumor diameters when the tumors are small. The mean tumor size of the treated and control animals at the time of death was 2.4 and 2.3 cm., respectively.

**TABLE 3**

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>NO. ANIMALS</th>
<th>TRANSPANTATION TIME</th>
<th>DEATH TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>14 days</td>
<td>45 days</td>
</tr>
<tr>
<td>Ether</td>
<td>13</td>
<td>29 days</td>
<td>66 days</td>
</tr>
<tr>
<td>Ether residue</td>
<td>8</td>
<td>45 days</td>
<td>55 days</td>
</tr>
<tr>
<td>Acetone</td>
<td>13</td>
<td>55 days</td>
<td>72 days</td>
</tr>
<tr>
<td>Acetone residue</td>
<td>7</td>
<td>55 days</td>
<td>57 days</td>
</tr>
</tbody>
</table>

* Obtained from fourfold x² table (51).

**TABLE 4**

<table>
<thead>
<tr>
<th>TUMOR ANIMALS</th>
<th>TUMOR PER CENT INCREASE/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. A</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>Controls</td>
</tr>
<tr>
<td>No.</td>
<td>(cm.)</td>
</tr>
<tr>
<td>14</td>
<td>45.1</td>
</tr>
<tr>
<td>10</td>
<td>41.0</td>
</tr>
<tr>
<td>11</td>
<td>21.4</td>
</tr>
<tr>
<td>10</td>
<td>14.2</td>
</tr>
<tr>
<td>10</td>
<td>14.5</td>
</tr>
</tbody>
</table>

* Obtained from fourfold x² table (51).

**TABLE 5**

<table>
<thead>
<tr>
<th>TUMOR DIAMETER</th>
<th>Treated</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cm.)</td>
<td>(10)*</td>
<td>(10)*</td>
</tr>
<tr>
<td>1.2</td>
<td>8.6</td>
<td>5.8</td>
</tr>
<tr>
<td>1.4</td>
<td>8.8</td>
<td>5.9</td>
</tr>
<tr>
<td>1.6</td>
<td>7.6</td>
<td>5.2</td>
</tr>
<tr>
<td>1.8</td>
<td>8.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Obtained from student's "t" value (51).
DISCUSSION

The presence of an active accelerating substance in heat-denatured tumor tissue, extractable with either ether or acetone, strongly suggests that this material is lipid in nature. The accelerant in the ether or acetone fraction gave negative biuret, Molisch, and Fehling tests, which indicates the absence of protein and carbohydrate in the accelerating material. The accelerating effect was small or absent when normal tissues were used in the preparation of the accelerant, indicating that the accelerant is, if not peculiar to tumor tissue, at least present in greater quantity in mammary tumors.

In the studies described in this paper, ZBC mice of the same age and sex were used as host animals. Careful selection of the tumor material for the preparation of the accelerant and challenging injections was necessary, as it had been shown that these factors affected the final results (14).

It appears that the tumors were altered either directly by the accelerant or indirectly by the accelerant through the host (Table 4). The accelerated tumors, when retransplanted into untreated ZBC hosts, maintained their acceleration. Repeated retransplantations of the accelerated and control tumors into untreated hosts resulted in a diminution of the acceleration. This decline in the acceleration was due to a gradual diminution of the transplantation and killing times of the control tumors with repeated passages and not due to a deacceleration of the accelerated tumors.

The accelerant material appears to be effective in increasing the growth rate of tumors (Table 5); however, the mean tumor diameters at the time of death of the treated and untreated groups are not significantly different (2.3 and 2.4 cm., respectively).

Acceleration of transplanted tumors has previously been described with the use of frozen tumor tissue (8), lyophilized tumor tissue (7, 18, 19, 20), and aqueous extracts of fresh tumor tissue (13, 17) as the accelerating material. The method of assay of the acceleration effect differs among the various investigators in the field and thus makes direct comparison of results difficult. Casey, Kaliss, Day, and Snell utilized the abrogation of resistance to heterologous transplantation. Shear employed the 50 per cent death time of tumors indigenous to the recipient host. In the present experiments the method of assay is similar to that of Shear et al., but in addition to the 50 per cent death point we have employed the 50 per cent take time. The transplantation time appeared to be a more accurate measure of the acceleration effect, since the measurement is not complicated by such factors as necrosis and the ensuing infection of the tumor and host, cachexia, metastasis, and a generalized deterioration of the host just prior to death.

It will be observed from Tables 1 and 4 that the variability as indicated by the P values, is smaller when the transplantation time is utilized as an index of acceleration. However, it will be observed that, in spite of the complications in the terminal stages of tumor growth contributing to the death of the animals, the per cent acceleration of the transplantation and death times was not very different.

The heat stability and the solubility of the accelerant in organic reagents indicates a qualitative difference of this material from that described by others (3, 7, 18, 20). The fresh tumor tissue accelerant described by Shear may be lipid in nature, as indicated by his data (16), but the heat-lability sets it apart from the accelerant described in this paper. The heat-stable accelerant in the doses employed (1 mg. dry weight) does not alter the resistance of the host to heterologous tumor transplants. In this respect also the heat-stable accelerant differs from the accelerant described by Casey, Snell, Kaliss, and Day but is similar to that described by Shear.

From reports in the literature describing the high level of cholesterol in tumor tissue (10, 12) and the accelerating effect of cholesterol on tumor growth (2, 15), it was thought that this accelerant, because of its solubility characteristics, may conceivably be cholesterol. However, injection of 0.2 cc. of 0.26 per cent cholesterol in 0.9 per cent NaCl in a manner identical to that used for the injection of the accelerant material failed to produce acceleration.

Experiments are now in progress endeavoring to isolate and identify the accelerating material and to determine the mechanism of its action.

SUMMARY

Heat-denatured tumor tissues contain an accelerating material which reduces the transplantation and killing times of subsequently inoculated tumors.

Tumors that have been accelerated by the tumor-accelerant material continue to show acceleration with repeated retransplantation into untreated hosts.

REFERENCES

5. ————. Variations in Growth of Jensen's Rat Sarcoma and Influence of Technique. Ibid., 33:558-61, 1930.
Acceleration in the Transplantation and Killing Time of Mammary Tumors in Mice Pretreated with a Heat-stable Tumor Tissue Preparation

George Miroff, Carlos Martinez and John J. Bittner


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/15/7/437

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.