Assay of Spontaneous and Transplanted Mammary Tumors for the Mammary Tumor Agent*

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The mammary tumor agent, one of the primary causes of mammary cancer in mice (5, 6), has the characteristics of an infectious agent or virus.

The presence of the agent in infected hosts may be demonstrated by making extracts of either normal or cancerous tissues and injecting these into susceptible animals which do not carry the agent. It has been assumed that the percentage of mice developing tumors and the average cancer age are indicative of the concentration and/or activity of the agent.

In several studies (2, 4, 9, 11) transplanted tumors have been tested for the presence and propagation of the mammary tumor agent. However, in most studies the activity of the agent from transplants of one tumor was compared to that from spontaneous tumors which developed in other animals of the same or a different cancerous stock. Such results do not give definite information as to the best source of the agent for biological work, especially where large amounts of material may be required.

When the concentration and/or activity of the agent from transplanted and from spontaneous mammary tumors are compared, the data should be based upon studies where spontaneous and transplanted tumors descended from the same tumor are used. By doing so, information may be obtained on the survival of the agent in the transplants over a number of passages.

An attempt has been made to do so in this report, where the final observations (10, 11, 13) are presented on the tumor-inducing ability of the agent secured from several spontaneous mammary tumors and from the transplants of those which were continued for a number of passages in mice which did not possess the agent. In some experiments, normal tissues were also assayed from the same donors which were bearing either spontaneous or transplanted mammary tumors.

MATERIALS AND METHODS

Five spontaneous adenocarcinomas of the mammary gland, which developed in breeding females, were used, of which three were transplanted by the trocar technic.

Tumor No. Z8044 appeared in a female of the cancerous Z (CSH) stock when the animal was 278 days of age. The mouse was a member of the 53d successive generation to have mammary cancer.

The other four tumors developed in females of the B (C57BL) stock, subline 6, either in mice which had been fostered by females of cancerous strains or in their progeny.

Tumors Nos. B7476az and B7477aj, were from B females which had been nursed by females of the Z stock, and the tumors were observed when the mice were 482 and 500 days of age, respectively. Litter-mates give rise to the other two tumors, Nos. B7476az and B7477aj, at the respective ages of 348 and 320 days. Their mother, fostered by a female of the A stock (A1), also had a mammary cancer when she was 287 days of age.

The tissue to be assayed was weighed, ground in a mortar with sand, and suspended in either physiological saline or distilled water, 1:10. Distilled water was used except for the four spontaneous tumors from the B females. The tumor suspensions were centrifuged twice, for periods of 10 minutes each, at approximately 2,500 r.p.m. The final supernatant was removed and either used at the same concentration or diluted so that the intraperitoneal injection of 1 cc. contained the agent extracted from an equivalent weight of tumor tissue as given in the tables.

The test animals were ZBC hybrids, derived by matings between mice of the fostered (without the agent) lines of the A and Z stocks (11), with an incidence in controls of less than 1 per cent. The age of the mice at the time of the administration of extracts of tumor Z8044 are given in the table; in the other studies they were from 30 to 97 days. All experimental mice were continued as breeders, and few of those which died noncancerous had less than five litters. Only noncancerous mice which survived for longer than 250 days have been included in the tabulations.

The mice were maintained on Purina Fox Chow and tap water.
RESULTS

The results obtained following the injection of extracts of the five spontaneous mammary tumors are recorded in Table 1.

TABLE 1
BIOLOGICAL ASSAY IN ZBC HYBRID MICE FOR THE MAMMARY TUMOR AGENT IN SPONTANEOUS MAMMARY TUMORS FROM THE B (C57BL) AND Z (C3H) STOCKS

<table>
<thead>
<tr>
<th>Tumor no.</th>
<th>GM equiv.</th>
<th>No. mice</th>
<th>Per cent</th>
<th>Av. age in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>injected</td>
<td></td>
<td>with cancer</td>
<td></td>
</tr>
<tr>
<td>B7476a*</td>
<td>2×10^{-4}</td>
<td>20</td>
<td>80</td>
<td>455</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>15</td>
<td>46</td>
<td>429</td>
</tr>
<tr>
<td>B7477a2</td>
<td>2×10^{-3}</td>
<td>99</td>
<td>59</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>10^{-3}</td>
<td>25</td>
<td>64</td>
<td>408</td>
</tr>
<tr>
<td>B7478z2</td>
<td>10^{-3}</td>
<td>81</td>
<td>81</td>
<td>461</td>
</tr>
<tr>
<td>B7788z2</td>
<td>2×10^{-3}</td>
<td>41</td>
<td>95</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>10^{-3}</td>
<td>49</td>
<td>88</td>
<td>352</td>
</tr>
<tr>
<td>Z8044</td>
<td>10^{-4}</td>
<td>38</td>
<td>76</td>
<td>528</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>33</td>
<td>70</td>
<td>511</td>
</tr>
</tbody>
</table>

* A (a) or Z (z) agent, and generations in B stock.

The earliest average cancer ages to be found in the injected ZBC mice were seen in those which received extracts of the mammary tumor from a female of the cancerous Z stock, Z8044; the next for one tumor from a B mouse with the agent from the Z stock (B7788z2). Considerable variation was encountered when the two tumors from females of the B stock with the agent derived from the A strain were assayed, although these tumors developed in litter-mates and were tested the same day. However, at some concentrations, extracts of these tumors were as active as another cancer with the Z agent, B7478z2.

Transplants of the first and tenth passages of tumors B7476a* and B7477a2 were also studied, and the data have been tabulated in Table 2. That the tumors possessed the mammary tumor agent, after being transplanted for ten passages in mice of the B stock, is evident from the results.

Tumor Z8044 was continued for 23 months through 30 transplant generations to investigate the propagation of the mammary tumor agent, and had been transplanted for 41 passages when the extensive amount of necrosis in the tumors discouraged further study. The hosts were F1 hybrids, produced by reciprocal matings between fostered A and Z animals, and the grafts grew progressively in all.

The intraperitoneal injection of extracts of tumor Z8044, of either the spontaneous or transplanted tumors, gave the results recorded in Table 3, where the data are arranged according to the concentration of the extracts. As noted, two series were tested with transplants of the first passage using tumors either 51 or 53 days following inoculation. Details about the data will be considered later.

The liver and spleen from the cancerous mouse bearing tumor Z8044 were also extracted and tested for the activity of the mammary tumor agent. Owing to the number of fractions that were used in this study, different groups of mice were injected with the tumor extracts to ascertain if there might be any loss of activity during the time required for administration. These are listed in the order they were injected in the tabulations (Table 4). The first and seventh series received the amount of the agent derived from 10^{-4}-gm. equivalents of tumor tissue, and no significant variation was observed in the results. This concentration of tumor was as active as the extract containing 10^{-2}-gm. equivalents of tissue. At a dilution of 100-fold, the tumor-inducing ability of spleen was approximately the same as that of the tumor; when 10^{-4}-gm. equivalents were used, the activity of the former was greatly reduced. At all dilutions tested, extracts of liver were much less active than the other tissues obtained from the cancerous donor.

Thirty-one days following the first passage of tumor Z8044, at the time the transplanted tumors were assayed, spleen and liver from tumor-bearing mice were also used. As compared to the transplanted tumors, spleen and liver were relatively inactive (Table 5).
DISCUSSION

Since 1944 (8), it has been known that the mammary tumor agent could be recovered from transplanted mammary tumors, even after ten (10-13, 17), 30 (13), and 42 (15) serial passages in animals which did not themselves carry the agent. Although the results obtained by various workers have not been in accord as to the advisability of employing this material as the source of the agent for various problems, only preliminary data (10, 11, 15) have been presented on some serial dilution studies comparing the activity of the agent in spontaneous mammary tumors, and in transplanted tumors descended from the same.

Andervont (1) reported that, although a spontaneous mammary tumor from a C3H female and the fourth passage transplants carried in C3H hosts possessed the agent, when this tumor was transplanted into agent-free hybrids, the third and 23rd transfer tumors failed to induce mammary tumors in suitable test animals, from which it was concluded that the agent was absent in these transplants. Dmochowski (14) was unable to demonstrate the agent in the transplants of the spontaneous tumors did not possess the same degree of activity, due to the presence of the agent, is evident from the results (Table 1). The one with the greatest tumor-inducing ability in the test animals, B7788zi, appeared in a B mouse with the Z agent, and yet another tumor (B7478zi) from a mouse fostered by a female of the same strain was no more active than a tumor with the A agent after it had been propagated for one generation in mice of the B stock.

The time of appearance of the spontaneous mammary tumors in the B mice did not bear any relationship to the activity of its mammary tumor agent, since the tumors with the A agent developed

TABLE 3

ASSAY OF TUMOR Z8044, WITH EXTRACTS FROM THE SPONTANEOUS AND TRANSPLANTED TUMORS

The data are arranged according to the concentration of the extracts administered.

<table>
<thead>
<tr>
<th>Passage</th>
<th>Gm. Equiv.</th>
<th>Age When</th>
<th>Per. Ave. Age in Days</th>
<th>Sp. T.*</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>48th</td>
<td>10^4</td>
<td>36-37</td>
<td>18-78</td>
<td>362</td>
<td>385</td>
<td>385</td>
</tr>
<tr>
<td>7th</td>
<td>10^4</td>
<td>37-50</td>
<td>15-75</td>
<td>347</td>
<td>438</td>
<td>438</td>
</tr>
</tbody>
</table>

* Sp. T. = spontaneous tumor.
† Used 31 days after inoculation; others same passage after 33 days.
before the mice were 350 days of age, while the most active tumor, B7788x, appeared when the mouse was 500 days of age. In some tests, the amount of the agent derived from 10−4-gm. equivalents of tissue produced a higher frequency in the test mice than did extracts containing a larger amount of material. While the extracts of the tumor from the Z stock, Z8044, did not cause more of the ZBC test mice to become cancerous, their tumors appeared at an earlier average age than was seen following the administration of extracts of the tumors from the B mice.

The two mammary tumors from litter-mates of the B stock possessing the A agent were transplanted for ten passages in unfostered B mice. Since the spontaneous tumors and transplants of the first and tenth passages were tested for the agent, these data, at some dilutions, may be compared for activity (Table 2). At all concentrations, extracts of the first-passage tumors were as active as the spontaneous tumors; after ten passages, some were more active, others perhaps less active.

With the exception of the eighteenth passage, extracts of the transplants of the Z tumor, Z8044, that were tested between the first and 30th passages showed approximately the same tumor-inducing ability at a dilution of 100-fold as did the spontaneous tumor, and comparable results were obtained for transplants of the seventh and 24th passages with 10−4-gm. equivalents of tissue. When dilution was 10,000 times, there was little difference between the activity of extracts of either the spontaneous tumor or transplants of the first passage, and an extract of the latter containing 10−4-gm. equivalents produced an incidence of 43 per cent in the test animals (Table 3).

All extracts of transplants of the eighteenth passage, regardless of the amount of material administered, showed lower activity, and it was not restored in later transplants except when the concentration was no more than 10−4 gm. equivalents. Some data for tumors of the seventh passage suggested that this “decrease in activity” might have occurred earlier.

Some variation was encountered when transplants of the same passage, the first (Table 3), were tested at different intervals following inoculation, and these results could not be correlated either with the concentration of the extract, the size of the tumors, or a slight difference in the age of the mice at the time of treatment.

Extracts of spleens from cancerous donors were found to be a poorer source of the mammary tumor agent, in general, than most spontaneous and transplanted tumors have been. When the spontaneous tumor, Z8044, and the spleen and liver from the cancerous animal were tested, spleen and tumor showed approximately the same tumor-inducing ability when the extracts were diluted 100-fold. If the extracts contained 10−4 gm. equivalents, the activity of the tumor remained approximately the same, while that of spleen was greatly reduced at this concentration. At both dilutions, liver proved to be relatively inactive (Table 4).

Previously, it was found (7) that, after small pieces of spleen from young donors were grafted, the presence of the agent in the grafts produced mammary cancer in over 20 per cent of the inoculated BAF1 females, and the agent was transferred by the inoculated females to their progeny. The agent could also be transferred by the transplantation of other normal tissues.

Prehm (18) has recently reported upon the transfer of the agent by the inoculation of either normal or cancerous tissue. When spleens from mice which possessed the agent were grafted, in several series at least 40 per cent of the females later developed mammary tumors, and others became “infected.” The agent could also be recovered from the spleens of the inoculated animals and could be detected within a period of 3 weeks, or earlier, after the grafts had been transplanted.

The spleens and livers from mice bearing the first-passage transplants of tumor Z8044 were tested 31 days following the inoculation of the tumors. Extracts of these tissues showed little activity, and the low incidences, together with the late average cancer ages, suggested that the agent probably could not be demonstrated (Table 5). At a concentration of 10−4-gm. equivalents, the tumor produced an incidence of 88 per cent in the test mice.

By the subcutaneous transplantation of mammary tumors to susceptible animals, and by excising tumors after 26–55 days, Andervont (2) tested this technic for the transfer of the agent from the tumor to the inoculated host. Only 4 per cent of the animals later had mammary tumors. Hummel and Little (17) used a similar method but used the ear for the inoculation site, and, after the tumors had developed, the ears were amputated. Fifty-two per cent of the inoculated hybrids, BAF1 (B9 × A), which received transplants of the first, fifth, and tenth passages of a tumor from the A stock showed progressively growing tumors. Among this group, 6 per cent had mammary cancer, while of those in which the ear transplants regressed, the incidence of mammary cancer was found to be 18 per cent. Extracts of the transplants, of unstated concentration, were also administered, and evidence was obtained that the agent had survived ten passages.
A higher incidence was observed by Prehm (15) following the removal of subcutaneous transplanted tumors, for in one series 40 per cent later gave rise to spontaneous mammary cancer. In a group of seven hybrids resistant to grafts of the tumor, six had mammary cancer due to the transfer of the agent from the transplanted graft.

In a previous publication (5), an attempt was made to ascertain the most active source of the mammary tumor agent by comparing the results found in test animals following the injection of extracts of various tissues. With material from donors of the A stock, lactating mammary glands were found to have a greater tumor-inducing potency than did spontaneous mammary cancer, and spontaneous mammary cancer was a better source of the agent than were transplants of the first passage. Lactating mammary glands from females of the Z (C3H) stock were less potent than mammary glands from the A strain. ABC mice were used as test animals for these studies, derived by matings between mice of the B and fostered A (Ax) stocks.

The evaluation of the observations on the concentration and/or activity of the mammary tumor agent, obtained following biological assay, must be made with many factors in mind.

The tumor-inducing ability either of the same material from comparable donors of the same inbred stock, as cancerous donors (Table 1), or of different tissues from the same donor (Tables 4 and 5), need not be the same, even when assayed in litter-mate controls. Also, when extracts of the same tissue have been tested by serial dilution, the amount of the agent to be administered may not be the determining factor, as has been shown in several studies (see 3, 4, 11).

Likewise, it has been demonstrated in reciprocal crosses between different inbred cancerous strains that the mammary tumor agent transferred by females of these strains need not have the same activity in offspring with the same genetic make-up. When females of the same maternal tissues are used to produce hybrids with other genetic constitutions, different incidences and average cancer ages may be obtained (see 11). This would imply that, to assay the tumor-inducing activity of material from two or more strains, test animals should be used which bear the same genetic relationship to the donor strains; and, based upon the results of using several tumors from a single stock (Table 1), multiple tests should be made.

It seems probable that adequate tests have not been completed, to date, to assay various tissues from either the same or different inbred stocks as to the best source of the mammary tumor agent for experimental studies. Because of the individual variation between the material tested in these studies, either tissues from the same donor or comparable tissues from litter-mates, it is doubtful if comparisons of all of these data would be meaningful; for this reason, each study should be considered separately.

It is to be recognized that aqueous extracts of tissues may carry inhibiting and/or inactivating factors for the mammary tumor agent. For example, intracellular enzymes liberated by maceration may influence the amount of activity shown by an extract.

Under the experimental conditions outlined in this report, it seems probable that for the first passage and, perhaps, for ten passages, transplanted mammary cancer may possess the mammary tumor agent with activity equal to the original spontaneous mammary cancer. For some studies, where a large amount of material with approximately the same tumor-producing activity may be needed over a period of days, transplanted mammary cancer might be the most satisfactory source of the agent for biological studies.

SUMMARY

In biological assays on the tumor-producing ability of the mammary tumor agent, considerable variation was observed in the activity of several spontaneous mammary tumors from the same inbred stock, including those from litter-mates.

The results could not be correlated with either the source of the mammary tumor agent or the time of development of the tumors.

Extracts of three transplanted tumors were as active after one passage, and in some cases ten, as the original spontaneous tumor.

A decrease in the activity of one transplanted tumor was seen in the eighteenth passage, and it was not restored in later passages except when extracts of high concentrations were administered.

The mammary tumor agent survived for as long as the tumors were transplanted—namely, ten and 30 passages.

Spontaneous mammary cancer proved to be a better source of the mammary tumor agent than did either spleen or liver from the cancerous donor.

In one study, it is probable that the mammary tumor agent could not be demonstrated in the liver and spleen from donors bearing transplanted tumors, although the tumors were very active.

For some experiments, transplanted mammary cancer may be the most satisfactory source of the mammary tumor agent.
REFERENCES

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