Stromal Malignancy in Mouse-Grown Transplants of Egg-Cultivated Mouse Mammary Carcinoma

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Several papers have been published from this laboratory giving the results of investigations designed to demonstrate tumor production in mice with cell-free extracts of materials from eggs containing implants of mouse mammary carcinoma. Successful experiments with both Berkefeld filtered and lyophilized yolk from eggs containing yolk sac implanted mouse mammary tumor have been reported (6, 10, 11). It has also been shown that mouse tumor tissue inoculated into the anterior chamber of the rat's eye may induce malignancy in cells of rat origin (9, 10).

While these investigations have furnished evidence for the tumor agent or virus concept of cancer causation, the methods used cannot be depended upon to demonstrate regularly the presence of the tumor agent in the various extracts under investigation. Many hundreds of experiments have been entirely negative and other laboratories which have as far as possible followed the procedures used here have failed to obtain positive results (1, 3, 13).

The conclusion, however, of Twombly and Meisel (13) that such tumors as have been produced from the various extracts of tumor-bearing eggs were due to the presence in the material injected of viable tumor cells is untenable. The 20 malignant tumors obtained from lyophilized extracts cannot be explained on this basis since the mouse tumor tissue inoculated into the anterior chamber of the rat's eye may induce malignancy in cells of rat origin (9, 10).

The present paper is concerned with a different approach to what is believed to be the same phenomena of tumor induction by a tumor agent. Unlike the previous reports, the method involved gives results which are repeatable.

In association with the study of methods for demonstrating the presence of a tumor-inducing agent in tumor tissue, the technique of cultivating cancer tissue in eggs by the yolk sac method has been perfected (8, 12). Mouse mammary carcinoma has been carried in eggs continuously in this manner for the past 2 years and 11 months.

It has been previously noted that extracts of yolk material from tumor-bearing eggs quite regularly produce tumors when injected into mice. This was not surprising since there were reasons for believing these extracts contained tumor cells. However, in some instances the tumors produced in this manner differed in cell origin from the donor mammary carcinoma (11). More recently it has been found that when tumor tissue from the egg-grown series was injected into mice the resulting tumor was frequently a mixed carcinoma-sarcoma. Since the egg-cultivated tumor has always remained histologically stable, transplants into mice can be made with the same basic material and the induction of malignancy in the normal cells of the stroma studied for long periods and under various conditions.
Instances of sarcomatous transformation of the stroma of mouse transplants have been reported. Recently Ludford and Barlow (4) reviewed the literature and described this phenomenon in a group of mouse carcinomas maintained by mouse transplants.

MATERIALS AND METHODS

Tumor tissue was obtained from egg-cultivated mouse mammary carcinoma. In most experiments the tumor tissue used had been kept continuously in eggs for 30 to 70 transplant-generations. A few experiments were carried out with tumor tissue grown in eggs for one or a few transplant-generations.

The mouse mammary carcinoma used in these experiments occurred spontaneously in breeder stock in 1941 and has since been designated as dba mammary carcinoma 1 (6). During the period of approximately 2 years it was carried in mice, and for the 2 years and 11 months it has been kept continuously in eggs, this tumor has maintained the histological appearance typical of a fast-growing homogenous mammary carcinoma.

The egg series is maintained by the yolk sac method essentially as previously described (8, 12). More experience with this technic has rendered it a simple and effective means for the continuous production of tumors suitable for egg cultivation. Yolk-sac-grown dba carcinomas average 1 to 2 gm. 13 days after inoculation. Since the blood and stroma are supplied by the chick, the mouse cancer tissue is contiguous to normal cells of chick origin only. This probably accounts for the stability of the egg-grown tumors.

Male and female dba mice 3 to 4 months old were used for carrying the transplants in mice. Inoculation was made subdermally into 2 or 3 mice by hypodermatic injection of a 1:4 saline suspension of tumor tissue. Transplants grown in mice were harvested and re-implanted after a period of 15 to 20 days at which time the tumors averaged 3 to 4 gm. Tumor tissue from each transplant-generation was prepared for histological study. The various sections of tumor tissue were evaluated with regard to the cytological characteristics and extent of any sarcomatous tissue which might be present.

The data presented are based on the results obtained in a series of 186 experiments involving the use of 2,440 mice.

RESULTS

Data are given in Tables I and II and Figs. 1 to 10. Sarcomatous cells were first observed in isolated patches in the stroma of the transplant. Later when the proportion of new tumor to original mammary tumor had increased, the separate groups became linked together. Histological sections at this stage presented irregular masses of mammary tumor enclosed by narrow strips of sarcomatous tissue (Figs. 2 and 9).

TABLE I: IMPLANTS IN MICE OF EGG-GROWN MAMMARY CARCINOMA. PER CENT OF TRANSPLANTS CONTAINING AREAS OF SARCOMATOUS TISSUE OR COMPLETE REPLACEMENT OF THE ORIGINAL TUMOR FOR EACH TRANSPLANT-GENERATION

| Transplant-generation in mice | No. of exper. | No. of mice | No. of areas of sarcoma | Per cent exper. showing areas of sarcoma
|-----------------------------|-------------|-------------|------------------------|----------------------------------------
| 1                          | 186         | 547         | 71                     | 38.1                                   |
| 2                          | 186         | 366         | 124                    | 66.7                                   |
| 3                          | 180         | 362         | 125                    | 69.4                                   |
| 4                          | 178         | 361         | 125                    | 70.2                                   |
| 5                          | 145         | 202         | 109                    | 75.2                                   |
| 6                          | 104         | 212         | 77                     | 74.0                                   |
| 7                          | 62          | 128         | 36                     | 58.1                                   |
| 8                          | 42          | 88          | 27                     | 64.3                                   |
| 9                          | 23          | 50          | 13                     | 56.5                                   |
| 10                         | 15          | 34          | 10                     | 66.7                                   |

The mammary tumor used in these experiments has a very limited stroma, and there was no observable tendency for stromal hyperplasia to develop prior to the appearance of sarcomatous tissue.

Successive generations of mouse transplants tended toward increase in the amount of new tumor present. In some instances, however, after more than 30 per cent

TABLE II: LIST OF EGG-GROWN MAMMARY CARCINOMAS COMPLETELY REPLACED BY SARCOMAS

<table>
<thead>
<tr>
<th>Exper. no.</th>
<th>Total no.</th>
<th>Transplant-</th>
<th>Transplant-</th>
<th>Description of sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>transplant-</td>
<td>generation-</td>
<td>generation-</td>
<td>Round-cell sarcoma with nuclear debris</td>
</tr>
<tr>
<td></td>
<td>generations</td>
<td>showing areas</td>
<td>showing complete</td>
<td>(Not classified) with nuclear debris</td>
</tr>
<tr>
<td></td>
<td>in mice</td>
<td>of sarcoma</td>
<td>replacement by sarcoma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>Spindle-cell sarcoma</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>1</td>
<td>2</td>
<td>Spindle-cell sarcoma</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>Spindle-cell sarcoma with nuclear debris</td>
</tr>
<tr>
<td>43</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>(Not classified) with nuclear debris, giant cells, mitotic aberrations</td>
</tr>
<tr>
<td>46</td>
<td>9</td>
<td>2</td>
<td>8</td>
<td>Spindle-cell sarcoma</td>
</tr>
<tr>
<td>58</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>Spindle-cell sarcoma</td>
</tr>
<tr>
<td>66</td>
<td>13</td>
<td>7</td>
<td>9</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td>67</td>
<td>11</td>
<td>3</td>
<td>5</td>
<td>Unclassified sarcoma</td>
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<td>1</td>
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<tr>
<td>115</td>
<td>15</td>
<td>2</td>
<td>13</td>
<td>Unclassified sarcoma</td>
</tr>
</tbody>
</table>
of the tumor was of the sarcoma type, it reverted to the original mammary carcinoma in subsequent transplants. The ultimate status of the new tumor in relation to the original carcinoma could be evaluated to some extent by its relative rate of cell division. When the newly induced tumor had a relatively high rate of division, it quickly replaced the original mammary tumor. In experiments 1 and 2, the second mouse transplant-generation was made up entirely of new tumor (Fig. 5). This tumor has been carried through more than 45 generations of transplants and has retained the characteristics it had in its first appearance. It is an exceedingly fast-growing tumor, hence its quick replacement of the original mammary tumor.

When the growth rate of the new tumor was relatively low, sections of transplants showed a mixed tumor through many months of transplant propagation. In many experiments, however, the new tumor presented the appearance of a fast-growing tissue in which no unusual cell aberrations appeared. Giant cells and nuclear debris were not present in the host mammary tumor. (Fig. 7).

Centrifugation of suspensions of mammary tumor tissue which contained areas of sarcomatous tissue resulted in two layers—the upper layer contained the sarcomatous cells.

No effort has been made to make more than a very
FIG. 2.—Mammary carcinoma transplant. Dark masses of cells in upper and lower portions are mammary carcinoma cells. Middle lighter portion is stromal tissue which is undergoing marked mitotic activity. Mag. X 960.

general classification of the new tumors obtained. In Table II, where the diagnosis indicates spindle-cell sarcoma, the sections indicate several subdivisions in type. The same is true for the round-cell sarcomas with the exception of the lymphosarcoma which is a specific type. This tumor appeared to develop in the right inguinal lymph node which was enveloped by the transplant.

The new tumors tended to be more malignant than the host tissue on the basis of rate of growth of transplants and the time required to kill the mouse bearing the tumor. Transplants of all these new tumors grew
readily and no further change in histology was noted even after as much as 45 transplant-generations (Fig. 5).

As Table I shows, the induction of malignancy in normal cells of the mouse by implants of egg-cultivated tumor could be demonstrated quite regularly. However, as the data given in Fig. 1 discloses, there were periods when the inducing effect was much intensified and other times when it became relatively mild.

DISCUSSION

The results show that induction of malignancy in normal contiguous cells was a frequent occurrence when
egg-cultured mouse mammary carcinoma was implanted back into mice. Sarcomatous tissue could be observed in 38 per cent of the first mouse transplant-generations with an increasing tendency toward stromal malignancy in succeeding transplants.

The egg-grown tumor tissue does not come into contact with normal cells of mouse origin so that under these conditions the mouse mammary carcinoma retains indefinitely the histology it possessed at the time the continuous egg culture was initiated. Accordingly a relatively pure line of mammary carcinoma is available for repeated implants into mice.

Previous reports of mouse mammary carcinomas which showed the development of sarcomatous tissue...
after a varying number of transplant-generations were based on a study of a few individual tumors. Each carcinoma behaving in this manner could be the subject of only one experiment in this regard, since, when transformation to a sarcoma was complete, the original tumor was lost. The objection can be made that the sarcomatous tissue was present from the beginning and became evident as a result of changes in the relative growth rates of the two types of tumor tissue. Such an explanation cannot be used to explain the present results. The egg-grown tumor when implanted in mice was associated with the development of sarcomas of different
cell origins. It is unlikely that several types of sarcomatous tissue could have been present in the original mammary tumor and escaped observation during a period of nearly 5 years in which time hundreds of sections of this tumor were given careful study. Further, in some instances, no sarcomatous tissue was observed in the mammary tumor after several transplant-generations in mice.

It can be objected that these malignant growths are the original mammary carcinoma which has undergone...
superficial changes in structure. There are several reasons for discarding this possibility. These can be summarized as follows:

1. The new tumors have a higher growth rate. In some instances 11 to 12 day implants are as large as 15 or 17 day implants of the donor tumor.
2. The histological appearances of the new tumors are of many types. To account for this it would have to be postulated that the mammary carcinoma was capable of assuming many different forms. Also, the histology of each tumor remains constant through numerous generations of transplants which is contrary to what happens in the instance of superficial structure changes.
3. The tumors are observed to originate in the stroma in most instances.
4. The new tumor tissue quite commonly contains giant cells, nuclear debris and mitotic aberrations not observed in the host tissue.
5. Centrifugation of mixed donor and new tumor separates the two types into a lower and upper layer respectively.

The new tumors were at least as malignant as the host tissue. Transplants in mice were 100 per cent effective and the tumor could be maintained for an indefinite period. One tumor (Fig. 5) has been carried by serial transplants in mice for more than 2 years. Many of these tumors also were cultivated successfully in eggs by the yolk sac method. Passage through eggs did not change their histology.

The speed with which this process of tumor induction in the stroma occurs is comparable to the time required for cell-free extracts to induce malignancy in normal cells (11). Implants were allowed to grow in the mouse for about 15 days and this period was sufficient for the establishment of numerous areas of sarcomatous tissue in the first transplant-generation of the source egg material.

On the basis of a large number of experiments with cell-free extracts of materials from tumor-bearing eggs, it was suspected that there was a periodicity in the activity of the tumor agent. This idea has received support in the present investigation. However, it will be necessary to investigate this aspect of the problem much more extensively before coming to a definite conclusion.

The simplest explanation for the results obtained would seem to be the assumption that normal cells of the connective tissue have been infected by the tumor agent as a result of contiguity with cancer cells. Apparently the process is essentially the same as the production of tumors by cell-free extracts of egg-grown tumor tissue. This is especially likely in view of the close similarity between sarcomas produced by implants of fresh egg-grown tumor tissue and those induced by lyophilized extracts of materials from tumor-bearing eggs. In both instances, giant cells, unusual mitotic aberrations and nuclear debris were common (11). The probability that the tumor agent concerned in these experiments is a virus has been considered elsewhere (7). The ability of the agent as obtained from living carcinoma cells to infect and induce malignancy in connective tissue cells may appear to contradict this thesis. The Rous tumor virus, for example, appears to infect only a definite type of tissue (5). However, many viruses do not show this degree of specificity. Also viruses in general are exceedingly prone to transformations which affect their behavior in this regard. It is possible in these experiments that the tumor virus does undergo some change before being capable of infecting the cells of stroma. In many experiments there was no sign of sarcomatous transformations in the connective tissue after many mouse transplant-generations. In those instances, the tumor agent appeared to be incapable of inducing malignancy in the cells of the stroma.

One noteworthy aspect of the data is contained in the diversity of the tumors obtained. Figs. 4, 5, 6, 8 and 10 show various spindle-cell sarcomas, and two unclassified sarcomas which were carried through numerous generations of mouse transplants without showing any deviation from their initial histology. If the tumor virus from one type of carcinoma is capable of producing such definite types of tumors, then the virus concept of cancer causation may not require the postulation of as many different types of tumor agents as has been supposed.

SUMMARY

1. A mammary carcinoma from a dba mouse (dba mammary carcinoma 1) has been cultivated continuously in eggs for 2 years and 11 months, or 90 transplant-generations. During that period, there has been no observable change in the histology of the tumor tissue. The stroma of these egg-grown tumors is chick tissue.
2. Implants of the egg-grown mammary carcinoma into mice resulted in mixed carcinoma-sarcomas in 38 per cent of the first mouse transplants. The new tumor type usually arose as isolated patches in the stroma which in subsequent generations coalesced, dividing and encircling the original carcinoma. The proportion of the transplants containing sarcomatous tissue increased with further transplant-generations in mice. A total of 186 experiments involving the use of 2,440 mice were used in the study.
3. Sarcomas produced in this manner were of different cell origins, varying growth rates and cytological characteristics. Many contained giant cells, nuclear debris, and unusual mitotic aberrations.
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4. When egg-grown tumor is implanted back into the mouse, the stroma of the resulting tumor is composed of mouse tissue. It is concluded that the induction of malignancy in normal cells of the stroma is due to contiguity with the cancer cells of the implant, and consequent infection with the tumor agent or virus. The process is considered to be essentially the same as that involved in the production of malignant tumors by injection into the mouse of cell-free extracts of materials from mouse tumor-bearing eggs.

REFERENCES


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