BREAST CANCER IN MICE

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Many of the advances in medical research, especially in the cancer field, may be attributed directly or indirectly to the favorable animal material available for purposes of experimentation. Widely used at the present time are inbred strains of mice developed by various workers, especially Little and Strong. The most exacting physiological tests—the transplantation of normal and neoplastic tissues—have demonstrated the homogeneity of individuals within inbred lines. By using the same stocks, workers in different laboratories have obtained analogous results. A noteworthy example is the extra-chromosomal theory of breast cancer in mice advanced by the members of the staff of the Jackson Memorial Laboratory in 1933 and confirmed by Korteweg in 1934 (1–2). The same stocks were used in each experiment. Results have been published for seven different crosses, all verifying the original observations.

In the work with high-tumor and low-tumor stocks of mice it has been assumed in the past that the genetic constitution of the stock determined whether or not the individuals would develop spontaneous tumors of various types. That is, a strain had breast tumors because they had inherited a breast tumor susceptibility. Strong (3), applying the pure-line theory of Johannsen, showed that selection within a high-breast-tumor stock has no influence on the tumor incidence in future generations. Within an inbred line it has been observed by Strong (4) and the author that the incidence of mammary gland tumors is usually as high among the progeny of non-tumor mothers as among those from cancerous mothers.

In this report we shall present evidence showing that the breast tumor incidence may be increased or decreased without altering the inherited or genetic constitution of the animals.

Four strains of mice, inbred brother to sister, have been used in these experiments: the high-tumor lines A and Z or C3H, the low-tumor B or C57 Black, and the X or CBA line. In the tabulations only mice which developed mammary carcinoma are designated as cancerous. All others are termed non-cancerous, although many had primary lung cancer, lymphosarcoma, etc. Thus, only breast tumors are considered.

In the control series of experiments the young mice were permitted to nurse their mothers. In the fostered or experimental classes the young were removed from their mothers as soon as they were recorded each day and fostered by females of other stocks. Only one generation was fostered. The progeny from these fostered females were mated to obtain subsequent generations. When animals of the A stock were fostered they are designated here as

1 The investigations on foster-nursing have been supported by grants from the International Cancer Research Foundation and the National Cancer Institute.
A by B\(^q\), etc. In the hybrid classes the maternal stock is mentioned first, as \(ABF_1 = A^q \times B^p\).

The number of A stock breeding females which have been observed is now 1093. In this group 914, or 83.6 per cent, developed breast tumors and 179 died from other causes. The average age at which the mammary gland tumors were observed was 11.1 months. Non-breast-tumor mice died at an average age of twelve months. Breast tumors were observed in 4.9 per cent of 223 A stock virgin females. The average ages for the cancerous and non-cancerous groups were 18.5 months and 19.3 months respectively.

Only 9 A stock females were fostered by X (CBA) stock females, and 3 of these developed breast tumors. Approximately one-third of the females of the A strain which have been fostered by B (C\(_b\), Black) stock females have died. In this group of 86 mice 4, or 4.7 per cent, were cancerous. Combining the figures we have 7 breast tumors in 95 fostered A stock breeding females, or 7.4 per cent. The average breast-tumor age was 10.4 months while the average age at death for the non-tumor mice was 16.3 months.

The progeny of the first generation fostered mice of the A stock have been divided into two groups: (1) those which developed breast cancer; (2) those which died without breast cancer. No account is taken of the stock used for fostering in tabulating the data. Seventy-seven progeny of A by X\(q\) and A by B\(^p\) breast-cancer mothers may be reported. Of this number, 66.2 per cent had breast cancer at an average age of 11.7 months. The non-tumor mice lived to an average age of 15.9 months. First generation fostered females which were non-cancerous have had 286 progeny which have died. Twenty-seven or 9.8 per cent of these have had breast tumors and they were observed in mice living to an average age of 12.2 months; non-cancerous mice died at 16.1 months.

Some data are available for hybrid groups of breeding females (Table II). In the \(ABF_1\) \((A^q \times B^p)\) generation, which totaled 101 animals, all the mice have died. Of this number, 95 per cent developed breast tumors. In the reciprocal \(BAF_1\) generation 59 mice have died and 37 are living. The breast tumor incidence for the animals which have died has been 8 per cent. The average ages for the \(ABF_1\) and \(BAF_1\) generation mice are: tumor ages 11.3 and 11.4 months and non-tumor ages 10.3 and 20.2 months respectively.

Over 300 \(ABF_1\) females have been fostered by B stock females and are from seven to eleven months in age; no tumors have been recorded among these to date. Another small group of 6 mice can be reported. Three have died non-cancerous, one during the seventh month and two during the fourteenth month. The other 3 are living at 21 months. Thus, 5 of the 6 \(ABF_1\) by B\(q\) lived 14 months or more without breast cancer; in the unfostered group of \(ABF_1\) hybrids all the mice which died after living 13 months or longer developed breast cancer.

Thirty-nine breast tumors have been observed among the \(BAF_1\) by A\(q\) hybrids which are from 7 to 11 months in age. In an older group of 31 animals all but 4 living mice have had breast tumors; average age 10.2 months. The incidence for the total is 93 per cent. Thus, the breast tumor incidence of 8 per cent observed in the \(BAF_1\) unfostered group has been increased, by fostering another series of the same generation, to a ratio approaching the
incidence observed in the ABF₁ hybrids. The ABF₂ and BAF₂ generation tumor ratios are 81 per cent and 7 per cent respectively.²

Another group of F₁ breeding females may also be compared. They were obtained by crossing females of the B (C₅7 Black) low-tumor strain to males of the Z (C₃H) high-tumor line. The unfostered breeding BZF₁ hybrid females number 39. Nineteen have died, of which one had breast cancer. Forty-five females were fostered by Z females (BZF₁ by Z₂), of which 39 have died with a tumor incidence of 79 per cent. The percentages of animals living in the fostered and unfostered groups are 13 and 54 per cent respectively, although the youngest mouse in the latter group is two weeks older than the oldest mouse in the fostered class.

**Comparison of Susceptibility to Spontaneous and Transplantable Breast Carcinoma**

It has been demonstrated previously that neoplastic tissue (Little and Strong, 6) and normal tissue, such as spleen (7), obey the same genetic theory of transplantation when the hosts are representatives of inbred stocks of mice or hybrids derived by mating such animals. Strong (8) was the first to demonstrate that when two or more tumors arising in a single host are inoculated simultaneously radically different results are obtained. These observations have been confirmed by others (9, 10).

When a breast tumor which arose spontaneously in a mouse of an inbred strain was inoculated into controlled genetic material, all the susceptibility factors required for progressive growth of that tumor were observed to be contributed by the parental stock. Several tumors which arose in hybrid mice have also been tested. These hybrid mice were obtained in two ways: by mating members of two high-breast-tumor stocks and by mating females of a high-tumor stock to males from a low-tumor stock.

The tumors which arose in the first generation hybrid females obtained by mating two high-breast-tumor stocks of mice have given interesting and perhaps significant results following transplantation. Five tumors have been tested which developed in two ADF₁ females (A¥ X D or dBa¥). Two of the three tumors which arose in one mouse required the same number of factors for susceptibility, but the genetic make-up was not the same (11). The male parental stock contributed, for each tumor, a larger number of the genetic susceptibility factors than did the maternal parent, as shown in Table I. On the other hand, the maternal parental stock had a higher tumor incidence and the tumors were more malignant than observed in the paternal stock.

Four spontaneous breast tumors have been examined by the inoculation method after developing in hybrids following the mating of high-tumor females to low-tumor males (Z or C₅7H¥ X N§). In every case one or more of the transplantation susceptibility factors was contributed by the low-tumor stock male parent.

Similar experiments have been made with splenic tissue, using again the Z and N stocks (7). The grafts of normal tissue were obtained from donors representing both parental stocks and their reciprocal F₁ hybrids. The results following transplantation were similar to those obtained for neoplastic

² The data in the preceding paragraphs are for the most part presented in Tables I and II of an earlier paper (5).
tissue from similar donors. That is, when the tissue was taken from a pure stock individual all the factors necessary for susceptibility to that tissue were derived from that stock; when the tissue was from a first generation hybrid mouse the factors were contributed by the stocks mated to obtain the hybrid animal. There was not an equal number from each parental stock.

The extra-chromosomal or maternal influence has been observed to control, to a large extent, the development of spontaneous mammary gland tumors in the hybrid females of the A × D and the Z × N crosses (12, 13). Thus, what we have been designating as the inherited susceptibility to spontaneous breast cancer is largely determined by the extra-chromosomal or maternal influence, which is probably not genetic. Susceptibility to transplantable breast cancer is genetic in character and is determined and controlled, in the case of tumors which develop in hybrid animals, by factors contributed by both parental stocks. The paternal stock may even contribute more of the transplantation susceptibility factors than the maternal stock.

By fostering the young born to mothers of a high-breast-tumor stock of mice to females of a low-breast-tumor strain, it is possible to lower the breast tumor incidence in the fostered animals and their progeny (14, 15). If the young are permitted to nurse for two or three days before they are fostered they usually develop tumors. Further studies may determine that a much shorter time will produce the same effect.

Although these experiments are far from complete, the results suggest that some of the fostered mice and their progeny behave quite differently from the average female of the control high-tumor strain. Approximately 10 per cent of the young from potential breast-tumor mothers which were fostered developed breast tumors. In general, the breast-tumor incidence among their progeny approached the ratio for the unfostered animals. The progeny from

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**TABLE I: Summary of the Transplantation Susceptibility Factors Obtained by Inoculating Normal and Neoplastic Tissue (7 and 11)**

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of Factors in F₂ Generation</th>
<th>Factors from Maternal Stock</th>
<th>Factors from Paternal Stock</th>
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<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13714 AX</td>
<td>6 or 7</td>
<td>1 or 2</td>
<td>4 or 5</td>
</tr>
<tr>
<td>BX</td>
<td>4 or 5</td>
<td>1</td>
<td>3 or 4</td>
</tr>
<tr>
<td>19308 A</td>
<td>A♀ × D♂</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>3</td>
<td>4 or 5</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>2 or 3</td>
<td>4 to 5</td>
</tr>
<tr>
<td>ZNF₁ 945</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZNF₁ 1035</td>
<td>Z♀ × N♂</td>
<td>8 or 9</td>
<td>4 or 5</td>
</tr>
<tr>
<td>ZNF₁ 1037</td>
<td>5</td>
<td>3</td>
<td>?</td>
</tr>
<tr>
<td>ZNF₂ 883</td>
<td>ZNF₁♀ × ZNF₁♂</td>
<td>5 or 6</td>
<td>3 or 4</td>
</tr>
<tr>
<td>Splenic Tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>10 or 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7 or 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZNF₁</td>
<td>13</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>NZF₁</td>
<td>13</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

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a few of these mice have a much lower incidence, suggesting a difference in the ability to transmit breast tumors on the part of these fostered breast-tumor females. The fostered mice which die without breast tumors have progeny which show a breast tumor incidence of about 10 per cent. If these progeny with breast tumors are studied, it is found that their progeny do not have a high tumor incidence, the rate being no greater than for the entire group descended from non-breast-tumor mothers. A few mice die without breast cancers though they are potentially cancerous as determined by the incidence among their progeny. The age at death for these animals is usually early.

Similarly the incidence of breast tumors for mice of a low-tumor hybrid generation (BAF₁) may be increased to equal that of a high-breast-tumor stock by fostering the young to high-cancer females (A stock). Unfostered breeding females of this generation (nursed by their B stock mothers) have a low tumor incidence. Breeding females of the reciprocal hybrid generation (ABF₁) have a very high incidence when nursed by their A stock mothers but a low incidence when fostered by B stock females. A few observations may indicate that the breast tumor percentage of the B strain may be increased materially by permitting the young to be nursed by A stock mothers.

The unfostered (control) A stock mice and the females of this strain which were fostered to B stock females had the same genetic constitution. The same applies to the AB and BA first-generation mice—hybrids between two inbred strains. Transplantation studies have also demonstrated that the two groups of the respective pure stock and hybrid generations give the same results. The only difference, as far as can be determined, was the source of milk which these mice obtained. Females which were nursed by potentially cancer mothers had a high incidence of breast tumor; females which nursed mothers from a low-tumor strain showed a low incidence. A tentative theory has been advanced to account for these observations, namely that a “breast-cancer-producing influence” is transmitted through the milk of cancerous mothers. The source of milk is more important than the genetic constitution of the individual in the etiology of breast cancer. The nature of the “influence” remains to be determined.

It may be of interest to theorize as to how the so-called “breast-cancer-producing influence” may act, with or without other factors or agents, in the change from normal to neoplastic tissue. Normal as well as neoplastic tissue probably has a definite genetic constitution. The most accurate test for the determination of this constitution is by the process of transplantation from pedigreed donors into hosts of known ancestry. For susceptibility to grafts of splenic tissue a large number of factors is required. Unless the same factors are carried in more than one chromosome, all the chromosomes are not represented in the genetic complex. The number of genetic factors required for susceptibility to splenic tissue is in excess of the number needed for the progressive growth of most mammary tumors. Whether all normal tissues have the same genetic constitution is problematical, as no accurate genetic study has been made for the simultaneous inoculation of multiple grafts from the same donor.

Strong (16) and others (11) have observed that tumors may change or undergo mutation during the process of transplantation. Every tumor which
has undergone mutation requires a smaller number of factors for susceptibility after the change than before. At least one tumor has been observed which, after several changes, became non-specific and grew in all inoculated hosts. With each change the growth of the inoculated tumor was more rapid. The same workers have also noted that when more than one spontaneous tumor from a single individual has been inoculated simultaneously no two have ever proved to have identical genetic constitutions.

It is debatable whether or not there is a relationship between spontaneous and transplantable tumors. If we assume such a correlation and include transplanted normal tissue, we may speculate as to how the latter differs from neoplastic tissue and perhaps how the change occurred.

If we are correct in our assumption that a "breast-cancer-producing influence" is the active principle responsible for the cancerous transformation of normal breast tissue and that this is usually transmitted to the progeny through the milk, a latent period must elapse before the cancerous change ensues. In an inbred strain of mice the observed time of the appearance may vary from four to twenty-two months. As selection within a pure stock is without effect, as shown by Strong (3), it is probable that the concentration of the "influence" has little effect as a time-reducing element. Individual physiological cellular conditions may be of more significance, as only one or a few cells undergo transformation at any given time. In some stocks an irritation factor, such as the bearing of young, is also necessary. In other strains the incidence of breast tumors in virgin females is nearly as high as in the breeding females. In strains requiring the production of young it is plausible that this factor may more or less determine the state of the cell and the time when the "influence" is enabled to initiate the alteration to cancer. The stimulation factor would prepare or determine the condition of the cell for the transformation to cancerous tissue; the "breast-cancer-producing influence" would alter only those cells having such physiological characteristics.

Any relationship between normal tissue, spontaneous breast cancer, and transplantable breast cancer may be present as determined by the genetic constitution of the grafts of the tissue following transplantation. This may be expressed by the number of dominant factors required for susceptibility. If neoplastic tissue is more embryonic and less complex than adult differentiated tissues, we might expect this difference to be expressed in the requirement, by cancer tissue, of fewer susceptibility factors for continued growth. This has been generally observed in genetic transplantation studies. Thus, it may be that cancer tissue results following the loss of one or more of the factors of specificity. This reduction in the constitution complex may go on in cancerous tissue following continued transplantation until it becomes non-specific. This process has been declared by Strong to be analogous to somatic mutation. As we have preliminary observations (14b) which may indicate that the "breast-cancer-producing influence" is present in normal tissue, it is possible that it is present also in spontaneous breast tumors. If such is the case, any mutational change in the tumor cell following transplantation may result from the "influence" being active in the grafted cells.

In addition to the breast tumors resulting from the action of the "breast-tumor-producing influence," there may be a second group where such an ex-
planation is of little value. Those which occur in a low-tumor line undoubtedly result from other causes. These may be due to changes similar to somatic mutation only, as they are not transmitted to their progeny.

**Literature Cited**
1. **Staff of Jackson Memorial Laboratory**: Science 78: 465, 1933.