Observations on the Genetics of Susceptibility for the Development of Mammary Cancer in Mice*

J. J. Bittner, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

(Received for publication March 30, 1942)

As inbred strains of mice have been under observation for more than 50 generations of brother-to-sister matings, they should be satisfactory for genetic studies on the inherited susceptibility to cancer of the breast. In an effort to determine how this susceptibility might be transmitted, reciprocal matings were made between mice of the high mammary tumor A and the low mammary tumor B (C57 black) strains. Breeding females of the B stock have an incidence of mammary tumors of 0.5 per cent as reported by Little, Murray and Cloudman (9); in the author's line no mammary tumors have been observed in several hun-

dred mice. Only mice of one subline of the A stock, selected for a high incidence of mammary tumors, were used and they had an incidence of 96.8 per cent in a group of 527 mice. They represented mice of the 41 to 61 inbred generations. Preliminary reports have been published (4, 7).

All the hybrids considered in Table I nursed their mothers and were used as breeders. Those produced by mating females of the A strain to males of the B stock were called ABF₁ hybrids; hybrids resulting from the reciprocal cross were termed BAF₁. The

---

Table I: Incidence of Spontaneous Cancer of the Mammary Gland in Breeding Females of the A (High Cancer) and B (Low Cancer) Strains and Their F₁ to F₃ Hybrids.

<table>
<thead>
<tr>
<th>Strain of hybrid</th>
<th>Nursed by</th>
<th>Number</th>
<th>Cancer incidence, per cent</th>
<th>Cancer, months</th>
<th>Noncancer, months</th>
<th>Average age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (F₄₁–F₆₁)</td>
<td>High cancer ♀</td>
<td>527</td>
<td>96.8</td>
<td>9.6</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>C₅₇ black (B)</td>
<td>Low cancer ♀</td>
<td>568</td>
<td>0.5</td>
<td>21.4</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>ABF₁ (A² × B²)</td>
<td>High cancer ♀</td>
<td>141</td>
<td>95.0</td>
<td>11.2</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>ABF₂</td>
<td>High cancer ♀</td>
<td>300</td>
<td>76.3</td>
<td>13.2</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>ABF₃</td>
<td>Cancerous F₂ mothers</td>
<td>285</td>
<td>68.8</td>
<td>12.1</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>Noncancerous F₂ mothers</td>
<td></td>
<td>57</td>
<td>57.9</td>
<td>12.9</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>342</td>
<td>67.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAF₁ (B² × A²)</td>
<td>Low cancer ♀</td>
<td>148</td>
<td>1.4</td>
<td>12.5</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>BAF₂</td>
<td>Low cancer ♀</td>
<td>358</td>
<td>0.8</td>
<td>19.8</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>BAF₃</td>
<td>Low cancer ♀</td>
<td>228</td>
<td>0.9</td>
<td>19.0</td>
<td>21.1</td>
<td></td>
</tr>
</tbody>
</table>

The mice of the ABF₃ generation have been divided according to cancerous and noncancerous mothers of the F₂ generation. Those having cancerous mothers had an incidence of 68.8 per cent and the progeny of noncancerous mothers gave an incidence of 57.9 per cent. This difference was not statistically significant (1.6 × S. E.) as there were only 57 animals in the latter group. The incidence for the entire group of 342 ABF₃ mice was 67.0 per cent.

Preliminary data obtained by mating F₁ and F₂ females to males of the B strain are available. These mice are called backcross animals (BC) and since the cross was made to the B stock, the generations are termed the BBC generations. The prefix ABF₁, BAF₁, etc., designates the type of females used to make the cross.
Many of the mice are still living but most of them have passed the cancer age and it is probable that only a few additional tumors will be observed (Table II). Those which had ABF₁ mothers have given an incidence of 53.3 per cent. The progeny of noncancerous ABF₂ mothers have had an incidence of 5 per cent; those with cancerous mothers an incidence of 47.4 per cent. The incidence of mammary tumors recorded in the BA F₁-BBC and BA F₂-BBC generations was 0.5 and 1.5 per cent respectively for a total progeny are included in the tabulation by litters and the number of mice in some groups may differ from the figures given in Table I.

To determine further if the age at which the mothers developed tumors might have any effect on the incidence of mammary tumors in their progeny, the F₂ and F₃ data were arranged as presented in Table III and Fig. 2. The 962 mice include hybrids of the AB F₁, AB F₂, BA F₁, and BA F₂ generations nursed by females of the A stock. The BA groups of 496 mice. The living mice are from 17 to 28 months of age.

To obtain mice of the hybrid generations it is possible either to use a large number of mothers and to mate a small number of progeny from each female or to use a small number of mothers and secure as many young as possible from each animal. The latter method was used in this work as it gives a greater opportunity to study the makeup of the individual parents. From 15 to 20 mothers were used in each group and the average number of female progeny observed from each was 18.

The incidence of tumors in the hybrids was greater for mice born in the 3rd and succeeding litters than for members of the 1st and 2nd litters. The data are represented graphically in Fig. 1 for the AB F₁, AB F₂, and AB F₃ generations. Only mice having 8 or more are not given in Table I as they were not used to make BBC hybrids, but they have been reported elsewhere (7).

The mice whose mothers developed tumors when they were from 200 to 300 days of age had a higher incidence of mammary tumors than did the young of mothers which developed tumors at later ages. These differences were not significant. In every group, however, the incidence was higher in the mice born in the 3rd and following litters than for those of previous litters, and in every group the difference was significant (Table III).

To ascertain if fathers of the C57 black strain might exert some influence, the mice of the fostered BA F₁ generation were considered. They had mothers of the B stock, fathers from the A stock, and were nursed by females of the A strain. They are tabulated accord-
ing to the litters in which they were born to these B
stock females and to the litters thrown by females of
the A stock before they were used as foster mothers
(Table IV).

The mice nursed by females of the A strain follow-
ing their first litters gave an incidence of 78.6 per
female and BAFa by A female generations were tabu-
lated (Table V and Fig. 3). The former group was
descended from matings between A female and B
male and the latter resulted from the cross between
B female and A male. The mice of each group were
descended from 1st generation animals which nursed

### Table III: Incidence of Mammary Cancer in ABF, BAF, BAFa, and ABF, Hybrids Nursed by Females of the A Strain, Tabulated by Litters According to the Age at Which the Mothers Developed Tumors, or by Noncancerous F, Mothers

<table>
<thead>
<tr>
<th>Cancer age of mothers, days</th>
<th>1-2 litters</th>
<th>3+ litters</th>
<th>Total</th>
<th>Difference in incidence between 1-2 and 3+ litters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>200-300</td>
<td>74</td>
<td>130</td>
<td>100</td>
<td>18.7% or 3.2 X S.E.</td>
</tr>
<tr>
<td>300-400</td>
<td>115</td>
<td>236</td>
<td>236</td>
<td>14.6% or 2.8 X S.E.</td>
</tr>
<tr>
<td>400+</td>
<td>92</td>
<td>199</td>
<td>199</td>
<td>16.5% or 3.0 X S.E.</td>
</tr>
<tr>
<td>Total</td>
<td>281</td>
<td>574</td>
<td>574</td>
<td>16.0% or 5.0 X S.E.</td>
</tr>
</tbody>
</table>

Cancerous F, mothers
- making F, hybrids: 154, 333, 81.1%
- making F1 hybrids: 43, 25.6, 64, 60.9%
- Total: 324, 58.3, 638, 77.4%

Noncancerous F, mothers
- making F, hybrids: 43, 25.6, 64, 60.9%
- Total: 324, 58.3, 638, 77.4%

### Table IV: Incidence of Mammary Cancer in BAF, Male by A Female Hybrids, Tabulated According to Litters Born to Their Mothers (B Stock) and Foster Mothers (A Stock)

<table>
<thead>
<tr>
<th>BAF, by A male</th>
<th>1st litter</th>
<th>2nd litter</th>
<th>3+ litter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born to B female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1, 27</td>
<td>23</td>
<td>22</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>5, 80</td>
<td>15</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>3+</td>
<td>10, 80</td>
<td>20</td>
<td>24</td>
<td>54</td>
</tr>
<tr>
<td>Total</td>
<td>42, 78.6</td>
<td>58</td>
<td>49</td>
<td>149</td>
</tr>
</tbody>
</table>

### Table V: Incidence of Mammary Cancer by Litters, in Mice of the ABF, Male by A Female and BAF, Male by A Female Generations. Only the Progeny of Mothers Having 8 or More Young Are Tabulated

<table>
<thead>
<tr>
<th>ABF, by A female</th>
<th>Number</th>
<th>Cancer, per cent</th>
<th>Number</th>
<th>Cancer, per cent</th>
<th>Number</th>
<th>Cancer, per cent</th>
<th>Difference in incidence between 1-2 and 3+ litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancerous and noncancerous</td>
<td>84</td>
<td>51.2</td>
<td>238</td>
<td>73.5</td>
<td>322</td>
<td>67.7</td>
<td>22.5% or 3.8 X S.E.</td>
</tr>
<tr>
<td>BAF, by A female</td>
<td>116</td>
<td>46.6</td>
<td>126</td>
<td>78.4</td>
<td>278</td>
<td>65.1</td>
<td>31.8% or 5.5 X S.E.</td>
</tr>
<tr>
<td>Total F, Cancerous</td>
<td>157</td>
<td>54.8</td>
<td>336</td>
<td>78.3</td>
<td>493</td>
<td>70.8</td>
<td>35.3% or 3.6 X S.E.</td>
</tr>
<tr>
<td>Total F, Noncancerous</td>
<td>43</td>
<td>25.6</td>
<td>64</td>
<td>60.9</td>
<td>167</td>
<td>46.7</td>
<td>23.5% or 6.4 X S.E.</td>
</tr>
<tr>
<td>Total F,</td>
<td>200</td>
<td>48.5</td>
<td>400</td>
<td>75.5</td>
<td>600</td>
<td>66.5</td>
<td>27.0% or 6.6 X S.E.</td>
</tr>
</tbody>
</table>

cent and those nursed by females following their 3rd
litters had an incidence of 95.9 per cent. There was
little variation in the mice born in successive litters
to females of the B stock. The difference in incidences
was 17.3 per cent and may possibly be statistically sig-
ificant (2.0 X S.E.). The incidence for the entire
group (89.7 per cent) was intermediate to those given
above.

For further comparisons the data obtained from
observations made on the mice of the ABF, by A
females of the high mammary cancerous A stock.
The number of mice was 322 and 272 and the inci-
dence of mammary tumors 69.3 and 68.4 per cent
respectively. Also there was no significant difference
in the mice born in different litters.

The difference in incidence of tumors between the
ABF, hybrids born in the 1-2 and 3+ litters was
22.8 per cent; for the BAF, mice the difference was
32.2 per cent. These differences are significant.
DISCUSSION

As stated in previous publications there are intrinsic and extrinsic influences operating in the development of mammary cancer in mice (4). Those which have been recognized are: (a) genetic susceptibility, (b) hormonal stimulation, and (c) an active milk influence obtained while nursing from females of stocks having a high incidence of mammary tumors.

Susceptibility to the development of mammary cancer may be transmitted by males and females of the susceptible strain and is an intrinsic factor. Shimkin and Andervont (10) found that the amount of estrogenic hormones secreted by mice of various strains was not causally related to the formation of mammary cancer but was due to strain differences. This might be considered as resulting from intrinsic causes but others must be considered.

In a strain of mice with a high incidence of mammary tumors in breeding and virgin females (1) the amount of estrogenic hormones needed for the development of these tumors may result from intrinsic factors. In other strains, the A stock for example, the amount produced as the result of intrinsic factors is not sufficient to elicit tumors in more than 5 per cent of the virgin females. If hybrids are produced by mating females of the A stock to males of the C57 black stock, the hybrid females maintained as virgins have the same incidence of mammary tumors as the virgin females of the A stock (3). The increased amount associated with the production of young is necessary before a high incidence of tumors will be observed. In such a stock the extrinsic causes (breeding) of hormonal stimulation would have to supplement the amount produced by intrinsic factors before tumors would be expected. In other strains breeding plays a role in causing the tumors to appear at an earlier age (1).

The influence normally obtained in the milk of females of mammary cancer strains is an extrinsic cause in the production of mammary tumors, as it may be eliminated entirely by foster nursing (5). An active influence may also be supplied by feeding or injecting extracts containing this influence, and the amount thus obtained may determine the incidence of tumors and the age at which they appear.

Few spontaneous mammary tumors occurred in hybrids with mothers from a strain of mice with a low incidence. Mice of 3 hybrid and 2 backcross generations were observed (1,230 mice). These mice would not obtain the active milk influence.

In the reciprocal cross (mothers from a cancerous and fathers from a low cancerous stock) the incidence of tumors recorded in hybrids of the 1st and 2nd generations is in accord with the genetic theory that susceptibility to mammary cancer is inherited as a single dominant factor. Preliminary data secured in the backcross generations to males of the low cancer stock also support this theory.

After mating females of the 2nd hybrid generation to produce mice of the 3rd hybrid generation it was noted that the progeny of cancerous females had a higher incidence than did the progeny of noncancerous mothers. Noncancerous F2 females mated to males of the resistant strain had progeny with a very low incidence of tumors; the incidence of cancer among the progeny of cancerous females was higher, as would be expected according to the theory.

To determine the number of factors involved for susceptibility to mammary cancer, the incidences observed should be compared with theoretical incidences which would be expected for different numbers of factors. The application of statistical methods should then enable one to determine the genetic constitution of the character being studied.

In the present investigation it is impossible to make these comparisons with any degree of accuracy because, in the hybrids, different incidences of tumors were observed in the mice of successive litters. In every group the incidence in the 1st and 2nd litters was less than that for mice born in the following litters. The incidence for the total number in each generation represents a figure intermediate between the incidence as tabulated by litters and may or may not be a true incidence for the entire group unless the mice are equally distributed by litters.

From a genetic standpoint there is no reason to expect that the mice born in successive litters would differ in their susceptibility to the development of carcinoma of the mamma. Also there was no evidence in our hybridization cross that the father exerted any influence, as indicated by Andervont (2).

There is some evidence, however, that the age at
which a female develops a tumor may have some bearing on the incidence of tumors to be expected in her progeny. The younger the mother at the time she develops the tumor, the higher will be the incidence in her daughters. The average age at which the young developed tumors has not been tabulated.

The only explanation we can advance for these findings is that they may be due to different concentrations of the active milk influence in mice of various ages. If we assume that the active influence present in the milk of females of cancerous stocks may be, as has been suggested (6,11), a virus, this influence would have the ability to multiply with the increasing age of the mice. Thus we might expect that mice born in the first litters would obtain a smaller amount than those born in the following litters. The incidence of mammary tumors would be associated with the amount of the influence received.

While the data observed in reciprocal hybrids indicate that susceptibility to mammary tumors may be a single factor, different incidences observed in successive litters of mice and/or the age at which the mothers develop tumors make it impossible to state the exact nature of the susceptibility to spontaneous mammary cancer. That there is an inherited susceptibility has been demonstrated by various workers and has been substantiated in these studies. The observation of different incidences of mammary tumors among the progeny of noncancerous females of the second hybrid generation when mated to males of the same generation or males of the resistant strain is of importance in demonstrating this susceptibility.

Until a few years ago mammary tumors were believed to result from a combination of estrogenic stimulation and genetic susceptibility. After it was demonstrated that an extrachromosomal (milk) influence was involved, the role of inherited susceptibility was said to be of minor consequence. With the use of larger numbers, and as the result of observations on hybrid mice, the significance of the genetic constitution of the host was again recognized. In the meantime, however, a few workers have neglected to mention the function of estrogenic hormones in preparing mammary tissue so the cancerous change may take place.

Most workers are of the opinion that inherited susceptibility for mammary tumors is dominant, but the use of different inbred strains of mice has not resulted in a uniform theory regarding the exact constitution of this susceptibility. This is what we might expect, as it has been noticed many times that the same strain of mice may not show the same incidence of tumors in different laboratories and that the same strain may even show different incidences in the same laboratory at various times. No genetic comparison has been made with the same strains of mice in hybrid crosses.

Using females from a single strain of high mammary cancer mice and males from different strains with a low incidence, Andervont (2) concluded that the fathers exerted some influence on the susceptibility to these tumors and that genetic factors exerted their influence by controlling the degree of susceptibility. The data secured from crosses between the C3H females and males of the I and C57 black stocks would support the theory for a single factor; those obtained with males of the Y (yellow) strain would not. It is of interest that Little (8) observed in a cross between dilute brown and yellow strains that the yellow mice had a significantly lower incidence of mammary tumors than did the nonyellow mice. This may account for the variations observed by Andervont.

Since the mothers in Andervont's experiments were members of the same inbred strain, their contribution to the inherited susceptibility for mammary tumors should have been the same regardless of the fathers. It is possible, however, that the male parents contributed factors which modified susceptibility transmitted by the females of the cancer-susceptible strain.

Another possibility, suggested by the work of Shim-kin and Andervont (10), is that intrinsic factors transmitted by the fathers may have produced different levels of estrogenic secretion in their hybrid female offspring. The hybrids secreting the smallest amount of hormones would be expected to develop tumors later and to have the lowest incidence of mammary tumors. Andervont observed that hybrids with the lower incidences developed tumors at a later age than those with higher incidences.

In general the results reported by Andervont and the data given above agree except for minor details, as might be expected with the use of different stocks in different laboratories.

**CONCLUSIONS**

Data obtained on the incidence of mammary tumors in hybrid mice show that inherited susceptibility is transmitted as a dominant.

The results are in accord with the genetic theory that it is inherited as a single factor.

Mice which develop tumors at an early age have progeny with a higher incidence of mammary tumors than mice born to mothers which develop their tumors at later ages.

The incidence of mammary cancer recorded among the progeny of cancerous females is higher than the incidence for the progeny of noncancerous females of the second hybrid generation.

That the milk influence may be more concentrated
in older mice is suggested by the incidence of tumors when the young are tabulated by litters.

REFERENCES


Observations on the Genetics of Susceptibility for the Development of Mammary Cancer in Mice

J. J. Bittner

Cancer Res 1942;2:540-545.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/2/8/540.citation

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, use this link http://cancerres.aacrjournals.org/content/2/8/540.citation. Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.