The Genetics and Linkage Relationship of the Inherited Susceptibility to Mammary Cancer in Mice*

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(Materials and Methods)

Studies on the etiology of mammary cancer in mice may be divided into two groups: (a) Those made on heterozygous animals, and (b) Those made on inbred strains and their reciprocal hybrids. Haldane (11) stated that exact work is impossible unless homozygous animals are used, because no two individuals might have the same genetic constitution when other types of animals were tested and the results might not be the same in various experiments. Moreover, other conditions must also be controlled in order to obtain reliable data.

While a great amount of our knowledge concerning the problem of cancer may be attributed directly or indirectly to the use of "pure" strains of mice, some strains have diverged into sublines that may differ genetically from one another sufficiently to be considered as distinct strains [Andervont (2), Bittner (7, and unpublished data)]. Thus all experimental and control animals should be representatives of the same line and should be observed in the same laboratory during the same period.

The early studies of Bashford, J. A. Murray, Tyzzer, Haaland, Slye, and Lathrop and Loeb were of importance because they established the fact that some lines or families of mice produced more tumors than did others, and indicated a genetic transmission of the disease; for references see Loeb (22) and Little (13).

By studying the incidence in hybrids Lathrop and Loeb (18-20) could find no explanation that would account for the results in all the reciprocal crosses. In some hybrids the higher rate of the maternal strain predominated; in others the hybrids showed the high incidence characteristic of the paternal line. Although their results suggested that the maternal strain may possibly be of greater importance, they believed that there might be a partial linkage of the factors determining tumor incidence with the sex determinants. Later Loeb (21) and Tureen and Loeb (24) assumed that two sets of factors controlled the tumor rate and the tumor age and that it was possible to dissociate these two sets, especially through hybridization. However, in 1940, Loeb (22) stated that "The so-called 'spontaneous' mammary-gland carcinoma is a cancer produced by the estrogen given off rhythmically in mice hereditarily predisposed to this disease." Of more significance were the observations of Lathrop and Loeb (18-20) on the role of the estrogenic hormones in the genesis of mammary cancer in mice.

Following the discovery of the maternal influence in the etiology of these tumors, various theories were advanced on how it might function and on its importance (12, 15-17), but foster-nursing experiments demonstrated that it is transferred in the milk (4, 9). Following the foster nursing of inbred strains and their reciprocal hybrids, the role of inherited susceptibility was again evident. In one cross the observations were in accord with the genetic theory that the susceptibility for mammary cancer is transmitted as a single dominant factor, but other explanations were possible (5, 6, 8, 10). Andervont (1) suggested "that genetic factors exert their influence in the occurrence of spontaneous mammary cancer in mice by controlling the degree of susceptibility of the animal or the concentration of the extrachromosomal, or milk influence," but that in some crosses the paternal parents introduced factors that influenced the incidence of cancer in the progeny (3).

Thus, from the data now available, three “inciting” influences have been recognized in the production of mammary cancer in mice: the agent transferred in the milk, a dominant inherited susceptibility, and the estrogenic and associated hormones. The possible relationship of these causative influences has been discussed previously (9).

In this report we wish to consider the possible linkage relationship between one of the factors for the inherited susceptibility for spontaneous mammary cancer and one of the genes controlling coat color in mice.

MATERIALS AND METHODS

The two inbred strains used in these experiments were the B (C57 black) and the A stocks.

*These experiments were started at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and were assisted by grants from the National Cancer Institute and The Jane Coffin Childs Memorial Fund for Medical Research.
Less than 1 per cent of the breeding females of the B stock have spontaneous mammary cancer in the end (14). Several sublines of this strain are being carried that trace back to single pairs brought from Ann Arbor, Michigan, to Bar Harbor, Maine, in 1929. None of the breeding females of the B strain serving as controls in this experiment developed mammary tumors (10.)

Approximately 95 per cent of the breeding females of the inbred A strain ultimately have mammary tumors (10). Sublines of the A cancerous strain have not been maintained; the strain has been inbred for more than 70 generations by brother-to-sister matings, mice, all hybrids nursed their maternal parents but are designated by the strain used to nurse the F1 animals (10).

Two groups of backcross generation hybrids are also considered that were produced by mating either ABF1 by A or ABF2 by A females to males of the B stock. These were called BBC ("B" backcross) animals.

To secure the experimental animals of the hybrid generations, only a small number of mothers was used and as many progeny as possible were continued from each one. The mice of the reciprocal generations were studied during the same period. All the females were and over 40 consecutive generations of mammary tumors have been recorded.

In this report only the hybrids that were nursed by females of the cancerous A stock are included. Those that did not receive the mammary tumor milk inciter had a low incidence of tumors, and are of little value in a discussion of the inherited susceptibility for mammary cancer (5, 10).

The hybrids that had maternal parents from the A strain and paternal parents from the B stock, and nursed A females, were called ABF1 by A. The hybrids made by reciprocal matings (B × A) were termed BAF1. Because they were transferred to females of the A stock soon after birth, they are designated as BAF1 by A. The hybrids of the F1 generations were mated inter se to produce the F2 hybrids, etc. With the exception of the BAF1 by A continued as breeders and they were permitted to nurse their progeny.

The animals received Purina fox chow and an unlimited amount of water.

Only carcinoma of the mammary glands is considered in this report. The incidence was determined by the percentage of mice in each group living beyond the age at which the earliest tumor was observed.

All animals of the A stock, F1 and F2 hybrid and F2 backcross generations had cancerous mothers; mice of the F2 hybrid and F2 backcross generations had either cancerous or noncancerous mothers.

**EXPERIMENTAL RESULTS**

The incidence of mammary cancer in mice of the inbred strains and their hybrids is given in Table I.
with the average ages expressed in days. No significant difference was encountered for the incidence in any of the reciprocal groups, which permits the totaling of the mice of the respective generations. Likewise, the variation in the incidence of cancer for the mice of the A stock and the total number of F\(_1\) by A hybrids was not of statistical significance (1.83\(\times\)S.E.). There were, however, differences in the average ages at which the mice of these classes developed cancer.

If there is an inherited susceptibility to mammary cancer in mice, the segregation of this factor (or factors) should be evident in mice of the second hybrid generation. Whereas the incidence in mice of the F\(_2\) generation was 92.3 per cent, that for mice of stock are black. The expected ratios in the F\(_2\) generation would be 9 black : 3 brown : 4 albino mice. The relationship between the coat color of the F\(_2\) by A hybrids and the incidence of mammary cancer is presented in Table I.

Brown mice of the F\(_2\) by A generation were observed to have a higher incidence (88.1 per cent) of cancer than did the albino (76.5 per cent) and black (68.9 per cent) animals. Comparisons showed that the variations between the browns and mice of other coat colors were probably significant.

The mice of the ABF\(_2\) by A—BBC generation had mothers with various coat colors (all BBC animals were black). The offspring of brown cancerous mothers had a higher incidence (68.2 per cent) than

<table>
<thead>
<tr>
<th>TABLE II: INCIDENCE OF MAMMARY CANCER IN THE F(_2) BY A GROUP WHEN MICE ARE TABULATED BY COAT COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total F(_2) by A: No.</td>
</tr>
<tr>
<td>Brown 84</td>
</tr>
<tr>
<td>Black 209</td>
</tr>
<tr>
<td>Albino 98</td>
</tr>
<tr>
<td>Total 391</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>TABLE III: INCIDENCE OF MAMMARY CANCER IN THE ABF(_2) BY A—BBC (ABF(<em>2) by A(^{2})X B(</em>{5})(^{2})) HYBRIDS ACCORDING TO COAT COLOR AND WHETHER OR NOT THE ABF(_2) BY A MOTHERS DIED OF CANCER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABF(_2) by A mothers</td>
</tr>
<tr>
<td>Brown 110</td>
</tr>
<tr>
<td>Black 57</td>
</tr>
<tr>
<td>Albino 56</td>
</tr>
<tr>
<td>Total 223</td>
</tr>
</tbody>
</table>

Differences in incidence

Br. and Blk. Mothers: 33.1% ± 7.0 or 4.8 \(\times\) S.E.
Br. and Al. Mothers: 23.5% ± 7.4 or 3.2 \(\times\) S.E.
Al. and Blk. Mothers: 9.7% ± 7.5 or 1.3 \(\times\) S.E.

the F\(_2\) generation was 74.9 per cent, which would be in accord with the genetic theory that the inherited susceptibility might be transmitted as a single dominant factor.

The total F\(_2\) by A population had an incidence of 66.5 per cent; the incidence for mice with cancerous mothers was 70.9 per cent, whereas those with noncancerous mothers had an incidence of 45.9 per cent (5.02 \(\times\) S.E.).

Hybrids of the ABF\(_1\) backcross generation had an incidence of 60.1 per cent, and mice of the ABF\(_2\) backcross generation an incidence of 50.5 per cent. Cancerous and noncancerous females were used to produce the ABF\(_2\)-BBC hybrids. Those with cancerous mothers had an incidence of 62.8 per cent, while the progeny of noncancerous mothers had an incidence of 5.0 per cent.

Mice of the A strain are albino in coat color and carry the brown factor, while the animals of the B did those having black (59.6 per cent) or albino (55.4 per cent) cancerous mothers. With the number of mice studied, these differences were not significant. All the F\(_2\) by A brown mice used to produce hybrids of the ABF\(_2\) by A—BBC generation died cancerous, but some albino and black F\(_2\) by A mothers died noncancerous. When their progeny were grouped with those of cancerous females with like coat colors, significant differences were found (Table III) between the progeny of the brown and black mothers (4.77 \(\times\) S.E.), and the brown and albino mothers (3.19 \(\times\) S.E.), but not for the black and albino mothers (1.30 \(\times\) S.E.).

An analysis of the F\(_3\) by A data from the standpoint of linkage was made difficult because in certain matings it was not possible to determine what factors for coat color were transmitted by the parents of the second hybrid generation. Many of the matings were between albino animals, and these parents might
have carried black and/or brown. However, mice born of brown parents had an incidence of 77.5 per cent, while other mice with cancerous mothers had an incidence of 70.3 per cent.

Of interest were the results obtained from mating 5 F₁ by A females to the F₂ by A black male, No. 65280 (Table IV). Three of the females (2 brown and 1 black) died cancerous and their progeny showed a high incidence of cancer for mice of the F₂ generation. One brown noncancerous female had progeny with an incidence of 13 per cent, while the progeny of a noncancerous black female had an incidence of 32 per cent. If the progeny born to the noncancerous brown female are not included, the incidence for the other progeny of the black male, No. 65289, was: 33 blacks, 48.5 per cent; 13 browns, 76.9 per cent; and 15 albinos, 66.7 per cent. Again we note a higher incidence among the brown mice, but the differences are not significant because of the small numbers.

From the incidence of mammary cancer observed in the various hybrid generations in this study, a single dominant factor could reasonably explain the transmission of the inherited susceptibility. In most groups, either the incidence was in accord with this hypothesis or more tumors were recorded than would be expected. If, however, the incidence was considered according to the coat color of the mice of the F₂ by A generation, the theory for a single factor would not apply, as more brown mice had tumors than would be expected with independent assortment, but the incidence for the brown mice was lower than would be expected for linkage. Thus other theories must be considered to explain the results.

It will be recalled that 18.4 per cent of the mice of the C57 black strain with the active milk agent died cancerous but none of the mice of this stock that did not have this agent developed tumors. If animals of this strain (mice of several sublines were probably included in our data) were not susceptible to mammary cancer, these mice would be genetically low cancerous but somatically cancerous. If this was the case, it would also be assumed that some of the nonsusceptible mice of the hybrid generations also died cancerous. The observed incidence for cancer would then include not only animals that had the inherited susceptibility but also those that developed tumors because of other inciting influences. These findings would tend to show that the inherited susceptibility to mammary cancer might be transmitted by multiple factors. Also corroborating this view was the fact that some brown females of the F₂ by A generation died noncancerous and apparently did not transmit the inherited susceptibility to their progeny. However, the ratios based on two dominant factors and corrected on this basis (18.4 per cent of the nonsusceptible mice developing cancer) were lower than the observed data.

When two dominant factors are transmitted, a certain number of the hybrids would carry one of the factors, either in the homozygous or heterozygous condition, but have the recessive alleles of the other. If we assume that the mice of the C57 black stock did not have any of the susceptibility factors, it might be

<table>
<thead>
<tr>
<th>BAF₂ by A Females</th>
<th>Total progeny</th>
<th>Black</th>
<th>Brown</th>
<th>Albino</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% Ca.</td>
<td>No.</td>
<td>% Ca.</td>
</tr>
<tr>
<td>63355 Brown</td>
<td>+ 379</td>
<td>16</td>
<td>81</td>
<td>5</td>
</tr>
<tr>
<td>63503 Brown</td>
<td>+ 474</td>
<td>10</td>
<td>70</td>
<td>6</td>
</tr>
<tr>
<td>63504 Albino</td>
<td>+ 358</td>
<td>10</td>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>63354 Brown</td>
<td>— 780</td>
<td>15</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>63356 Black</td>
<td>— 423</td>
<td>25</td>
<td>32</td>
<td>19</td>
</tr>
</tbody>
</table>

Females mated to BAF₂ by A black male, No. 65280

DISCUSSION

An analysis of the genetic susceptibility for spontaneous mammary cancer in these experimental data, obtained by crossing mice of a cancerous and a low-cancer stock, was made difficult because:

A. As previously reported (10), 18.4 per cent of the mice of the low cancer strain developed mammary cancer following foster nursing by females of the same cancerous strain. This would indicate that if the mice of the resistant stock were genetically nonsusceptible, the inciting influences of the milk agent and the hormones were sufficient to overcome the threshold of nonsusceptibility in some animals and produce tumors. It would also follow that some of the cancerous hybrids were probably genetically low cancerous.

B. The incidence of spontaneous cancer varied in many groups with the age at which the mothers became cancerous. Some of the differences encountered, for instance in the F₂ by A hybrids, were of statistical significance.

C. Many of the hybrids born in the third and following litters showed higher incidences than did the mice of the same groups born in the first and second litters.

From the incidence of mammary cancer observed in the various hybrid generations in this study, a single dominant factor could reasonably explain the transmission of the inherited susceptibility. In most groups, either the incidence was in accord with this hypothesis or more tumors were recorded than would be expected. If, however, the incidence was considered according to the coat color of the mice of the F₂ by A generation, the theory for a single factor would not apply, as more brown mice had tumors than would be expected with independent assortment, but the incidence for the brown mice was lower than would be expected for linkage. Thus other theories must be considered to explain the results.

It will be recalled that 18.4 per cent of the mice of the C57 black strain with the active milk agent died cancerous but none of the mice of this stock that did not have this agent developed tumors. If animals of this strain (mice of several sublines were probably included in our data) were not susceptible to mammary cancer, these mice would be genetically low cancerous but somatically cancerous. If this was the case, it would also be assumed that some of the nonsusceptible mice of the hybrid generations also died cancerous. The observed incidence for cancer would then include not only animals that had the inherited susceptibility but also those that developed tumors because of other inciting influences. These findings would tend to show that the inherited susceptibility to mammary cancer might be transmitted by multiple factors. Also corroborating this view was the fact that some brown females of the F₂ by A generation died noncancerous and apparently did not transmit the inherited susceptibility to their progeny. However, the ratios based on two dominant factors and corrected on this basis (18.4 per cent of the nonsusceptible mice developing cancer) were lower than the observed data.

When two dominant factors are transmitted, a certain number of the hybrids would carry one of the factors, either in the homozygous or heterozygous condition, but have the recessive alleles of the other. If we assume that the mice of the C57 black stock did not have any of the susceptibility factors, it might be
possible that the hybrids with one of the susceptibility factors would have a higher incidence than the fostered B mice. This figure would also have to take into consideration the difference in the incidence for mice born to mothers that developed tumors at various ages and for those born in succeeding litters.

With these assumptions, it would be possible to explain the data on the basis that the inherited susceptibility for mammary cancer in mice is transmitted by multiple factors, one of which is linked with the brown gene.

Before the number of factors can be determined it will be necessary to evaluate further the roles of extrinsic and metabolic influences in the development of mammary cancer in mice. These may be determined in part by the genetic constitution of the individual. That is, if multiple factors are transmitted, the mice without any of these factors might be expected to have a greater “threshold” to overcome, by the action of the milk agent and the estrogenic hormones, than would animals that had one or more of the susceptibility factors. Other genetic factors may operate in the development of mammary cancer that are not associated with the inherited susceptibility.

Although most mammary tumors in mice result from the combined inciting influences of the inherited susceptibility, the estrogenic hormones, and the milk agent, they do develop in some animals that have not all these mammary tumor inciters. Other influences may be involved that have not been recognized. Because of these facts it has not been possible, in these experiments, to determine definitely the number of genetic factors characteristic of the inherited susceptibility to mammary cancer.

CONCLUSIONS

Reciprocal F1 hybrids produced by mating mice of one low cancer (C57 black) and one high cancer (A) strain and nursed by females of the cancerous stock gave, in breeding females, approximately the same incidence of spontaneous mammary cancer.

No evidence was secured to suggest any intrauterine influence for the development of mammary cancer in mice.

The incidence in the total number of F1 hybrids nursed by females of the A stock did not differ significantly from the incidence secured in mice of the cancerous A stock.

Although the pooled data obtained in all the hybrid generations could be accounted for on the genetic theory that the inherited susceptibility for mammary cancer in this cross was transmitted as a single dominant factor, it becomes evident on analysis of the incidence of cancer in the several subgroups that such a simple interpretation is inadequate and that other factors are probably involved. Detailed analysis shows:

1. That the incidence of mammary carcinoma in the F2 hybrids with brown coat color was significantly higher than in their litter mates with black or albino coats.
2. That not all brown mice became cancerous, nor was the incidence in brown mice as high as in mice of the high cancerous A stock.
3. The progeny of brown mothers had a higher incidence than did the progeny of black or albino mothers, but not all brown mothers transmitted the inherited susceptibility to their progeny.
4. The incidence of cancer was significantly greater, in some groups, in the mice born in the third and later litters than in mice born in the earlier litters from the same mothers.
5. The incidence in the progeny was influenced also by the age at which the mothers developed mammary cancer.

It has previously been shown that a considerable proportion of mice of the so-called nonsusceptible stock developed mammary cancer when given the active milk agent. Therefore even mice of these strains are not totally resistant, and either they must possess certain factors conducive to mammary carcinoma production, or the tumors must result from extrinsic and/or metabolic inciting influences. Obviously the mice of low cancer strains do not have all the inciting factors that are present in mice of the high cancer strains.

If some mice of a genetically nonsusceptible strain develop mammary cancer, it is probable that some cancerous hybrid animals are likewise nonsusceptible. Thus the incidence would not represent the true percentage of susceptible animals, and should not serve as the only basis for a genetic interpretation of the data.

It is concluded that the inherited susceptibility for spontaneous mammary cancer in mice, as transmitted by mice of the cancerous A stock, probably depends upon multiple genetic factors, one of which may be linked with the gene for brown coat color.

REFERENCES

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