INCREASE IN MAMMARY CARCINOMA INCIDENCE FOLLOWING INOCULATIONS OF WHOLE BLOOD*

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This is a report of an investigation of the possible role of blood in influencing the incidence of mammary carcinoma in mice. It is part of some work started in 1941 to try to open up new leads in and to further the study of a non-mendelian (though transferable from generation to generation maternally) influence affecting the incidence of mammary carcinoma.

In this particular experiment we have been able to conclude observations on the first group of mice. The last animals have just died at 736 days of age. Since the earlier tabulations the results have become increasingly significant. The probability that the results are due to chance alone shows odds of 1 to 10,390, that a random sample would give as great or greater deviation.

The recipient and control mice were of the second inbred generation following the foster nursing of inbred "high tumor" Jackson Laboratory C3H mice on "low tumor" inbred C57 black mice.

\[
\begin{align*}
\text{C3H Strain with High Percentage of Mammary Carcinoma} & \quad \text{Inoculated with} \\
\text{Foster nursed on "low tumor" C57 black strain} & \quad \text{"high tumor" C3H blood} \\
\text{C3H Strain with Low Percentage of Mammary Carcinoma} & \\
\end{align*}
\]

**Figure 1**

Plan of experiment showing method of securing "low tumor" C3H strain and the setup for attempting to return it to "high tumor" strain.

At weaning time a male and four litter-mate females were placed in each compartment of a mouse box. Breeding was allowed to proceed normally and the young of each litter were removed by the time they were four weeks of age. Two females of each group of four litter-mates were injected subcutaneously with 0.5 cc of whole blood diluted with an equal part of distilled water. The recipients were 1 to 3 months of age. Normal males and females of the Jackson Laboratory C3H high tumor strain were used as donors. Most of the donors were young, non-lactating females, a few were breeding females and the balance were young males. The donors were killed with massive doses of nembutal and the blood secured from the thoracic cavity with a syringe, diluted with warm distilled water and injected
as quickly as possible. The blood of each animal was used individually and not pooled. Following injection into subcutaneous tissue of the back, the blood was spread under the skin with slight pressure. There were 118 mice in the experimental group and 111 in the control group at the time that the first tumor appeared. Animals dying before this age are not included.

**Results.**—In the control group of 111 females, 11 carcinomas of the mammary glands have appeared at an average age of 351.3 days; 100 mice have died tumor free at an average age of 508.7 days.

In the experimental group of 118 mice, 36 had mammary carcinoma at an average age of 354.0 days; 82 died tumor free at an average age of 460.5 days.

All of the tumors were examined histologically and were found to be adenocarcinomas. There were several mammary gland infections which might grossly have been mistaken for mammary gland tumors. These appeared in both the control and the experimental groups.

There were more than three times as many tumors in the experimental as in the control group. The percentage difference between the two groups was 20.6 with a standard error of the difference of 5.34. An analysis of the difference gives the difference over the standard error of 3.9. The *P* value was 0.000096. The odds are only 1 to 10,390 that a random sample would give as great or greater deviation. From analysis of the data it was ascertained that there was no litter-mate correlation and thus it was justifiable to use the whole number for comparison.

<table>
<thead>
<tr>
<th>MICE</th>
<th>NO. OF ANIMALS</th>
<th>NO. OF TUMORS</th>
<th>% OF TUMORS</th>
<th>AVERAGE AGE AT APPEARANCE OF TUMOR</th>
<th>NO. DYING, TUMOR FREE</th>
<th>AGE DYING, TUMOR FREE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>118</td>
<td>36</td>
<td>30.51</td>
<td>354.0</td>
<td>82</td>
<td>460.5</td>
</tr>
<tr>
<td>Control</td>
<td>111</td>
<td>11</td>
<td>9.91</td>
<td>351.3</td>
<td>100</td>
<td>508.7</td>
</tr>
</tbody>
</table>

20.60 ± 5.34  \( D/\sigma = 3.9 \quad P = 0.000096 \)

**Discussion.**—In testing for the significance the question arises whether both groups should be considered from the same date although the first tumor appeared a little later in one group than in the other. The first tumor in the control group appeared at 204 days and in the experimental group at 144 days. The data were examined by starting the control and experimental tabulations at the time of the earliest tumor of either group, that is, at 144 days, and again by starting each tabulation when the first tumor of that group appeared. The results remained significant with either method of calculation.

It is evident that the factor need not come only from breeding or from lactating females, as is shown from the age and sex of the animals used as donors. About four-fifths of the experimental mice received blood from males or immature females. Blood from the immature, non-lactating females, as well as from males, increased the incidence of breast carcinoma.
The blood injection did not return the incidence of mammary carcinoma to the high level of tumor incidence occurring in the Jackson Laboratory C3H strain itself. Instead of the 30% tumor incidence there should have been an incidence of 80% or more had this been so. It might be useful to hypothesize that that age of donor or recipient had something to do with this result. Another possibility is that the amount of blood injected was not sufficient to return the animals to the former high level of incidence. In line with this idea, Bittner, using fostered A strain mice, found that with the feeding of 0.9 cc. of milk 3 out of 10 developed mammary tumors, while with 1.7 cc. of milk, 8 out of 10 developed tumors. If the blood contained the influence in a quantity approximately equal to that in the milk, the 0.5 cc. of whole blood would not be expected to return the tumor incidence to the normal high level. There is some further evidence for interpretation of the data on a quantitative basis. Andervont has shown that high tumor young, suckling their own mothers for 17 hours or less, have a significantly lower mammary tumor incidence following foster nursing by low tumor females than young which have suckled for 24 to 48 hours. Twenty-eight mice suckling less than 17 hours had a tumor incidence of 25% while 23 sucking 24 to 48 hours had an incidence of 69.6%.

Other Tumors.—The second most frequent tumor to appear has been lymphoid leukemia which has now appeared in 25 animals. Fifteen of these were in the control group and at an average age of 585.4 days. Ten were in the treated group at an average age of 543.5 days. The general incidence, 10.1%, is a higher percentage of lymphatic tumors than has usually been recorded for the C3H strain. Appearing at the average age of 568.6 days it is evident that normally many animals which might have produced them were probably eliminated by the earlier appearing mammary gland tumors. Their appearance seems to have no relation to this experimental procedure except the fact that in general the mammary gland tumor incidence was fairly low thus leaving more than a normal proportion of animals to reach the upper age groups.

Summary.—Jackson Laboratory C3H mice which have had their tumor incidence lowered by foster nursing were injected at 1–3 months of age with 0.5 cc. of whole blood from normal high tumor C3H lactating females, young females and males. Significant differences are present between the inoculated mice and their litter-mate controls.

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