RELATION OF NURSING TO THE EXTRA-CHROMOSOMAL THEORY OF BREAST CANCER IN MICE

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Since it has been observed that some maternal "influence" is of more importance than any paternal contribution as a determining factor of breast cancer in mice, various experiments have been made to determine, if possible, some explanation for this difference in parental effect. The effect of nursing as a possible cause has been considered in previous publications (1-5). As much time is required for an exhaustive study along this line, this paper, like the earlier ones, must be considered as a preliminary report. It records observations on hybrid mice which have not been considered before.

The A inbred high-cancer line has served as the control stock in most of our experiments. The new-born young of A stock mothers were removed and fostered by females of the low-tumor C57 Black (B) or CBA lines. As this change was made the first thing each morning, the young had the opportunity of nursing their A stock mothers from the time of birth until the transfer was effected. Only breeding females have been included in these studies, and the animals are tabulated as breast-cancerous or non-breast-cancerous. Mice with other types of growths, usually primary lung cancer, are placed in the latter group.

Only one generation of A stock mice was fostered by low-tumor stock females. The females fostered were all used as breeders and many had progeny which were mated to obtain young for subsequent generations. The progeny of the fostered females which developed breast cancer are tabulated separately, for comparative purposes, from the young of non-cancerous fostered mice. Due to the limited laboratory space many fostered lines were not continued; others are in the tenth generation.

FIG. 1. METHOD OF TABULATING CONTROL AND FOSTERED BREEDING FEMALES OF THE A HIGH-CANCER STOCK

The lines under each class correspond to the curves used in the following figures.

1 Read before the American Association for Cancer Research, Atlantic City, N. J., May 2, 1938.
2 These investigations have been supported by grants from the International Cancer Research Foundation and the National Cancer Institute.
Table I: Cancer Incidence in Control and Fostered Mice and in the Progeny of the Fostered Females

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Per cent Cancerous</th>
<th>Average Age at Appearance of Cancer</th>
<th>Average Age at Death Non-cancerous</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Stock: Controls</td>
<td>1093</td>
<td>83.6%</td>
<td>11.1</td>
<td>12.1</td>
</tr>
<tr>
<td>A Stock: Fostered</td>
<td>95</td>
<td>7.4%</td>
<td>10.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Progeny of fostered cancerous females</td>
<td>77</td>
<td>66.2%</td>
<td>11.7</td>
<td>15.9</td>
</tr>
<tr>
<td>Progeny of fostered non-cancerous females</td>
<td>286</td>
<td>9.8%</td>
<td>12.2</td>
<td>16.1</td>
</tr>
</tbody>
</table>

Fig. 1 gives the method of grouping the animals and the types of curves employed in all A stock graphs. Fig. 2 shows the percentage of each group living to the beginning of each bimonthly age period or longer. It is apparent that the fostered mice and the progeny of the non-cancerous fostered animals lived considerably longer on the average than did the members of the other two classes.

The group of graphs in Fig. 3 represents the proportion of the total number dying at each age period as follows: (a) from all causes of death, (b) from breast cancer, and (c) from causes other than breast cancer. The mode of the curve for the control mice is at the ninth month and for the progeny of the cancerous fostered mice at the eleventh month, due in both instances to cancer deaths during these intervals (Fig. 3b). For the fostered mice and the progeny of the non-cancer fostered animals the modes are between the fifteenth and nineteenth months. Most of the mice in these two groups lived longer than the average controls and died non-cancerous (Fig. 3c).

Fig. 4 represents diagrammatically the average cancer age and the average
FIG. 3. PERCENTAGE OF TOTAL A STOCK MICE Dying IN EACH AGE PERIOD (A) FROM ALL CAUSES, (B) FROM BREAST CANCER, AND (C) WITHOUT BREAST CANCER
For explanation of curves, see Fig. 1.

AVERAGE AGE
CANCER  TOTAL

FIG. 4. AVERAGE CANCER AGE AND AVERAGE AGE AT DEATH OF THE A STOCK ANIMALS
age at death for the entire group in the different classes. It is obvious that the average age at death of the fostered group was considerably higher than for the control series.

Table I shows the number of animals observed thus far in each group, the breast cancer ratios, the average age when breast tumors were observed and the age at death of the non-tumor-bearing mice. The controls number 1093, with a tumor incidence of 83.6 per cent. Ninety-five fostered mice have died, of which number 7.4 per cent had breast tumors. Seventy-seven progeny of cancerous fostered females and 286 descendants from non-cancerous fostered mothers have succumbed, with tumor ratios of 66.2 per cent and 9.8 per cent respectively. Little variation was observed in the average breast-tumor age of the various classes. The average span of life of the non-cancerous controls was considerably less than for similar mice of the other groups.

The proportion of the mice living to the beginning of each age period or longer, in each group, to develop breast tumors is represented in Fig. 5. The curves for the fostered series and the progeny of the non-cancerous fostered mice are very similar. The curve for the progeny of the fostered mice with breast cancer is more like the control line, up to the fifteenth month. In the control group only 8.5 per cent of the mice lived to the beginning of the seventeenth month or longer. The breast-tumor ratio for the mice dying before

![Figure 5](image-url)

**Fig. 5.** Percentage of A Stock Mice Living to the Beginning of Each Age Period or Longer to Develop Breast Cancer

For explanation of curves, see Fig. 1.

![Figure 6](image-url)

**Fig. 6.** Representation of Matings and Fostering to Produce the AB and BA First Generation Control and Fostered Hybrids

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this age period was 86.2 per cent and for the animals living seventeen months or more 55.9 per cent. Comparative tumor percentages for the progeny descended from the fostered mice which developed tumors were 83.9 per cent and 19.0 per cent, with 27.3 per cent of the mice living at least seventeen months.

An outcross between the A high-cancer line and the B (C57 Black) low-cancer stock is represented in Fig. 6. When the maternal parent was from the A stock, the first generation mice were termed ABF₁ hybrids. If, however, the ABF₁ mice were removed from their A stock mothers and fostered by B stock females, the hybrids were designated as ABF₁ by B♀. The reciprocal cross was made to obtain the BAF₁ and BAF₁ by A♀ mice. ABF₂ and BAF₂ control breeding females were also observed, but not fostered animals of these generations.

The number of animals in each hybrid group is given in Table II. The cancer ratios are based only on the animals which have died from various causes. The age range of the living mice is given but not the average cancer age, as in some groups most of the mice are still alive.
None of the ABF, breeding females lived to the twenty-first month (Fig. 7), whereas 65 per cent of the BAF, mice reached that age. In the former group, 95 per cent of the mice developed spontaneous breast cancer as compared with 8 per cent in the BAF, mice. The reciprocal first-generation fostered hybrids were born at approximately the same time. Two per cent of the ABF, by B♂ and 38 per cent of the BAF, by A♀ animals have died. The tumor ratios in the respective groups have been, to date, 0 and 93 per cent. Seven per cent of the BAF, and 81 per cent of the ABF, mice have died cancerous.

Fig. 7 gives the age curves and Fig. 8 the cancer curves for the first generation hybrid classes. The cancer percentages for the fostered and control ani-
mals are represented in Fig. 9. Fig. 10 tabulates data for a few control and fostered BZF, breeding females (C57 Black ♀ × C3H ♂). The youngest control animal is older than the oldest fostered mouse. Forty-six per cent of the unfostered and 87 per cent of the fostered mice have died and the cancer ratios have been 6 per cent and 79 per cent.

**Discussion**

Three experiments testing the relationship of foster nursing to the incidence of breast tumors in mice have demonstrated thus far that the source of milk exerts a decided influence on the development of breast tumors. (Nursing has little or no bearing on the development of primary lung carcinoma.) Mice nursed by low-tumor stock females show a low percentage and mice nursed by high-tumor stock females have a high ratio. In another group of 8 C57 Black females nursed by A stock mothers, 3 developed tumors (2). This work is now being repeated on a much larger scale.

In the A high-breast-tumor stock 83.6 per cent of the breeding females become cancerous. As far as can be determined, the tumor percentage is nearly as high among the progeny of females without cancer as for the young of mothers which develop breast tumors. When the young have been removed as soon after birth as they are recorded and have been placed with low-tumor stock lactating females, the breast tumor incidence has been reduced, in the experiments completed to date, to 7.4 per cent. By this method of fostering, the young are permitted to nurse their mothers from birth until they are recorded, or up to twenty-four hours. They thus obtain some milk from high-cancer stock animals. The average tumor age for the control and fostered mice remained about the same, but the non-cancerous fostered mice survived over four months longer than the control mice. Thus, breast cancer possibilities had greater opportunities of being expressed.

The progeny of the fostered mice have been tabulated according to the cause of death of their mothers. In general, if the mother had a breast tumor the incidence among its progeny was nearly as high as for the control stock. Young descended from non-cancerous mothers usually have a low ratio. Exceptions are too common, however, to make these assertions definite. Non-cancerous mothers which do not follow the general rule usually come from litters in which there is at least one cancerous individual. A few cancerous fostered mice behave as non-cancerous individuals. Such exceptions have not been observed among the control mice and may account for the difference in the observed tumor ratios. If a female descended from a proved non-cancerous fostered animal develops a tumor, its progeny do not have a higher incidence than the average for this group.

In previous work we have assumed that a "breast-cancer-producing influence" was transmitted by potential breast-cancer mothers to their progeny through the milk. If such is the case, in an inbred strain of mice having a high tumor incidence a high ratio would be maintained regardless of whether or not a mother died of cancer. Selection, as observed by Strong (7), would be without effect. Evidently the first milk which a high-cancer stock female secretes does not have a higher concentration of the "influence" than that of
the remaining lactation period. Some A stock females had suckled young for four days before they were used as foster mothers. Tumors, however, developed in the fostered young.

The extra-chromosomal theory of breast cancer in mice was based primarily on the tumor incidence in reciprocal hybrids made by crossing high-tumor and low-tumor stock mice. When the maternal parent was from the high-tumor stock, the F₁ and F₂ generation mice gave ratios which approached the maternal stock. In the reciprocal generations the percentages were very low.

Reciprocal matings between the A and C57 Black stocks have confirmed the above observations in the F₁ and probably the F₂ generation. In the ABF₂ generation, however, the incidence is not as high as in the ABF₁ generation, although the average length of life of the second generation mice was considerably longer. Further studies must be made to determine if there will be a gradual reduction in the cancer incidence with subsequent hybrid generations descended from the maternal high-tumor stock and an increase in the hybrids descended from the maternal low-tumor stock. We do know that the pedigree relationship is very important in hybrid mice.

A few observations on fostered ABF₁ and BAF₁ animals of similar age are available to test the association of nursing and the extra-chromosomal influence. Most of the animals are still living and are between seven and eleven months old. In the control ABF₁ mice, 38 per cent of all tumors occurred in animals which were from seven to nine months in age. The incidence in ABF₁ breeding females, all of which have died, was 95 per cent. In fostered ABF₁ by B♀ mice no tumors have been recorded but a few are to be expected. Thirty-eight per cent of the BAF₁ by A♀ hybrids have died and these have had a tumor ratio of 93 per cent. To date 65 tumors have developed in fostered BAF₁ breeding females (62 per cent living), and 5 have been observed in unfostered mice of this generation (39 per cent living). In the BZF₁ hybrids 6 per cent of the controls and 79 per cent of the fostered mice have developed similar growths.

The maternal influence in breast tumor etiology observed in hybrid mice produced by high- and low-breast-tumor crosses may be explained by a "breast-cancer-producing influence" transmitted in the milk of potentially high-tumor mothers. There is some evidence that a sufficient amount may be transferred by the inoculation of a graft of normal tissue to initiate the development of mammary cancer in some cases. The nature of this "influence"—hormone, chemical or virus—has not been determined.

**Literature Cited**