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Further Studies on Active Milk Influence in Breast Cancer Production in Mice.*

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The purpose of this article is to summarize the published evidence and to present new material that the milk influence in inherited breast tumor production in mice is an actual and active influence.

In this paper the term tumor or cancer signifies breast carcinoma and unless otherwise stated, all the females were used as breeders.

Various experiments have been reported by the author[1-16] on the rôle played by the milk influence, genetic susceptibility and/or hormonal stimulation in the etiology of inherited breast cancer in mice. Others have confirmed the work regarding the milk influence in different strains.[17-20]

The analysis of the data also indicated that another type of breast carcinoma may develop in mice which apparently is not genetic since the progeny do not show tumors. The milk influence is generally inactive in such animals and the other etiological factors are not clear. No difference has been noted in the histological structure of these tumors.

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In the earlier papers it was stated that the incidence of breast tumor in mice ("A" stock) might be reduced from 90% (over 500 animals) to 8% (127 mice) by fostering the young of high breast tumor stock mothers to females of low breast tumor strains. The young were transferred before they were 24 hours old, and only one generation was fostered.

However, some of the young which were permitted to remain with their potentially tumorous mothers for less than 24 hours developed tumors as did some of their progeny. Other mice of the same fostered litter did not develop tumors and had descendants which likewise gave a low incidence of breast tumors, the incidence being similar to that observed in virgin females of the stock.

Fifteen young which were fostered not before from 1-5 days after birth gave a tumor incidence of 87%.

Twenty-seven young of fostered "non-breast cancerous" females were given to control (high cancer) "A" stock females the day of birth; tumors resulted in 89% of these mice. The fostered "non-breast cancerous" females nursed 29 of their progeny and no tumors were recorded.[10]

To determine if the milk influence was present only in the first milk secreted by a lactating female, some high tumor stock females were permitted to nurse their litters for 4 days before being used as foster mothers.[6, 9] The young fostered were BAF_1 hybrids (C_{57} black or "B" ♀♀ x "A" ♂♂). When the high tumor stock females were used as foster mothers the same day their own litters were born, the fostered F_1 females had a tumor incidence of 87.3% (63 animals). Females used one day after their litters were born nursed 54 BAF_1 mice and these females showed a breast tumor incidence of 85.2%. Two mice in each group developed internal tumors and if they are not included the respective ratios were 90.2% and 88.5%. Twelve BAF_1 females were given to "A" stock females which had nursed for 4 days; all of the hybrids developed breast tumors.

Hybrid females (BAF_1) which had maternal parents from a low breast cancer stock and paternal parents from a high breast cancer stock have a spontaneous breast tumor incidence of 2% if they are nursed by their maternal parents.[13, 16] The number of mice observed was 118. If inoculated with grafts of spleen or thymus from 4-week-old "A" stock donors, or lactating mammary tissue from older animals, the observed incidence was 21.7% for 46 mice. BAF_2 control breeding females (246 animals) had a tumor incidence of 0.6% and 11 inoculated mice of the same generation gave 18.2% cancer. Seventeen BAF_2 mice descended from inoculated BAF_1 females showed a mammary gland tumor incidence of

29.4%.[6, 11, 16] The mice of the inoculated groups were older at the time of tumor development than the control animals.

ABF_1 ("A" ♀♀ x "B" ♀♀) females, totaling 122, nursed by "A" stock females showed an incidence of 94.3%, whereas hybrids which had fostered "A" stock (low tumor)† maternal parents gave 0.6% cancer. Another group of 37 ABF_1 females was taken from their high tumor stock mothers and given to inbred C_{57} black stock females with a resulting breast tumor incidence of 8.1% for 37 individuals. Similar mice nursed by "B" stock females which in turn had been suckled by low tumor BZF_1 by B♀ hybrids (2.6% cancer, 39 mice) gave 4.2% cancer for 48 mice. If "B" stock females which had been nursed by high tumor hybrids (BZF_1 by Z or C_3H♀ —79.1% cancer for 43 animals) were used as foster mothers for ABF_1 females, these mice developed tumors in 78.4% of the total or 51 animals.

C_{57} black stock mice when nursed by high tumor stock females ("A" or C_3H) have a breast tumor incidence of approximately 10%.[15, 17] The number tested was 104.

Ten mice of a sub-line of "A" stock fostered mice, having a tumor incidence of 4.5% for 245 breeding females, were fed an average of 5 cc of macerated liver per animal when they were 4 weeks of age. Tumors have not developed and the living animals are 16 months old. Ten additional females received by mouth an average of 1.7 cc of milk obtained from lactating females ("A" stock) 11 to 19 days after their litters were born. One animal died at 356 days of age, 8 have developed tumors, average age 276 days, and the remaining animal is living at 16 months of age. Ten other females, also 4 weeks of age, consumed an average of 0.9 cc of milk from the same source. Seven of the group are living (16 months) and 3 have developed tumors, average age 303 days. This work is being repeated and additional animals are under observation. The "A" stock donors (milk and liver) were from 92 to 155 days of age.

The injection of estrogenic hormones has failed to produce breast tumors in control C_{57} black stock mice.[21, 22] Males of this strain nursed by females of the Paris R III high breast tumor stock will give rise to breast tumor following the implantation of crystals of estrone (Twombly[22]). Paris R III males nursed by C_{57} black stock

† Made by fostering "A" stock young to "B" stock females. The use of such animals eliminated the possibility of the hybrid young from obtaining milk containing the active influence.

21 Suntzeff, V., Burns, C. L., Moskop, M., and Loeb, L., *Am. J. Cancer*, 1936, **27**, 229.

22 Twombly, G. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 617.

females had a lower incidence of induced tumors than did the control animals.

Pellets of theelin (average weight 0.2 mg) and estradiol benzoate (average weight 0.15 mg) were implanted subcutaneously into 6 males and 13 females of the "A" high tumor and 19 males and 24 females of the fostered "A" low tumor stock. Animals dying from too much hormone before the appearance of the earliest tumor, 147 days following injection, are not included. The results for the 2 hormones are combined as they were very similar. Six high tumor females and 3 high tumor males have developed tumors (47.4% of the total). The average tumor age for the females was 189 days and for the males 185 days following injection.

Eleven fostered "A" stock females and 12 males are still alive, all being over 330 days of age. The other females survived an average of 242 days and the deceased males 248 days. No tumors were observed in either group.

Three induced tumors have been noticed among 12 high tumor C_3H stock males and females whereas 18 fostered C_3H animals have been negative. Hybrids have also been injected with estrogenic hormones but many are still living. Tumors have appeared only in the animals which were nursed by high breast tumor stock females.

Discussion. The foster-nursing experiments were started in an attempt to explain the maternal influence observed in breast tumor development in mice.[23]

Since 1936[1] it has been apparent that the incidence of breast tumors observed in high cancer strains of inbred mice might be reduced as the result of foster-nursing the young of such mothers by females of a low breast tumor strain. To account for these results it was assumed that a "breast cancer-producing influence" was transferred through the milk of breast cancer stock mothers to their progeny. Others stated, however, that the absence of an active influence from the milk of cancerous strain females would produce similar results. The importance of the inherited breast cancer susceptibility factor and hormonal stimulation of the mammary tissue were emphasized in later work[8, 9] but need not be considered in detail here.

It was noted in the first experiments that if the fostered progeny of cancerous stock mothers were non-breast cancerous, few tumors were observed in their progeny and descendants. The progeny of "fostered non-cancerous" females nursed by cancerous stock females behaved as control or cancer stock individuals. If the fostered

[23] Staff, Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.

females of a cancerous stock were themselves cancerous, breast tumors were common among their progeny.

The importance of the time interval between birth and fostering was obvious when tumors were observed in the progeny of cancer stock mothers which were permitted to remain with their maternal parent longer than 24 hours. The incidence for young fostered within 24 hours after birth was low; the incidence for young fostered after 24 hours following birth approached the incidence recorded for the control animals. That the influence was active after the first milk had been secreted was manifested by the use of foster mothers of a cancer strain after they had nursed their own litters for 4 days.

Breast tumors have also resulted following the inoculation of lactating mammary gland tissue, spleen and thymus from donors of a high tumor stock and by feeding 4-week-old mice a small amount of milk obtained from lactating females 11 to 19 days after their litters were born. The incidence and average tumor age seemed to be determined by the amount of the active influence obtained by the animals. Macerated liver fed by mouth has failed to produce tumors although liver obtained from young donors has not been used.

Females of a low breast cancer stock when nursed by high tumor stock females ("A" or C_3H) have a breast tumor incidence of approximately 10%.[15, 17] The incidence is low, presumably, because they do not have the genetic breast cancer constitution. Fostered low breast tumor stock females do obtain the active milk influence when nursed by high tumor females and although they may not develop tumors, the influence may be transferred by nursing to other animals and tumors generally will develop in mice having the genetic breast cancer susceptibility.

The discussion of the relationship of the milk influence to induced estrogenic tumor induction must be delayed until further experimental data are available.

Thus, all the evidence indicates an actual active influence in the milk of cancerous strain females. It is probably present during the entire lactation period as it is active in the first milk, in the milk obtained by nursing 4 days after the birth of a litter and in that secured 11 to 19 days after the beginning of lactation.

Conclusions. The "breast cancer producing influence" is an actual active "influence" present in the milk of high cancer stock females. It is probably present and active during the entire lactation period.

The active influence may be transferred by the inoculation of spleen, thymus, and lactating mammary gland tissue from cancerous stock animals. The active influence probably is not present (or has been destroyed) in the liver of high tumor stock mice. The active influence may be given to 4-week-old females by feeding-by-mouth milk obtained from lactating females of a cancerous stock. Fostered females of low breast tumor strains need not develop breast tumors to transfer the active milk influence by nursing. An active milk influence may be necessary for the development of induced estrogenic breast tumors.

(A future publication will show that a low breast tumor strain of mice may "acquire" an active milk influence at any time, resulting in a high breast tumor strain.)

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Appearance of the Melanophore-Expanding Hormone of Pituitary Gland in Developing Chick Embryo.*

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The purpose of this investigation was to determine when the presence of the melanophore-dispersing hormone can first be demonstrated in the developing chick embryo with a view to correlating the appearance of the hormone with the structural development of the pituitary gland.

Material and Methods. Hybrid eggs (Red-Rock) were incubated for periods of from one to 13 days. In embryos of 5 days and over, the pituitary region was removed with the aid of a dissecting microscope. In the 2-, 3-, and 4-day embryos, the head of the embryo was utilized, and at the one-day stage the entire embryo was used. The material was ground up in a small volume of saline or .25% acetic acid together with a few drops of 2.5% $NaHCO_3$ to bring the material to about pH 8. The resulting suspension was injected into the ventral sac of an hypophysectomized frog. The hypophyseal region of 6 to 20 chicks was used, depending on the age of the embryo.

Certain technical precautions were observed. Only frogs which had been hypophysectomized for at least 2 weeks were used, and the sensitivity of these was insured by testing them with minute doses

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