Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice*  

John J. Bittner, Ph.D.  
(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)  
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At least three etiological factors have been recognized in the development of spontaneous mammary tumors in mice. These are: the inherited susceptibility or nonsusceptibility of the individual, sufficient hormonal stimulation of the mammary gland, and some influence in the milk which the animals receive while nursing. Others may be involved but have not been identified.

I. THE ROLE OF THE HORMONES  
A. INTRODUCTION

"Carcinoma can occur only in a mammary gland which has undergone a certain degree of development. The development of the mammary gland is dependent upon the estrogens and pituitary hormones. But whether these stimulating factors play a direct role in the cancerization process, or only produce the anatomical development of the mammary gland sufficient to allow the cancer process to manifest itself is still undetermined."—from Lacassagne (36).

Earlier it had been demonstrated by Loeb (39, 40), Cori (25), and Murray (43-46) that few tumors of the mammary gland developed in mice which had been ovariectomized. In general, the younger the mice were when spayed the lower the incidence of mammary tumors.

Woolley, Fekete, and Little (62-64) have recently observed that castration in strains of mice having a high incidence of mammary tumors may be followed by hyperplastic changes in the adrenal cortex. In the females stimulation of the uterus, vagina, and mammary glands was noticed. Mammary tumors developed in mice of both sexes and it was concluded that the hyperplastic adrenal may directly or indirectly simulate or replace ovarian activity in stimulating the growth of the mammary tissue and the development of mammary cancer.

The significance of the estrogenic hormones in the development of mammary tumors became still clearer when Lacassagne (35) found that tumors might appear following the injection of these hormones over an extended period. In some experiments carcinomas arose in both males and females. Gardner (28) and Loeb (41) have recently reviewed the literature in this important and growing field.

B. THE STRUCTURE OF THE MAMMARY GLANDS  
Several workers have studied the structure of the mammary glands of mice to determine if there might be some anatomical structural relationship associated with the development of neoplasia.

Gibson (33) found that the mammary glands of mice of a strain which developed tumors were in-

[1] The use of this term implies that, unless indicated, the pituitary hormones are also active (34).
clined to show metaplasia of the epithelial elements, rather than atrophy, as age advanced. Mice of a strain producing few tumors had glands which underwent involution and fibrosis in an orderly and normal manner.

Gardner and Strong (31) could find no significant structural difference in the mammary glands of virgin females of strains varying in their predisposition to mammary cancer.

In mice of the dilute brown stock (high incidence) and the C57 black strain (low incidence), Fekete (26) observed variations in the mammary glands. Animals of the strain which developed few tumors had uniform changes in the epithelium of these glands during the latter part of pregnancy; as function was discontinued their cells ceased to secrete and the alveoli regressed completely. The cells of the glands of mice belonging to the dilute brown (high tumor) stock, on the other hand, did not always stop multiplication toward the end of pregnancy; some continued to multiply. During regression of the glands, small groups of alveoli often persisted and some cells continued to secrete. This persistent mitotic activity in small groups of cells probably marked the site of early malignant changes.

Gardner, Strong, and Smith (32) likewise noticed that the mammary glands from mice of strains with different susceptibilities showed anatomical characteristics which could be associated with neoplastic changes. Localized hyperplastic nodules were more common in mice of strains having a high incidence of mammary tumors. The authors did not believe that the occurrence of these nodules indicated greater ovarian stimulation of the glands in mice from strains having many tumors but thought that the alteration might be a result of "factors passed from parent to offspring in association with other factors predisposing to cancer." The number of hyperplastic nodules tended to increase with the age of the animals.

Injecting theelin into normal male mice of different strains, Gardner, Diddle, Allen, and Strong (30) determined that even mammary rudiments could be made to respond to the stimulation. In general there was no strain variation.

Gomez, Turner, Gardner, and Hill (34) found that growth of the mammary glands of hypophysectomized mice did not occur following the injection of estrogens, whereas the glands of nonhypophysectomized male or ovariectomized immature female mice developed rapidly under treatment. This study confirmed the observations of Nelson and Pfiffner (49).

Gardner, Strong, and Smith (32) concluded that since the mammary glands of mice would not respond beyond a certain point to continued treatment with estrogic hormones, some factor or factors maintained them in equilibrium with other tissues or organs.

van Gulik and Korteweg (59) observed that the characteristic architecture of the mammary glands of mice of the dilute brown (high cancer) and the C57 black (low cancer) stocks might be altered as a result of foster nursing. Foster nursing on females of the resistant strain decreased the proliferation of the gland-trees and lowered the incidence of tumors; on the other hand, hyperplastic nodules were observed in the glands of mice belonging to the C57 black strain and nursed on females of the dilute brown stock. These authors believe that the architecture of the mammary glands is probably determined by both intrinsic factors and the type of milk received. Their observations have been confirmed by Shimkin, Grady, and Anderson (51) on mice of the C3H and C stocks.

Fostered mice of the A stock will develop mammary tumors if nursed on females from strains with a high incidence of mammary tumors, or if artificially fed, when 4 to 6 weeks of age, with milk from females of strains with a high incidence (13). If fostered mice are permitted to have several litters before they are given the milk with the active influence, apparently few mammary tumors will result (Duran-Reynals and Bittner, unpublished). Thus, the active milk influence may have to be present from the time the mammary glands start to develop in order to prepare them for the cancerous change.

C. Variations in the Degree of Estrogenic Stimulation in Different Inbred Strains of Mice

Inbred strains of mice which differ considerably in tumor incidence are being used for studies on the etiology of mammary tumors. Some develop few tumors (37); some have a low incidence in virgin females and a high incidence in breeding females (13), whereas other stocks have a high incidence in both virgin and breeding females (4, 5, 59). The greatest variations in susceptible stocks have been observed in C3H and A mice, virgin females of the C3H strain having a very high incidence (4-6) and virgin females of the A stock a very low one (13), whereas breeding females of the two strains have a high incidence. Mammary tumors develop at an earlier average age in breeding females than in virgin females of the C3H stock, an observation suggesting that the amount of estrogenic stimulation has some bearing on the time at which the normal gland may change to a cancerous gland.

It is of interest to speculate on the cause or causes of the difference in tumor incidence for virgin females of the A and the C3H strains, and it may be suggested...
that the high incidence of mammary cancer in virgin females of the latter, as compared with the low incidence for virgin mice of the A strain is perhaps the result of:

(a) An increased production of estrogenic hormones.

(b) A decrease in the rate of destruction of these hormones.

(c) A lower threshold sensitivity of the mammary tissue to neoplastic change.

It was found by van Gulik and Korteweg (58) that the degree of response of the genital organs to follicular hormones was characteristic for each of the five strains tested, and they thought it probably determined by intrinsic factors. The mice of the two strains with a high incidence of spontaneous mammary tumors showed very little response, probably because, it was concluded, they produced naturally a relatively large amount of the hormones. The authors suggested that if the mammary glands from strains of different incidence are equally susceptible to follicular hormones, the excess amount of such hormones secreted by mice of strains with a high incidence of mammary tumors might increase the probability that their glands would become cancerous. Thus there might exist a causal relation between the amount of the hormones secreted, as expressed by the low susceptibility of the genital organs to the hormones, and the disposition to mammary cancer.

Shimkin and Andervont (50), however, using control and foster nursed mice of strains with different incidences, found that regardless of changes in the incidence resulting from foster nursing the susceptibility of the different strains to estrone was not altered and thus was not causally related to the development of spontaneous mammary cancer. Variations in the response to estrone from different strains are strain differences and, as such, are probably caused by inherited factors. Thus the amount of estrogenic hormones secreted by virgin females of different strains would be determined by heredity, and the incidence of mammary tumors would be higher in virgin females of the C3H stock than in virgin females of the A stock because inherited characteristics produced a higher level of estrogenic stimulation of the mammary tissue. This difference has also been observed in the response of mice of these strains to the artificial administration of estrogens (27, 42).

If the amount of estrogenic hormones secreted by virgin females of various strains is the result of intrinsic (genetic) factors, mutations might alter this amount, change the degree of mammary stimulation, and so result in variations in the incidence of mammary carcinoma. This suggestion is supported by experimental observations.

Thus in 1928 Murray (44) reported that the occurrence of mammary tumors in virgin females of the dilute brown stock was 15.8 per cent; by 1941 (47) it had increased to 61.6 per cent, and mice from the same strain have lately shown an incidence of 85 per cent in virgin females raised in another laboratory (58). Again, virgin females of the A stock had an incidence of 4.9 per cent in 1939 (13) and Murray (48) has recently reported that the virgin females of his A stock now have a high incidence of mammary tumors.

The production of estrogenic hormones may be influenced by diet. Bagg (8) reported that it was necessary to supply a high protein diet to keep the animals breeding actively in his forced breeding experiments, and Visscher and his associates (61, 7) have recently demonstrated that the incidence of mammary tumors may be decreased by restricting the caloric intake. Definite changes in the structure of the ovaries and mammary glands were described, probably resulting from a reduction in the amount of estrogenic hormones secreted by the experimental animals. When an attempt was made to breed some of the mice it was noted that less than 40 per cent of the females were fertile and that few of these gave birth to more than one litter (61).

The amount of estrogenic stimulation of the mammary tissue may be further increased: (a) as the result of normal breeding, permitting the females to nurse their offspring; (b) by forced breeding, the functional test of Bagg (8–10), where the young are removed as soon after birth as possible and the mothers are returned to the breeding pens, to become pregnant usually within a few days; and (c) by the administration of estrogenic hormones (35). If the amount of hormones secreted in the virgin females of different strains may possibly be the result of intrinsic factors, the increased secretion of such hormones associated with breeding and injection would be a combination of intrinsic and/or extrinsic causes.2

The effects of an increased amount of estrogenic stimulation are apparent in causing mammary tumors to appear at an earlier average age. For example, Andervont (4) found that tumors developed in virgin females of the C3H stock at an average age of 10.4 months, whereas the normal breeding females developed tumors at an average age of 8.6 months.

The largest amount of hormones may be produced as the result of forced breeding since Gardner (29) determined that "the environment of mammary glands in females subjected to continuously repeated pregnancy predisposed to a higher incidence of mammary

2 Pregnancy is here listed as due to an extrinsic cause; namely, the introduction of sperm.
tumors than did the environment created by administration of estrogens.

D. Summary

The amount of estrogenic hormones produced in virgin females of various stocks is the result of strain differences and as such may result from intrinsic factors. In some susceptible strains of mice the amount of hormones secreted by virgin females will produce a sufficient development of the mammary tissue to result in a high incidence of mammary tumors. Other strains of susceptible mice have a low incidence in virgin females probably because their intrinsic factors are able to produce only a low level of estrogenic stimulation. The production of estrogenic hormones may be further increased as the result of extrinsic causes—the production of young, as in the case of normal breeding; forced breeding; or the injection of hormones over a long period of time. In susceptible mice the average tumor age is correlated with the amount of estrogenic stimulation, and in nonsusceptible animals with the active milk influence the incidence of mammary tumors and the average tumor age are also related to the level of estrogenic stimulation.

The amount of estrogenic stimulation may be influenced also by diet.

II. CHARACTERISTICS OF THE ACTIVE MILK INFLUENCE

In 1933 it was determined that some hitherto unrecognized maternal adjuvant also was instrumental in the development of mammary cancer in mice (52). This was soon discovered to be an active "influence" present during the entire lactation period in the milk of females from strains with a high incidence of mammary tumors (11, 20), and probably more concentrated in older animals. It may be eliminated by foster nursing on females of strains with a low incidence of mammary tumors. It is active in lyophilized tissue, in a filtrate (Seitz filter), and in desiccated and glycerolated tissue (21). Following ultracentrifugation (110,000 x g) for a period of 60 minutes, the active material appears in traces, if at all, in the fat fraction and in the final supernatant fluid (60). It is probably a colloid of high molecular weight.

The active milk influence may arise de novo in animals which have not been in contact with those having a high incidence of mammary cancer (17). If it should thus appear in mice susceptible to the development of spontaneous mammary carcinoma, the incidence may change from 5 per cent to 98 per cent (17).

III. INHERITED SUSCEPTIBILITY TO SPONTANEOUS MAMMARY CARCINOMA

The data obtained from matings between mice of strains with low and high incidences respectively are in accord with the genetic theory that susceptibility to mammary carcinoma may be transmitted as a single dominant factor (13, 2, 20, 22). The results are somewhat complicated, however, because a higher incidence has been observed in mice of successive litters born to the same mothers (20, 22).

Inherited susceptibility to the development of mammary cancer is not itself altered as a result of foster nursing, and may be inherited through males or females of any genetically susceptible stock.

IV. VARIATIONS IN INCIDENCE OF MAMMARY TUMORS FOLLOWING DIFFERENT EXPERIMENTAL PROCEDURES IN STRAINS OF LOW INCIDENCE

A. Discussion

Inbred strains of mice are to the biologist what pure chemicals are to the chemist. Following a sufficient number of generations of brother-to-sister matings all the mice of an inbred stock are theoretically homozygous as far as their inherited characteristics are concerned. Living material is, however, subject to mutations and some of these may be of such nature as not to become apparent for a number of generations.

It is assumed that every individual of an inbred strain, or subline of an inbred strain, is genetically like every other member of that particular stock. Thus, so far as its genetic susceptibility to the development of spontaneous mammary cancer is concerned, we may suppose that it will be either susceptible or nonsusceptible. Consequently it is probable that any differences observed in the incidence of mammary cancer are a result of different amounts of estrogenic stimulation and/or different concentrations of the active milk influence received while nursing or from forced feeding. The level of estrogenic stimulation may result from intrinsic causes (amount secreted in virgin females) supplemented by extrinsic causes (normal breeding, forced breeding, or injection).

The incidence of mammary tumors in virgin and breeding females of the C57 black strain is less than 1 per cent (37), but when fostered by females of the A stock (high incidence) the fostered females of the C57 black stock and their progeny had an incidence of 10.6 per cent (15). Andervont observed an incidence of 10.3 per cent in C57 black mice nursed by females of the C3H stock and continued as breeders (1, 6, 50). When animals of the C57 black stock were kept as virgins after having been nursed on females of the dilute brown stock, van Gulik and Korteweg (58) found that 13 per cent, and Murray (47) that 9.2 per cent, developed mammary tumors.

Bagg and his coworkers (8-10) have used mice of the C57 black stock in studies on forced breeding or the functional test. When mice of subline Y were used some developed mammary tumors, whereas none appeared in those of subline A (9). Bagg (9) fostered
18 females of subline A on mice of the C3H stock and found that 50 per cent of the fostered females developed mammary tumors while others were still living without tumors at the time the report was made.³

Little and Pearson (38) also subjected approximately 100 female mice of the C57 black stock to the functional test and did not observe the development of mammary tumors in any of the animals. These experimental animals were all nurset by their mothers.

When females of the C57 black stock which had been nursed by females of the A stock were used to nurse mice of a susceptible stock, a high incidence of tumors was noted in these susceptible animals (15), showing that the milk influence remained active in mice genetically nonsusceptible to spontaneous mammary tumors.

Strong (53, 54) found that breeding females of the CBA strain had an incidence of mammary tumors of approximately 4 per cent. A subline secured from Strong had an incidence of 13.5 per cent in 125 breeding females (12). Suntzeff and his associates (56) found that 5.9 per cent of 202 breeding females and 0.7 per cent of the virgin females of the CBA strain, descended from Bittner's line, had spontaneous mammary tumors.

Mammary tumors have been induced by estrogen injection in males and females of the CBA stock by Gardner (27, 29), using mice of Strong's stock. Mice of the same strain, but of a different subline (12), did not develop tumors in similar studies conducted by Bonser, Stickland, and Connal (23), Bonser and Robson (24), and Suntzeff and his associates (56). Gardner (29) also found that a high incidence of mammary tumors could be produced in females of the CBA stock by forced breeding; but Bagg (8) was unable to obtain any tumors in mice descended from Bittner's line in similar studies.

Breeding females of the A stock descended from a female fostered by a female of the CBA stock (12) had a low incidence of spontaneous mammary tumors (8, 17) and no tumors were induced by estrogens (16, 19). If, however, the members of this fostered subline of the A stock acquire an active milk influence, a high incidence of mammary tumors will result in breeding females (17) and in consequence of the injection of estrogens (19).

Gardner (29) stated that the CBA females used in his experiments transferred to their offspring a tendency to develop mammary tumors in breeding females and after the injection of estrogen. This maternal influence as regards spontaneous or induced mammary tumors was not evident in the mice of Bittner's line of CBA mice or in mice fostered by these females. Since an active milk influence may arise without contact with females from strains of high incidence, by changes in the inactive influence or de novo (17), it is possible that such a change occurred in the constitution of the milk influence in the sublines of CBA mice studied by Gardner. Our results following the development of an active milk influence in mice fostered by CBA females were similar to those reported by him.

Andervont (3) observed that approximately 2 per cent of breeding females of the C strain will develop spontaneous mammary tumors. None of the fostered females of the C stock nursed on C3H mice kept as virgins developed mammary tumors. Those tested by forced breeding had an increasing incidence according to the number of litters born to the mice of the various groups (3). The incidence was higher also in mice subjected to forced breeding and nonsuckling than in those treated with estrogens (50, 51). Andervont (personal communication) found that fostered females of the C stock which died without having developed cancer had progeny with a high incidence. These observations are characteristic for mice of strains with a high tumor incidence, which are considered to carry genetic susceptibility to the development of mammary tumors and which have, in addition, the active milk influence.

B. COMPARISON OF C57 BLACK, C, AND CBA STRAINS

When mice of the C57 black and C strains were nursed by their mothers (inactive milk influence) a low incidence of mammary tumors was obtained in virgin, breeding, and estrogen-treated females.

When mice of the C57 black stock were nursed by females from different strains of high incidence, the occurrence in the fostered C57 females, virgin and breeding, and the progeny of the latter, was approximately 10 per cent. The incidence was no higher among offspring of the fostered mice that died with cancer than among offspring of the fostered mice that died without developing cancer. The milk influence was active, however, because when the fostered C57 females were used to nurse mice susceptible to mammary cancer a high incidence was obtained in these susceptible mice. The presence of an active milk influence and the estrogenic stimulation elicited in virgin and normally breeding females were able to overcome the threshold of nonsusceptibility in only 10 per cent of the C57 black mice. If the amount of estrogenic stimulation was increased by forced breeding (or the administration of estrogens), a high incidence of tumors was noted.

³ Bagg (personal communication) is of the opinion that some of the mice of his C57 black stock, subline Y, developed an active milk influence. Some mice used in these tests were raised by foster mothers (8).
Females of the C stock with inactive milk influence have a low incidence of mammary tumors whether breeding or treated with estrogen. When they have the active milk influence, obtained by fostering them on females of strains with a high rate, virgin females of the C stock have a low incidence of tumors and breeding females a high one. Mice of this stock are probably genetically susceptible to the development of spontaneous mammary tumors, since the fostered mice that died without having developed cancer had offspring with a high incidence. These results are summarized in Table I.

Shimkin, Grady, and Andervont (51) have demonstrated that the foster nursing of mice from the insusceptible C strain by mothers of the susceptible C3H stock causes hyperplastic changes in the mammary epithelium.

The genetic constitution of CBA mice for the development of spontaneous mammary tumors has not been determined. Early work with this strain showed that they transferred the inactive milk influence, but more recent experiments by Gardner indicate that mice of the Strong CBA stock may have the active milk influence. Further study of the various sublines of this strain is needed.

**Table I: Incidence of Mammary Tumors in Female Mice of the A, C3H, C, and C57 Black Stock under Different Experimental Conditions**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Milk influence in nursing females</th>
<th>Genetic constitution for spontaneous mammary tumors</th>
<th>Estrogenic stimulation</th>
<th>Incidence of mammary tumors, per cent</th>
<th>Average tumor age, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (13)</td>
<td>Active</td>
<td>Susceptible</td>
<td>Virgin females</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>A (17)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Breeding females</td>
<td>96</td>
<td>10</td>
</tr>
<tr>
<td>A (17)</td>
<td>Inactive</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>A (19)</td>
<td>Active</td>
<td>&quot;</td>
<td>Estrone</td>
<td>75</td>
<td>&quot;</td>
</tr>
<tr>
<td>A (19)</td>
<td>Inactive</td>
<td>&quot;</td>
<td>Estradiol benzoate</td>
<td>44</td>
<td>&quot;</td>
</tr>
<tr>
<td>C3H (18)</td>
<td>Active</td>
<td>Susceptible</td>
<td>Breeding females</td>
<td>92</td>
<td>10</td>
</tr>
<tr>
<td>C3H (18)</td>
<td>Inactive</td>
<td>&quot;</td>
<td>Estrone</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>C3H (19)</td>
<td>Active</td>
<td>&quot;</td>
<td>&quot;</td>
<td>66</td>
<td>&quot;</td>
</tr>
<tr>
<td>C3H (34)</td>
<td>Active</td>
<td>&quot;</td>
<td>Virgin females</td>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td>C3H (4)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Breeding females</td>
<td>91</td>
<td>9</td>
</tr>
<tr>
<td>C (3)</td>
<td>Inactive</td>
<td>Susceptible</td>
<td>&quot;</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>C (3)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Breeding females</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>C (50, 51)</td>
<td>Inactive</td>
<td>&quot;</td>
<td>Stilbestrol</td>
<td>75*</td>
<td>9*</td>
</tr>
<tr>
<td>C (50, 51)</td>
<td>Active</td>
<td>&quot;</td>
<td>&quot;</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>C57 (38)</td>
<td>Inactive</td>
<td>Non-susceptible</td>
<td>Virgin females</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57 (38)</td>
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<td>&quot;</td>
<td>Breeding females</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>C57 (15)</td>
<td>Active</td>
<td>&quot;</td>
<td>Virgin females</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>C57 (15)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57 (59)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Forced breeding</td>
<td>90*</td>
<td>&quot;</td>
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<tr>
<td>C57 (47)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>50*</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57, line A (9)</td>
<td>Inactive</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57, line A (9)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Injection</td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57, female (55, 57)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Injection</td>
<td>33</td>
<td>&quot;</td>
</tr>
<tr>
<td>C57 females</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* Some living without tumors.

It is probable that mice of all inbred strains with a high incidence of spontaneous mammary tumors in spring with a high incidence. These results are summarized in Table I.

V. VARIATIONS IN INCIDENCE OF MAMMARY TUMORS FOLLOWING DIFFERENT EXPERIMENTAL PROCEDURES IN STRAINS OF HIGH INCIDENCE

It is probable that mice of all inbred strains with a high incidence of spontaneous mammary tumors in...
incidence of tumors in these strains may be reduced as the result of foster nursing the young to females of strains with an inactive milk influence. This transfer must take place within a few hours after birth. In only one study have observations been made on the progeny of fostered animals (A stock).

Females of the A strain must be used as breeders before a high incidence of spontaneous mammary tumors will be obtained (14). Thus only breeding females are considered in the following statements.

When the young born to females of the A stock were transferred within a few hours after birth to females of the C57 black or CBA stocks, a low incidence of mammary tumors was observed in the fostered mice. If, however, the fostered females obtained a small amount of the active milk influence from their maternal parents before they were fostered, tumors might result. The progeny of these cancerous fostered mothers gave an incidence which, while lower than that observed in the control stock, was higher than that recorded among the progeny of the noncancerous fostered females. The incidence rose with increasing age of the mothers, confirming the theory (20, 22) that the milk influence is more concentrated in older animals. Other evidence exists (48) for the occurrence of variations in the intensity of the milk influence in different strains, as well as at different ages within the same strain. In the control A stock a high incidence of mammary tumors was observed among the progeny of mothers which died without mammary tumors—suggesting that these mothers might have developed tumors had they lived longer (12).

The progeny of fostered mice that died without having developed cancer had progeny with a low incidence of mammary tumors. If any of these progeny had mammary tumors, their progeny in turn also had a low incidence, unless the cancerous mothers had developed an active milk influence (17, 20).

VI. PRODUCTION OF INDUCED ESTROGENIC MAMMARY TUMORS IN SUSCEPTIBLE AND NON-SUSCEPTIBLE STRAINS

A. DISCUSSION

Gardner (28) and Loeb (41) have published reviews on the development of mammary tumors following the administration of estrogenic hormones. Gardner gave the following summary of the work to 1939:

"The observations on mice with known incidences of tumor appearance indicate that intrinsic factors other than estrogens play a significant role in mammary carcinogenesis. In other strains of mice, however, the influence of these intrinsic factors may be largely dominated by the effects of estrogens administered for long periods. In other strains, characterized by very low incidence of spontaneous carcinogenesis, a few mammary tumors have been obtained by administration of estrogens."

More recent studies have demonstrated that the type of milk influence received also plays a role in the development of induced estrogenic as well as spontaneous mammary tumors (57, 16, 19, 59, 51).

These comparisons are confined to two strains of mice of high incidence and two strains with a low incidence of mammary tumors of known genetic constitution. The strains with a high incidence are: the C3H, with high incidence in virgin and breeding females (4–6), and the A, with low incidence in virgin females and high incidence in breeding females (13, 17). Control mice of these stocks have the active milk influence and inherit the genetic susceptibility for the development of spontaneous mammary tumors. The two strains of low incidence are the C57 black and the C. Mice of both strains probably transfer the inactive milk influence; mice of the C stock are genetically susceptible to the development of spontaneous mammary tumors (3, 50, 51), whereas mice of the C57 black stock are genetically nonsusceptible (14, 22).

Gardner (27) found that a higher incidence of estrogen-induced mammary tumors could be produced in mice of the C3H strain than in animals of the A stock. Loeb and Suntzeff (42) also noticed that the mammary glands of mice of the A strain were more resistant to the effects of estrogens than were those of the C3H mice. Mice of these two strains would have the active milk influence. Since the mice were not used as breeders it is probable that the normal intrinsic production of estrogens was greater in the mice of the C3H stock than those of the A strain. This conclusion is based on the instances observed in the virgin females.

If the mice of these two strains have the inactive milk influence as the result of foster nursing, tumors are rarely produced as a result of estrogen administration (16, 19, 50).

Less than 1 per cent of mice of the C57 black stock have developed mammary tumors after treatment with estrogenic hormones, as determined by Suntzeff and his associates (55, 56), by Haagensen and by Gardner as reported by Twombly (57), and by Bittner (16, 19) in animals proved to have the inactive milk influence. Twombly (57) fostered mice of the C57 black stock on females of the R III (high incidence) strain and found that 33 per cent of the fostered males developed mammary carcinoma following treatment with estrone.

In Andervont's experiments (3) approximately 33 per cent of breeding females of the C stock developed spontaneous mammary tumors, and about the same incidence was obtained following the injection of estrogenic hormones. When mice of the C stock were nursed by females of the C3H strain, Shimkin and Andervont (50) and Shimkin, Grady, and Andervon...
It has been demonstrated that genetically nonsusceptible mice nursed by females of strains with a high incidence of mammary tumors actually do have the active milk influence, because when they are used to nurse susceptible mice a high incidence results in the fostered susceptible animals (15). The active milk influence and the amount of estrogenic stimulation produced in virgin or breeding females was able to overcome the threshold of genetic nonsusceptibility of the mammary glands in only a few animals. If the amount of estrogenic stimulation is increased by forced breeding or injection, a larger percentage of fostered genetically nonsusceptible animals develop tumors.

VIII. POSSIBLE ROLE OF EACH "INFLUENCE" IN THE ETIOLOGY OF MAMMARY CANCER

Since 1939 (14) three influences have been recognized in the etiology of the inherited type of mammary tumors in mice. May one of these be the "inciting influence" which causes the normal gland to become cancerous, or should they all be considered as "inciting influences"?

A summary of the results obtained under different experimental conditions in four strains of mice is given in Table I. Most of the observations were made on females but in a few cases it has been necessary to use the data obtained from males. Two of the strains had a high incidence and the others a low one when nursed by their maternal parents of the control stocks.

In experiments made to determine the role of each influence, the genetic constitution for inherited susceptibility or resistance to spontaneous tumors would probably remain constant during the life of the animals. The concentration of the active milk influence (20, 22) increases with the age of the females, particularly in mice which are used as breeders. It is likely, too, that if estrogenic hormones are secreted, causing development of the mammary tissue, the level of estrogenic stimulation also will increase as the mice of reproductive age become older.

If mice which are genetically susceptible (A) or genetically nonsusceptible (C57 black) are nursed by females of a strain with a high incidence of mammary tumors (active milk influence), they should obtain approximately the same amount of the milk influence and the concentration should remain the same in those of comparable ages. Furthermore, there is little reason to expect that the amount of estrogenic hormones secreted as the result of normal breeding would differ greatly in the females of these two strains. Thus it is probable that the concentration of the active milk influence and the estrogenic stimulation would be as

4 When nonsusceptible animals with the active milk influence were used to nurse susceptible animals, a high incidence of mammary tumors was observed in the susceptible mice (15).
nearly alike as is possible of attainment under experimental conditions. The chief variable would be genetic. In the genetically susceptible animals the incidence was 96 per cent (17) and in the genetically nonsusceptible mice 11 per cent (15). This difference must result directly or indirectly from variations in the genetic constitution of the susceptible and nonsusceptible animals. Whether or not the genetic susceptibility is "the mammary tumor inciter" in these animals cannot be determined. If the amount of estrogenic stimulation in the nonsusceptible animals was increased by forced breeding, the incidence was 50 per cent (9), and males of the C57 black strain with the active milk influence had an incidence of 33 per cent following the administration of estrogens (57).

While it is known that mammary carcinoma can arise only in a gland which has undergone a certain degree of development, it has not been determined whether the hormones merely prepare the gland for its initiation by some other influence or influences or act as a direct cause (36).

Since the initial stimulus resulting from the estrogenic hormones may take place months before a mammary tumor appears, it would seem that the hormones only prepare or condition the glands for the malignant change. An increase in the amount of estrogenic stimulation will prepare the glands for this transformation in a shorter period and indirectly increase the incidence of tumors, and the same results may be obtained by feeding mice various amounts of milk containing the active milk influence (16).

The architecture of the mammary glands of mice which have received the active milk influence differs from that seen in mice which have received the inactive milk influence (59, 51).

If mice which are susceptible to the development of spontaneous mammary tumors are permitted to have two or three litters before they are given milk containing the active milk influence, few tumors result; but if mice of the same stock are given milk with the active influence when they are 4 to 6 weeks of age, mammary tumors will develop in a high percentage, depending upon the amount and concentration of the active influence (16). The amount determines also the average tumor age. When milk containing the active influence is fed to adult females with mammary glands developed on the basis of an architecture resulting from an inactive milk influence, the active influence may be unable to take part in the development of mammary cancer. In fact it cannot do so unless it is able to affect the glands as soon as they start to develop. Thus the estrogenic hormones and the active milk influence would play similar roles in the etiology of mammary cancer in mice. Finally, the inherited susceptibility or nonsusceptibility to spontaneous mammary cancer must be considered as a determinant of the structure of the mammary glands (59).

Thus each of three influences in the etiology of the inherited type of mammary cancer in mice may be considered as a "mammary tumor preparatory influence or inciter." If one influence is not present or is inactive, the noninherited type of mammary cancer generally results. It is more likely, however, that the milk influence, genetic susceptibility, and estrogenic stimulation should be considered as complementary the one to the other and, as such, they might be called the "mammary tumor milk inciter (MTMI)," the "mammary tumor inherited inciter (MTII)" or "mammary tumor susceptibility inciter (MTSI)," and the "mammary tumor estrogenic inciter (MTEI)."

These symbols are useful only as abbreviations for the various inciters. It would lead to confusion if they were used to describe the concentration of the MTMI or the amount of stimulation produced by the MTEI resulting from intrinsic or extrinsic causes.

**IX. CONCLUSIONS**

For the development of mammary carcinoma in virgin and breeding females three agents usually must be present and active:

(a) An active mammary tumor milk influence which is generally transferred by nursing.

(b) Hormonal stimulation of the mammary tissue resulting in growth suitable for the cancerous change.

(c) An inherited susceptibility to the development of spontaneous mammary tumors which may be transmitted by males and females of the susceptible stock.

The active milk influence may be greater in some strains than in others, and may be more concentrated in individual females after they have given birth to three or more litters. The amount of the active milk influence that mice obtain may determine the incidence of mammary tumors, the average tumor age, and the incidence of mammary tumors observed in the progeny of experimental animals.

The active milk influence may originate following changes in the "inactive" milk influence, or de novo. It remains active in lyophilized tumors, in glycerol-treated and desiccated tissue, and in a filtrate of material containing it.

In association with intrinsic factors the active milk influence may be instrumental in determining the architecture of the mammary glands.

The amount of estrogenic hormones secreted by virgin females of various strains of mice may be determined by intrinsic factors, and is not causally related to the development of mammary cancer. The amoun
secreted by virgin females of some strains is evidently sufficient to result in neoplasms provided the other necessary stimuli are present and active. In mice of other strains the amount resulting from intrinsic causes must be supplemented by that furnished through such extrinsic causes as breeding or infection before many mammary tumors will develop.

A restricted diet may lower, and a diet rich in protein may increase, the amount of hormonal secretion.

Mice of strains with a low incidence of mammary tumors in breeding females may have:

(a) An inactive milk influence and be susceptible or nonsusceptible to the development of spontaneous mammary tumors.

(b) An active milk influence and be nonsusceptible to the development of spontaneous mammary carcinoma. If this active milk influence is transferred to genetically susceptible mice by nursing a high incidence of tumors will result.

Mice of one subline of a nonsusceptible strain may have the inactive milk influence, and those of another subline of the same strain may have the active milk influence. Only by testing can this difference be demonstrated.

The administration of estrogenic hormones will produce mammary tumors in males and females from strains of mice with the active milk influence. The induced incidence is usually higher in females than in males.

The stimulation produced by large amounts of estrogenic hormones may overcome the threshold of genetic nonsusceptibility in the presence of an active milk influence.

The incidence of induced estrogenic tumors is higher in mice that are genetically susceptible to the production of mammary tumors.

Few induced tumors have been elicited in mice that were genetically susceptible or nonsusceptible to mammary tumors and that had the inactive milk influence. The increased estrogenic stimulation was unable to overcome the threshold resulting from the inactive milk influence in susceptible or nonsusceptible tissue.

In the same strain of mice forced breeding with nonsuckling (the functional test) has produced a higher incidence of mammary tumors than has the administration of estrogenic hormones. Mammary tumors have not been observed to develop in many force-bred mice which did not have the active milk influence.

Two or more types of mammary carcinoma may develop in mice. These may be termed the inherited and noninherited types.

The noninherited mammary tumors may arise in mice that have the inactive milk influence and are susceptible or nonsusceptible, or in those that have the active milk influence and are nonsusceptible. The incidence of mammary tumors among the progeny of these cancerous females is the same as that characteristic of the strain. Many, if not all, of these tumors may be described as developing by a process analogous to somatic mutation in that the progeny of their bearers have not a high incidence.

The inherited type of mammary tumors develops in mice of strains that have the inherited susceptibility and the other active influences. The progeny of both cancerous and noncancerous females of these strains usually have a high incidence.

It is possible that mammary tumors produced by estrogenic hormones in nonsusceptible tissues may be grouped with the noninherited mammary tumors, and those produced in susceptible tissue with the inherited.

Only by observing the incidence of mammary tumors in the progeny of cancerous females is it possible to determine, in many instances, the type which an animal may have.

The influences underlying the development of mammary tumors in mice may each be considered as a "mammary tumor preparatory influence" or a "mammary tumor inciter."

If they are called inciters, a distinction should be made between the different varieties as: the mammary tumor milk inciter (MTMI); the mammary tumor inherited inciter (MTII) or the amount of stimulation of the MTEI; and the mammary tumor susceptibility inciter (MTSI); and the mammary tumor estrogenic inciter (MTEI).

These symbols are useful only as abbreviations and could not be used to describe the concentration of the MTMI or the amount of stimulation of the MTEI resulting from intrinsic and/or extrinsic causes.

X. REFERENCES


Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice

John J. Bittner


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