Vaginal Sensitivity to Estrogen as Related to Mammary Tumor Incidence in Mice

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The demonstration of the importance of ovarian secretion or exogenous estrogen to a high mammary tumor incidence in mice was followed by numerous studies of the estrous cycles of mice of different strains, in an attempt to correlate high mammary tumor incidence with some peculiarity of the cycle. In general, no significant and consistent correlation could be demonstrated in this regard. Korteweg and co-workers, however, focused attention on the possibility that, whereas outward manifestations of the cycle might be relatively constant, fundamental differences may exist in the sensitivity of the genital tissues to estrogen, and that such differences may be related to the mammary tumor incidence. Van Gulik and Korteweg (5) reported that of one high tumor and two low tumor inbred strains, compared with regard to vaginal estrogen-sensitivity, the high tumor dba strain was the most resistant to exogenous estrogen stimulus, requiring roughly 3 times the amount of estrogen needed by the low tumor C57 strain for a comparable degree of vaginal stimulation. They pointed out also that Bonser (1) observed a somewhat higher percentage of positive vaginal smears in a low mammary tumor strain than in a high mammary tumor strain when both were injected with equal but varying doses of estrogen. On the basis of such findings, they theorized that, since a normal estrous cycle is essential for continued existence, those strains of low vaginal estrogen-sensitivity probably produce larger amounts of estrogen and that this may be causally related to a high mammary tumor incidence. They reported also that reciprocal hybrids between the dba and C57 strains showed an almost equal vaginal sensitivity to estrogen, intermediate between that of the parental strains, indicating that vaginal susceptibility to estrogen bears no relation to the maternal extrachromosomal factor.

Mühlbock (2, 3), from the same laboratory, confirmed the higher estrogen requirement (5–7 times as much) of the high tumor dba strain as compared to the low tumor C57 and 020 strains for a comparable degree of vaginal stimulation by either intravaginal or subcutaneous administration of estrogen.

Shimkin and Andervont (4) also reported on the vaginal estrogen-sensitivity of three strains of mice of known mammary tumor incidence—the C57, C, and C3H strains. Again the high tumor strain was more resistant, the C57 strain requiring twice as much estrogen as the low tumor C57 and C strains for a positive response in 50 per cent of the mice. It was also reported that, whereas foster nursing of the high tumor strain by a low tumor strain, or vice versa, altered the mammary tumor incidence, it did not alter vaginal sensitivity to estrogen.

The present investigation was undertaken to determine whether the reported inverse relationship of vaginal estrogen-sensitivity and mammary tumor incidence is of universal occurrence or restricted to the few inbred strains of mice studied thus far. In view of the apparent lack of effect of milk factor on vaginal estrogen-sensitivity, it would seem improbable that such an inverse relationship could apply to all strains of mice, since some strains may have a low mammary tumor incidence only because of the absence of the milk factor. Nevertheless, such an inverse relationship might conceivably apply when comparison is made only between strains known to possess the milk factor.

MATERIALS AND METHODS

Included in the present study are mice of three strains of high mammary tumor incidence, the C3H, CBA, and A strains, and three of low tumor incidence, the C57, JK, and N strains. In addition, mice of two high tumor strains free of milk factor...
as a result of foster nursing are included. These are the C3H and A strains, designated, respectively, BL and AL. Also studied were three groups of F1 hybrids, the CC1 (C57 ♂ X CBA ♀), the CC2 (CBA ♀ X C57 ♂), and the AB2 (CBA ♀ X A♂).  

The mammary tumor incidence of breeding females of the inbred strains is presented in Table 1. These data are compiled largely from Dr. Gardner's colony over the period of approximately the past ten years and from the relatively fewer animals of the author's colony during the past two years. Both colonies were maintained under comparable conditions. Breeding mice received Purina laboratory chow, and nonbreeding experimental animals received Purina Fox Chow. Adequate numbers of animals are not available to report mammary tumor incidence for the N strain under comparable conditions. However, mammary tumors are rare in mice of this strain in Dr. Strong's colony, and none have been observed in the few breeding females used for the present experiment.

Groups of approximately twenty virgin female mice of each of the stocks mentioned previously were ovariectomized at approximately 1 year of age (8–14 months range), allowed to rest for 0.5–7 weeks, then smeared 4 times on alternate days. In the JK strain many of these smears contained, in addition to leukocytes, numerous small nucleated epithelial cells, indicating a possible extra-ovarian source of estrogen. After estrogen administration, the smears became fully cornified, followed by a completely diestrous type of smear. It is probable, therefore, that during the course of the assay, involving repeated estrogen injections, the amount of endogenous estrogen was negligible. Nevertheless, the JK strain is included in the present report with the reservation that it may possess a post-castration extra-ovarian source of estrogen, in the absence of which responsiveness to exogenous estrogen might have been lower.

A single injection of estradiol benzoate in 0.05 cc. of sesame oil was used. Smears were taken by lavage, and only a fully cornified smear with no more than occasional leukocytes was considered positive. The mice were primed with 0.1 μg of estradiol benzoate, and assays were performed at 1–2-week intervals thereafter, depending on the amount of the previous injection and the length of time required for a return to a negative smear. A graded series of estrogen dosages were administered, and different dose levels repeated as often as necessary to obtain a fairly smooth sigmoid type dose-response curve for each strain.

The dams of the mice used for vaginal assay were kept to determine mammary tumor incidence. Two of the BL dams developed mammary tumors—one an adenocarcinoma and the other a squamous-cell carcinoma with small scattered areas of adenocarcinoma. Progeny of both of these mice were eliminated from the experimental series.

The incidence of mammary adenocarcinomas in the BL strain, since it was obtained from Dr. Bittner in 1941, has been 6.2 per cent. These tumors have appeared relatively more frequently in recent years, raising some question as to the complete absence of milk factor in this strain at the present time. The data for the BL strain are nevertheless presented, since the tumor incidence is still much below that of the C3H strain, and progeny of tumor-bearing animals have been eliminated.

RESULTS AND DISCUSSION

The percentage of positive vaginal smears obtained from mice of the different strains at the various levels of estrogen are presented in Table 2. The dose-response curves for the different strains are presented graphically in Figures 1, 2, 3, and 4. Table 3 lists the different strains in decreasing order of vaginal sensitivity, as measured by the minimum dose of estrogen required for a positive response in approximately 50 per cent of the mice.

The dose-response curves of the three high tumor and the three low tumor inbred strains do not affirm the reported inverse relationship of vaginal estrogen-sensitivity and mammary tumor incidence. The range of sensitivity represented by the two groups of curves overlaps considerably. It is apparent why an inverse relationship might be indicated in the comparison of a smaller number of strains, one of which is the low tumor C57.
strain. This proved to be the most sensitive of the several strains tested.

If one considers only those inbred strains known to possess milk factor, the C3H, CBA, and A strains, the mammary tumor incidence of these strains is directly proportional, rather than inversely proportional, to vaginal estrogen-sensitivity. Even this relationship does not apply if one also considers the fact that the C57 strain, after exposure to the milk factor, generally shows a relatively low incidence of mammary tumors, while vaginal estrogen-sensitivity remains unaltered.

### TABLE 2

Percentage of positive vaginal smears obtained from mice of different strains treated with graded doses of estrogen

<table>
<thead>
<tr>
<th>Strain of hybrid group</th>
<th>Micrograms of estradiol benzoate</th>
<th>Per cent positive/Number of smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>0.4</td>
<td>95/25</td>
</tr>
<tr>
<td>JK</td>
<td>0.3</td>
<td>85/25</td>
</tr>
<tr>
<td>C3H</td>
<td>0.27</td>
<td>86/25</td>
</tr>
<tr>
<td>BL</td>
<td>0.25</td>
<td>87/25</td>
</tr>
<tr>
<td>CC1</td>
<td>0.22</td>
<td>89/19</td>
</tr>
<tr>
<td>CC2</td>
<td>0.20</td>
<td>91/23</td>
</tr>
<tr>
<td>CBA</td>
<td>0.15</td>
<td>94/17</td>
</tr>
<tr>
<td>AB+</td>
<td>0.12</td>
<td>91/21</td>
</tr>
<tr>
<td>N</td>
<td>0.10</td>
<td>96/24</td>
</tr>
<tr>
<td>A</td>
<td>0.08</td>
<td>96/26</td>
</tr>
<tr>
<td>AL</td>
<td>0.06</td>
<td>96/24</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>96/24</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>96/24</td>
</tr>
</tbody>
</table>

### TABLE 3

Minimum amount of estrogen giving positive vaginal smears in approximately 50 per cent of the mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Micrograms of estradiol benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>0.06</td>
</tr>
<tr>
<td>JK</td>
<td>0.08</td>
</tr>
<tr>
<td>C3H†</td>
<td>0.1</td>
</tr>
<tr>
<td>BL</td>
<td>0.08–0.1</td>
</tr>
<tr>
<td>CC1‡</td>
<td>0.08–0.1</td>
</tr>
<tr>
<td>CC2‡</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Low mammary tumor inbred strains.
† High mammary tumor inbred strains.

![Vaginal Response to Estrogen](image)

Fig. 1.—Percentage of positive vaginal smears from mice of three high tumor and three low tumor strains treated with various levels of estrogen.

![Vaginal Response to Estrogen](image)

Fig. 2.—Percentage of positive vaginal smears from mice of the A strain and fostered A strain treated with various levels of estrogen.

In short, no consistent correlation is demonstrable between mammary tumor incidence and vaginal sensitivity to estrogen.

Mice of the A and AL strains, and of the C3H and BL strains, showed remarkably similar vaginal response to the different levels of estrogen. The presence or absence of milk factor as a result of foster nursing did not significantly alter vaginal sensitivity to estrogen. This is in agreement with the findings of Shimkin and Andervont (4). The similarity of these two sets of dose-response curves is also of interest in view of the many years during which the lines of descent of the fostered and non-fostered were maintained.
fostered mice of these two stocks have been separated.

The dose-response curves for the three groups of hybrids used and for the three parental inbred strains from which they were derived are presented in Figure 4. The reciprocal hybrids between the high tumor CBA and the low tumor C57 strains show a vaginal sensitivity intermediate between that of the two parental stocks; whether the female parent was from the high tumor or low tumor strain made very little difference. As mentioned above, similar results have been reported by Van Gulik and Korteweg (5), using reciprocal hybrids between the dba and C57 strains.

The AB2 hybrids between the two high tumor strains, CBA and A, also show a vaginal estrogen-sensitivity intermediate between that of the parental strains.

SUMMARY

1. The inverse relationship between vaginal sensitivity to estrogen and mammary tumor incidence that has been reported for limited numbers of strains of mice was found not to apply when larger numbers of strains were studied. This was the case when either all strains or only those known to possess milk factor were considered.

2. The presence or absence of milk factor, obtained by foster-nursing or by reciprocal crossing of a high and a low tumor strain, did not alter vaginal sensitivity to estrogen.

REFERENCES


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