Establishment of Mice Strains with Special Reference to Mammary Growth Response

VI. Sensitivity of mammary glands

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The sensitivity of the mammary glands of mice has been studied by many investigators, especially in connection with mammary tumorigenesis. TRENTIN reported that in castrated male mice of strains highly susceptible to mammary tumor development, the duct system of the mammary glands was more responsive to estrogen than in those of less susceptible strains. MÜHLBOCK reported that the mammary glands were equally sensitive to estrone in gonadectomized mice of certain high and low tumor strains.

Establishment of mice strains with special reference to mammary growth response has been conducted in the author's department. In this breeding experiment, normal immature mice treated with a standard dose of estrogen were used because of mainly operational efficiency in conducting the experiment. Based on the value of the mammary duct area determined at 35 days of age, value being called mammary growth response, the mice were divided into two groups.

The present work was carried out for the purpose of computing the portion (degree of participation) of mammary gland sensitivity in the mammary growth response of female mice and comparing such portion in the mammary duct area between treated and intact female mice of the same age.

Materials and Methods

Analytical method: The mammary growth of an animal may be defined as the sum of the effects of factors contributing to the mammary growth. As pointed out by NAITO et al., such two factors as the sensitivity of the mammary gland (S) and the hormonal quantity concerned (H) should take part in the mammary growth. Moreover, an error factor (E) concerning the procedure to prepare the mammary gland and to measure the mammary growth should be considered as an additional factor. Thus the aggregate value of the mammary growth (M) of an animal is

\[ M = S + H + E. \]

where \( M, S, H, \) and \( E \) represent deviations from means due to each agency.

If no correlation is assumed between the effects of factors, variance \( M (V_M) \) equals to the sum of three variances of the effects; that is,

\[ V_M = V_S + V_H + V_E. \]

From this formula, the portion due to the mammary gland sensitivity in the mammary growth may be expressed as

\[ \frac{V_S}{V_M} \times 100 \% . \]

Measuring the mammary duct area: The third right mammary glands were removed from the skin and prepared by the whole mount method. The mounted glands were projected on a screen glass at 10\( \times \) magnification, so that the perimeter of each gland might be traced. The areas in \( \text{mm}^2 \) were determined with a planimeter.
Experiment 1. Computing the portion due to estrogen factor: Randomly bred, female Kasukabe mice (K, KII) were used. Litter females were divided into two of three groups consisting of (1) gonadectomized and estrogen-treated, (2) estrogen-treated, and (3) intact ones, respectively. Gonadectomy was carried out at 19 days of age. Estrogen treatment was exactly the same as described in the previous paper\(^3\). Measurement of the mammary duct area was made after mice were killed at 35 days of age.

Experiment 2. (a) Effect of the degree of skin extension on the mammary duct area: Skin specimens, including mammary glands, were collected from 9 female mice treated with a standard dose of estrogen and from 9 female mice without treatment and extended on a cork plate with a practically constant tension (Fig. 1). Immediately after the extension, the left half portion of a skin specimen was put off the plate and then re-extended to be 0.8 times as long as the original one. Then the specimen was fixed in Bouin solution and measurement of the mammary duct area was made.

(b) Reproducibility of skin extension: Eighteen male mice were divided into 6 groups. Under the condition of 17°C and 60% humidity, 3 mice of a group were killed successively. After a skin specimen was harvested from one of the 3 mice, extended on and put off the plate, skin specimens from a 2nd and 3rd mice were extended on and put off the plate. The same procedure was repeated 3 times for each group of mice. Measurement of the skin area was made for each extension.

Results

Experiment 1. Table 1 shows the variance of the mammary duct area in three groups. Variance in the intact female group seemed to be the largest among the three groups. The smallest variance in the spayed, estrogen-treated group consists of the sum of \(V_s\) and \(V_E\). On the other hand, the variance in the estrogen-treated normal group should consist of the sum of \(V_s\), \(V_E\) and \(V_{NE,SE}\), in which \(NE\) and \(SE\) mean the natural estrogenic substance secreted from the ovary and exogenous synthetic estrogen, respectively. On the basis of this partition of variance, the portion \(V_{NE,SE}\) was computed. In the K and the KII subgroup, the portion \(V_{NE,SE}\) was 6% of total variance (359.96) and 13% of total variance (491.24), respectively, the average being 10%. From these values, the portion \(V_s+V_E\) of the KII subgroup was computed to be 87~94% (357.87~387.53) of total variance (413.10). Thus, the portion \(V_{NE}\) in the intact female group may be computed as follows:

\[
\frac{535.34-387.53}{535.34} \times 100 = 28\% \quad \text{or} \quad \frac{535.34-357.87}{535.34} \times 100 = 33\% 
\]

Accordingly, the average of portion \(V_{NE}\) was 30% in the intact female group.
A graphical representation of the document content is not possible. The content is text-based and can be read as if naturally written. The text provides experimental results on the sensitivity of mammary gland area and the effect of skin extension on the measurement of mammary duct area. It includes tables summarizing the results of experiments involving the comparison of mammary duct areas in different groups of mice and the investigation of error factors due to skin extension.

**Table 1. Variance of mammary duct area in three groups of ovariectomized-estrogen-treated, estrogen-treated, and intact female mice.**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Mammary duct area</th>
<th>Component of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>10</td>
<td>45.9 (+33.6)</td>
<td>Vₛ + Vₑ</td>
</tr>
<tr>
<td>♂</td>
<td>16</td>
<td>53.0 (435.54)</td>
<td></td>
</tr>
<tr>
<td>♀</td>
<td>10</td>
<td>48.2 (+395.06)</td>
<td>Vₛ + Vₑ</td>
</tr>
</tbody>
</table>
| ♀     | 17          | 76.0 (491.24)     | Vₛ + Vₑ + Vₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉᵉ>e":141}
Generally, the word "sensitivity" (in a broad sense) seems to indicate the potential capacity, including such factors as permeability and distribution of blood vessels, of the mammary gland responding to the hormones concerned. It was assumed in the present work that the sensitivity was not correlated with the quantity of the stimulating hormones, since the sensitivity in a narrow sense of the word corresponds to an animal's genotype of mammary duct growth. The difference between the portion of sensitivity in the mammary growth response (0.86) and that of genotype in the mammary growth response (0.51) by NAGAI3) could be attributable to the effect of such factors as permeability and vascularity, provided that these portions were well estimated. These factors should be distinguished from the sensitivity in a narrow sense, because confusion might arise in explaining the possible phenomenon that animals kept under a low level of feeding often show low sensitivity in a broad sense of the word.

Although only exogenous and endogenous estrogenic substances were taken as hormonal stimulators of the mammary gland, it is well known that the hormones secreted from the pituitary and adrenal play an important role in the mammary duct growth as illustrated by LYONS6). Further studies are needed to take account of several hormones contributing to the mammary duct growth. Nevertheless, it is worthy to note that the portion of sensitivity was larger than that of the estrogenic factors in the intact female group, suggesting the importance of sensitivity when selection for mammary parenchyma was intended. It was also presumed that the selection for mammary parenchyma would be more effective in using treated female mice than normal female without treatment, because the portion of sensitivity was larger in the former. Two groups were established with special reference to mammary growth response in the breeding experiment in the author's department. These results here are interesting in comparison with the work by YOSHIDA7) who reported that a group of mice responding markedly to a standard dose of estrogen at the immature stage required a half dose at the stage prior to pregnancy to develop the same mammary duct area as in the other group of mice responding poorly at the immature stage.

NAITO et al.8) induced lactation in heifers treated with synthetic estrogen and discussed a possibility of predicting the milk yield of normal lactation from the record of induced lactation. It is very interesting to find out analogy regarding hormonal treatment between the studies on heifers and mice both treated with estrogen. In the studies using mice the portion of estrogen factor in the mammary growth response was approximately 10 per cent because of the situation that the quantity of estrogen administered was too much larger than that of the endogenous estrogenic substance secreted from the ovary. In the studies on heifers, the portion of estrogen in the induced milk yield should not be significant, because implication of selection for dairy performance in response to a certain standard dose of estrogen given would be made.

The mechanism of manifestation of the dairy performance was analyzed theoretically by NAITO et al.4). On the other hand, in the field of etiology, multiple factors leading to atherosclerosis were illustrated by FRIEDMAN et al.9). Such analytical discussion including all interacting factors, should be followed by investigations which place varying degrees of emphasis on any given factor without disturbing the basic integrity of the mechanism. In the present investigation an attempt was made to exploit a concept10) in population genetics to the mechanism of mammary duct growth, although its scheme was rather simple from the standpoint of endocrinology and needs further studies.
The sensitivity of mammary glands

Summary

For the purpose of computing the portion of sensitivity in the mammary duct area in mice 35 days of age, female mice were divided into three groups: (1) a spayed and estrogen-treated group, (2) an estrogen-treated group, and (3) an intact group. A standard dose of estrogen was given from 20 to 35 days of age. At 35 days of age areas of mammary ducts were measured. Based on the explanation of the variance of the mammary duct area in the three groups, analysis was made.

The portion of estrogen factor was approximately 10 per cent in the estrogen-treated group and 30 per cent in the intact group. The error portion was estimated to be 4 per cent in both groups. Thus the portion of sensitivity was estimated to be 86 per cent in the estrogen-treated group and 66 per cent in the intact group. The results obtained suggest primarily that emphasis should be placed on the sensitivity rather than the estrogen stimulator for mammary growth and that the use of estrogen-treated mice may be efficient than the use of intact female mice, when selection for mammary parenchyma is intended.

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References