Establishment of Mice Strains with Special Reference to Mammary Growth Response

VIII. Selection with inbreeding for the mammmary growth response

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It has long been known that lactation in livestock and laboratory animal is rather complicated character on its mechanism of manifestation. Many investigations have been carried out on the way how to improve it.

NAITO et al. (1955) analysed a dairy performance mainly from the standpoint of endocrinology and they considered that the dairy performance will be improved by the improvement of simple constitutive character of it. Based on the explanation, selection for the mammary growth response (mammary duct area in response to a standard dose of estrogen) has been carried out in mice. The mammary growth response shows the sensitivity of mammary gland to the hormone concerned (NAGAI 1960) and is considerably heritable (NAGAI 1959). Choice of the mammary growth response in order to improve lactational performance may be supported by the work of MIXNER and TURNER (1943) reporting that “considerable milk secretion can occur from a well extended duct system”.

Present study concerns with the rate of progress of the mammary growth response.

Material and Method

In 1954, seventy of Kasukabe mice were introduced in our Department which have been kept among farmers in northern district of Tokyo. From the date of arrival in our Department, full-sib mating (mating of one brother with two sisters as a rule) was carried out every generation after random breeding where mating of one male with one female was carried out.

On the basis of the mammary growth response, selection to modify population mean was carried out in the group as the positive directional selection group (nominated as KA group), with the control group (without the selection, nominated as KB group) and simultaneously the selection to reduce the variability of the mammary growth response was carried out in both groups. In the directional selection, the sense of sib-test was applied (NAGAI 1959). Based on the mean and variance of the mammary growth response of full-sibs which was recorded separately in both sexes, selection was carried out every third generation. Parents with superior offsprings over population mean and gains in the KA group were in detail tabulated in Table 1. Changes of the mammary

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Table 1. Data on the adopted dams with superior offspring over population mean and on gains in the KA group

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. of tested dams</th>
<th>No. of adopted dams for further reproduction</th>
<th>( \varphi )</th>
<th>( \delta )</th>
<th>( \varphi \delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>2</td>
<td>21.7</td>
<td>2.2</td>
<td>15.4</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>2</td>
<td>8.3</td>
<td>32.7</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>2</td>
<td>14.9</td>
<td>-0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>2</td>
<td>16.9</td>
<td>15.4</td>
<td>-0.8</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>3</td>
<td>24.1</td>
<td>9.7*</td>
<td>2.5</td>
</tr>
</tbody>
</table>

M: Mammary growth response. \( W_{35} \), \( W_{12} \): Body weight of 35-, 12-day-old mice

*: Difference of mean M between 12th and 15th generation

growth response and body weight on 35 days in successive generations in response to the selection in the KA group were plotted in Figure 1. These graphs were not only subjected to visual judgement and supplemented by statistical analysis. This can best be done by fitting regression lines to the generation means, since the regression line represents the best estimate of the true response from the actual data.

In order to exclude the effect of difference of body weight on the mammary growth response, corrected mammary growth response for mean body weight at generation concerned, using linear regression of mammary growth response on body weight in either sex was calculated and was used as a selection criterion at the 6th and 9th generation. Through the breeding experiment, litter size was increased or reduced to six approximately to omit the litter size difference on the mammary growth response.

Heritability was roughly estimated from the response to selection, using the formula,

\[
h^2 = \frac{\text{progress under selection}}{\text{average superiority over population mean}} = \frac{1}{2} \left( \text{superiority } \varphi + \text{superiority } \delta \right).
\]

Code number (1, 2, 3 and 4) was given for generation 6, 9, 12 and 15 and numerator was given by the slope of regression line of the mean mammary growth response on generation because little reliance could be placed on the means of individual generation, owing to the sampling errors. As a denominator, simple average superiority over population mean was adopted, although FALCONER and KING (1953) reported the use of the mean selection differential in generations weighted according to the number of litters contributed by each pair of parents to the measurement of the next generation. The variance of the heritability estimate was taken to be \( \sigma^2 / s^2 \), where \( b \) was the rate of response, and \( s \) the average superiority over population mean.

Genetic correlation \( (r_G) \) was estimated from the following formula described in the paper by FALCONER (1954),

\[
r_G = \frac{\Delta G'}{\Delta G} \frac{h}{h'} \frac{\sigma_p}{\sigma_p'},
\]

where \( \Delta G, h \) and \( \sigma_p \) stand for the genetic change, positive root of heritability and standard deviation respectively in the character selected, prime being those in the correlated character not directly selected.
Further, body weight of 12-day-old mice (mice of the 12th day of life) was recorded as an indicator of lactational performance in mother mice.

**Results**

Changes of mean and variance in the mammary growth response and body weight at autopsy in successive generations are shown in Table 2 and diagrammed in Figure 1.

Table 2. Mean (m) and variance (v) of the mammary growth response (M) and body weight (W) of 35-day-old mice in successive generations of full-sib mating

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>m</td>
<td>v</td>
<td>No.</td>
<td>m</td>
<td>v</td>
<td>No.</td>
</tr>
<tr>
<td>KA</td>
<td>M</td>
<td>58</td>
<td>46.7</td>
<td>705</td>
<td>178</td>
<td>68.4</td>
<td>553</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>58</td>
<td>11.3</td>
<td>8.56</td>
<td>178</td>
<td>13.5</td>
<td>5.23</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>M</td>
<td>56</td>
<td>29.9</td>
<td>374</td>
<td>131</td>
<td>45.3</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>56</td>
<td>12.0</td>
<td>16.44</td>
<td>131</td>
<td>14.5</td>
<td>5.57</td>
</tr>
<tr>
<td>KB</td>
<td>M</td>
<td>20</td>
<td>48.3</td>
<td>474</td>
<td>79</td>
<td>48.3</td>
<td>429</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>20</td>
<td>12.2</td>
<td>6.40</td>
<td>79</td>
<td>11.8</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>M</td>
<td>11</td>
<td>29.2</td>
<td>361</td>
<td>61</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>11</td>
<td>13.0</td>
<td>7.30</td>
<td>61</td>
<td>12.9</td>
<td>4.41</td>
</tr>
</tbody>
</table>

In the mammary growth response, there was a gradual increase up to the 15th generation in the KA group though the rate of increase was not so uniform. In the control group the mammary growth response fairly increased up to the 6th generation and was maintained constantly. Although the cause of the increase up to the 6th generation is not known exactly, the possible one may be the change of environment to which mice were exposed and also unconscious selections for body size at intermittent non-selection generations which is correlated with the mammary growth response both phenotypically and genotypically. The conjecture is based on the fact that the parents on the 2nd generation in the KB group which produced females with $30.7 \pm 7.7$ mm$^2$ in the mammary growth response and $10.2 \pm 1.0$ g in the body weight were used for further reproduction and the females on the 6th generation showed $73.6 \pm 5.1$ mm$^2$ in the mammary growth response and $16.8 \pm 0.7$ g in the body weight. Thus the change of the mammary growth response until the 6th generation in the KA group was not considered as the result of selection for the mammary growth response.

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Regression lines were fitted on the generation mean from the 6th to 15th generation in each group. The slopes of the regression lines in each sex and group were shown in the last column of Table 2, the slopes of the mammary growth response in the KA group being significantly positive in contrast with those in the KB group. From the change in the KA group it is clear that the effect of selection was appreciable though not so remarkable in the male and that the role of environment acting on the mammary growth response might be important. When computation was made to estimate heritability, the estimate was $0.497 \pm 0.324$ and $0.279 \pm 0.428$ in female and male respectively, the face value suggesting possibly of further genetic improvement in the mammary growth response.

On the other hand, body weight at autopsy increased gradually in both sexes in the KA group up to the 15th generation. In the KB group, the body weight increased up to the 6th generation and thereafter did not show further increase. It is interesting that the change of the body weight for which selection was not carried out was similar to the trends of change of the mammary growth response though the scale in Figure 1 was not adjusted so that one standard deviation never occupied the same vertical distance for each character. When regression lines were computed at the range from the 6th to 15th generation in the KA group, the slopes were 0.280 and 0.340 in female and male respectively although these were not significant statistically. When these values were applied to the formula used before, the estimate of heritability was $0.290 \pm 0.272$ and $0.379 \pm 0.050$ in female and male respectively. When average variance of last four generations and heritabilities were used, the genetic correlation between the mammary growth response and body weight at autopsy was estimated as 0.62 and 0.47 in female and male respectively.

Table 3 shows mean and variance of body weight on the 12th day of birth in the KA group. Figure 2 shows the change of average mean body weight of both sexes in the same group. As seen in the figure, the tendency of change of the body weight was not so uniform but similar to the tendency of the change in the mammary growth response and body weight at autopsy. When regression line on generation was computed, the slope was 0.092. The last column of Table 1 shows the superiority of the body weight of 12-day-old mice over population mean which were produced from parents adopted for further reproduction based on their progeny records on the mammary growth response. The fact that the slope was not significant statistically may be mainly dependent upon a single generation mean at the 9th generation and the slope may be considered to include some import.

Phenotypic correlation coefficient between the 12-day—

--- 45 ---
weight and mammary growth response both in terms of generation mean was 0.95 in the female of the KA group. As seen in Figure 3, the mammary growth response increased with increasing 12-day-weight in the KA group from the 6th to 15th generation.

Discussion

Continued inbreeding when supplemented by rigid selection is the quickest and surest method of fixing and perpetuating a desirable character or group of characters. Superior lines or strains of a variety of animals have been established by the use of inbreeding with selection. It is, however, general agreement that successive consanguine mating induces reduction in viability and reproductiveness and then results in extinction of a group of animals concerned. Protecting against or overcoming of the inbreeding depression has been reported by several workers. WATERS and LAMBERT (1936) developed a high degree of homozygosity in White Leghorns by inbreeding less intense than that of brother sister matings and maintained careful selection for hatchability. In the present study, the closest inbreeding was carried out for the purpose of yielding of genetic homozygosity of the strains of mice. But any remarkable decrease of viability in terms of body weight was not found until the 15th generation of full-sib mating. The reason why viable groups of mice were established may be mainly attributable to the selection for and maintenance of the mammary growth response which manifests predominantly the sensitivity of mammary gland to estrogen and may be one of the important factors in lactational performance of mice. In fact, the mammary growth response increased in one group and was maintained unaltered in the other group. Thus it may be probable that the groups of mice might give so favorable maternal environment to their offsprings that they grew well and composed of viable groups of mice.

McLAREN and MICHIE (1956) considered that for a trait physiologically correlated with vigour, high degree of homozygosity of individuals in colony was responsible to the large variability of a trait concerned. However, in the present study reduction of variability was seen in the KA and KB group of mice in spite of attaining at high degree of homozygosity of individuals to which the work by YOSHIDA (1961) is referred. The evidence suggests the existence of some important limiting factors in regulation of variability. It
is general conception that reduced viability of inbred individuals makes them more susceptible to uncontrolled fluctuations in their environment and thus produces a greater range of phenotypic expression of their metric traits (cited by Lerner 1954). According to the conception, one of the important factors may be viability of individuals which owes much to the selection for and maintenance of the mammary growth response.

The increase of the mammary growth response was seen in the directional selection group (KA). Although some fluctuations were shown, the mammary growth response increased to the extent of about 70% of standard deviation of the character during the stage from the 6th to 15th generation. The cause should be the selection applied to the group, in consideration of the fact that in the KB group, the mammary growth response remained constant. In the course of selection, the pressure applied was considerably intense as seen in Table 1 and the gains were rather larger in the successive generations. The reason for such improvement may be partly attributable to the accuracy of selection adopted. Though the heritability estimate of the mammary growth response was considerably high (Nagai 1959), the selection was carried out on the basis of more than two litters of full-sibs' record, depending on the situation that the estimate of heritability included the fraction of total variance that was not of importance for breeding scheme. Such method applied may contribute to the improvement of the mammary growth response, substantiated by the given vital force as noted before.

The other reason for the improvement may be an existence of genetic variability of the character concerned in spite of the use of a small number of foundation stock. Heritability roughly estimated from the consequence of selection during the 6-15th generation was unexpectedly considerable. Though the figures seem to encourage the hope in further improvement of the mammary growth response, the situation may not be so optimistic because of property of heritability i.e. heritability is, in addition to including sampling errors, subjected to variation whenever either the numerator or the denominator was altered. If similar tendency of decreasing variance of genotypic and environmental source was seen, heritability should be unaltered in spite of the decrease of genetic variance due to inbreeding. In such explanation, further improvement may not be expected from the apparent existing genetic variance.

Craft et al. (1951) suggested ingeniously that the expectations based on heritability analysis had not always been fully realized in the gains made by selection. According to the paper by Reeves and Robertson (1953) on the experiment on Drosophila, the heritability estimated from offspring-parent regressions was as high as 50% at the end of selection where increased wing length had reached a limit and remained unaltered for thirty generations. Lerner (1958) pointed out that after a population had been under fairly intense pressure for a number of generations, it is not uncommon that response to further selection become erratic. The probable explanation was gradual decay of additive genetic variability and the consequent increase in the importance of complex non-additive interactions might play main role. And also it cannot be overemphasized that the force of genetic homeostasis (Lerner 1954) is included as the significant influence although the force is only now beginning to be explored and the information on it is
currently not available. On these not yet unknown complications informations are necessary to predict the further response to selection within specifiable limits of sampling errors (Lerner 1958).

The cause of the improvement obtained may also be sought for the intermittent selection i.e. alternating periods of selection and no selection. There seems to be some indication from experiments to suggest that occasional relaxation of selection may be efficacious practice (Lerner 1958). In the experiment of Mather and Harrison (1949) with Drosophila, suspension of artificial selection pressure at a certain point in the phylogeny of a line selected for bristle number not only saved it from extinction but permitted the line eventually to reach a higher level of the selected trait. Scossioli (1954) who gave further relevant information on this subject based on the experiment of selection for bristle number in Drosophila where he applied artificial selection pressure only every other generation. The empirical evidence on the subject is admittedly meager, but it is not inconsistent with the possibility that intermittent selection may have practical value (Lerner 1958). Though it is a question whether the intermittent selection applied in the present study played an important role for the rise of the mammary growth response, the selection might contribute to the evidence obtained to some extent.

The body weight on 35 days was recorded as an indicator of viability of an individual. As seen in Figure 1, the weight increased to some extent corresponding to the increase of the mammary growth response. The evidence indicates clearly the existence of some relationship between them. They were considerably correlated in terms of phenotypic and genetic correlation, suggesting the existence of a general size factor acting on both characters. Though the description on the common causal factor was made in a separate paper (Nagai 1961), this is to state here that they belong to the category of growth and were positively correlated from beginning to the last stage of selection for the mammary growth response. In the course of inbreeding, a considerable number of mice was discarded mainly on the basis of the mammary growth response. Therefore, the change of phenotypic and genetic correlation in successive generations might theoretically seen. Besides the uncertainty whether or not the selection procedure altered the correlation between them, it is of interest to investigate actually the effect of selection for the body weight at autopsy on the mammary growth response, based on the correlation between them (Nagai 1959).

There is some ambiguity in the interpretation of 12-day-weight of young because the relative importance of the mother and young themselves is not precisely known. In 1954, Bateman, discussing the preweaning weight of litters of suckling mice in relation to the measurement of lactation, concluded that random environmental and genetic differences among the young and intra-uterine and postnatal maternal influences were all approximately equally important in determining the variations in individual 12-day-weights. This subject may be stated generally as follows: the 12-day-weight is determined predominantly by the maternal influence under a strictly controlled condition at the generation where close inbreeding has been continued for a long time so that genetic uniformity is endowed to the population concerned. In the present study, the 12-day-
weight in the KA group increased to some extent in correspondence to the increase of
the mammary growth response in successive generations although birth weight was not
altered (average birth weight was 1.55, 1.55, 1.62 and 1.56 g at the 6, 9, 12 and 15th ge-
neration respectively). Thus the results may be interpreted to mean that the improve-
ment of the mammary growth response evoked that of maternal influence including
lactation, provided that the genetic and random environmental variation were small.
But it is necessary to conduct further studies on this problem.

Summary

Evidences on the effect of directional selection for the mammary growth response were
reported together with the effect of the selection on the 12- and 35-day-weight of mice.
Full-sib mating was continued and the selection for the mammary growth response was
taken place every third generation. In the directional selection group, the mammary
growth response increased from the 6th to 15th generation by the selection under the
intense inbreeding, the body weights showing some increases. According to the estimate
of heritability computed from response to the selection, genetic variance in the mammary
growth response appeared to be present. Genetic associations of the mammary growth
response with the 35-day-weight were suggestive.

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Establishment of mouse strains different in
mammary growth response by selection with
乳腺発育反応の異なるマウス系統の育成 VIII.
選抜並びに兄姉交配の効果

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（東京大学農学部）

マウスにおける泌乳性と関係があると思われる。未成
熟マウスの乳腺発育反応に対する選抜を2代おきに行い
つつ、15 代にわたって兄姉交配をかきわたった。

選抜区において乳腺発育反応は增大し、雌において特
に著しかった。35 日齢体重は乳腺発育反応の変化の傾
向とかなりよく似ていた。各形質の遺伝力並びに形質間
の遺伝相関の推定を試みた。