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Relationship Between Incidence and Onset Age of Mammary Tumors and Reproductive Characteristics in Mice*

HIROSHI NAGASAWA and YUKO NAKAJIMA

Pharmacology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan.

Abstract—*Simple and partial correlation coefficients between incidence or onset age of spontaneous mammary tumors and several reproductive characteristics were examined during the period between the 2nd and the 30th generations of brother × sister mating in SLN female mice as a possible step to evaluate the role of reproductivity in mammary tumorigenesis. Mammary tumor incidence had a simple negative correlation with delivery interval and positive simple correlations with pup's weights at* days 12 *and* 20 *or pup's growth rate at* day 20 *as indices of lactational performance. However, the partial correlation coefficient between mammary tumor incidence and delivery interval was not statistically significant when any index of lactational performance was held constant and* vice versa. *These results indicate that it is difficult to determine which of these characteristics is more primary for mammary tumor incidence, while both participate in this process. No significant simple correlations were observed between onset age of mammary tumors and any reproductive characteristics, suggesting little dependence of progression of this type of mammary tumors on reproductivity,*

INTRODUCTION

IN OUR laboratory, a basal stock of Swiss albino mice, which was transferred from the National Institute of Genetics, Mishima, Japan, as a non-inbred closed colony, was prepared and mice were selected for high and low mammary tumorigenesis. Consequently, two new strains were established (SHN and SLN) [1]. Although SLN breeders developed mammary tumors with much lower incidence at older ages than did SHN breeders, both incidence and onset age fluctuated largely from generation to generation. It is plausible that these fluctuations may relate to the reproductivity at each generation, since several hormones which control reproduction have important roles in normal and neoplastic mammary development. Moreover, marked stimulation of mammary gland growth during pregnancy is favorable for mammary tumor development in mice [2, 3].

In this paper, the relationship between incidence and onset age of spontaneous mammary tumors and several reproductive characteristics were examined preliminarily in SLN breeders as a possible step to evaluate the participation of reproductivity in mammary tumorigenesis.

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MATERIALS AND METHODS

Animals

The data from SLN breeding females which have been selected and maintained by brother × sister mating over 30 generations [1] were used. Throughout these periods, animals were kept in the same animal room air-conditioned (24–25°C and 65–70% r.h.) and artificially illuminated (12 hr light from 8:00 a.m. to 8:00 p.m.) and were provided with a commercial diet (CA-1; CLEA Japan Inc., Tokyo, Japan) and tap water *ad libitum*. Other feeding conditions were also the same throughout these generations.

Pups were weaned at 20 days of age and kept six per cage in Teflon cages (15 × 30 × 12 cm). Female mice began to be mated with their brothers at 70–80 days of age and concurrent pregnancy was planned until mammary tumor appearance or the sixth lactation. Mice retired after the sixth lactation were kept six per cage again.

Reproductivity

Number of pups was reduced to eight when more than eight on the day of parturition (day 0 of lactation) and no other adjustment was performed. The following characteristics were measured in individual breeding females at each generation as the indices of reproductivity; delivery interval, breeding female weight at parturition, litter size (number of live pups) at day 0, average pup's weights at days 0, 12 and 20, pup's growth rates and rearing rates at days 12 and 20 and further the rates of still-birth and of still-born pups; numbers of still-birth and still-born pups as percentages of total numbers of birth and pups born, respectively [4].

At each generation, the generation mean of parameters was employed as the representative value of every characteristic.

Mammary tumorigenesis

Each breeding female mouse was checked for palpable mammary tumors every 7 days throughout their lives beginning 4 months of age. Mammary tumor incidence was expressed in terms of the number of mice with tumors as the percentage of total number of breeding female mice examined.

Correlation coefficients

Simple correlation coefficients [5] were calculated between the generation mean for incidence or onset age of mammary tumors and each generation mean for reproductive measurements during the periods between the second and the 30th generations of inbreeding.

In this study, litter size was not adjusted during lactation except for the reduction to eight when it was more than eight at day 0. Therefore, pup's weight and growth rate at day 12 or 20 as indices of lactational performance may conversely be influenced by litter size or rearing rate at each age. To examine this possibility, the simple correlation coefficients between these parameters were also calculated.

Delivery interval and indices of lactational performance which had significant simple correlations with mammary tumor incidence also significantly correlated each other. Thus, partial correlation coefficients [6] were calculated between mammary tumor incidence and delivery interval when each of indices are lactational performance was held constant and *vice versa*.

RESULTS

General time trend of mammary tumorigenesis and reproductivity

Changes with generation in incidence and onset age of mammary tumors and reproductive characteristics are illustrated in Fig. 1. Most parameters fluctuated largely from generation to generation and no marked difference in the pattern of fluctuation of any characteristics was observed before and after the 20th generation at which all loci are theoretically homozygous [7]. Meanwhile, delivery interval had a trend to decrease with the advance of generations and pup's weights and growth rates of days 12 and 20 increased after the 20th generation.

Simple correlations

As shown in Table 1, mammary tumor incidence had statistically significant correlations with delivery interval, average pup's weights at days 12 and 20 and pup's growth rate at day 20.

None of the correlations between onset age of mammary tumors and reproductive characteristics was statistically significant.

Pup's weights and growth rates at days 12 and 20 had no significant correlations with litter size at day 0. Meanwhile, there were significant positive correlations between pup's weight or growth rate and litter size or rearing rate at both days 12 and 20, except between pup's weight or growth rate and litter size at day 12 (Table 2). These show that good lactational performance results primarily in a slight loss of pups and, in its turn, a high rearing rate.

Partial correlations

The results are illustrated in Table 3. Partial correlations between mammary tumor incidence and delivery interval were not statistically significant when any of indices of lactational performance was held constant. No parameters as the indices of lactational performance except for pup's weight at day 20 had also statistically significant partial correlations with mammary tumor incidence when delivery interval was held constant. The results indicate that both delivery interval and lactational performance interact with each other on mammary tumorigenesis.

DISCUSSION

The present findings show that delivery interval has a negative correlation with mammary tumor incidence, indicating that the

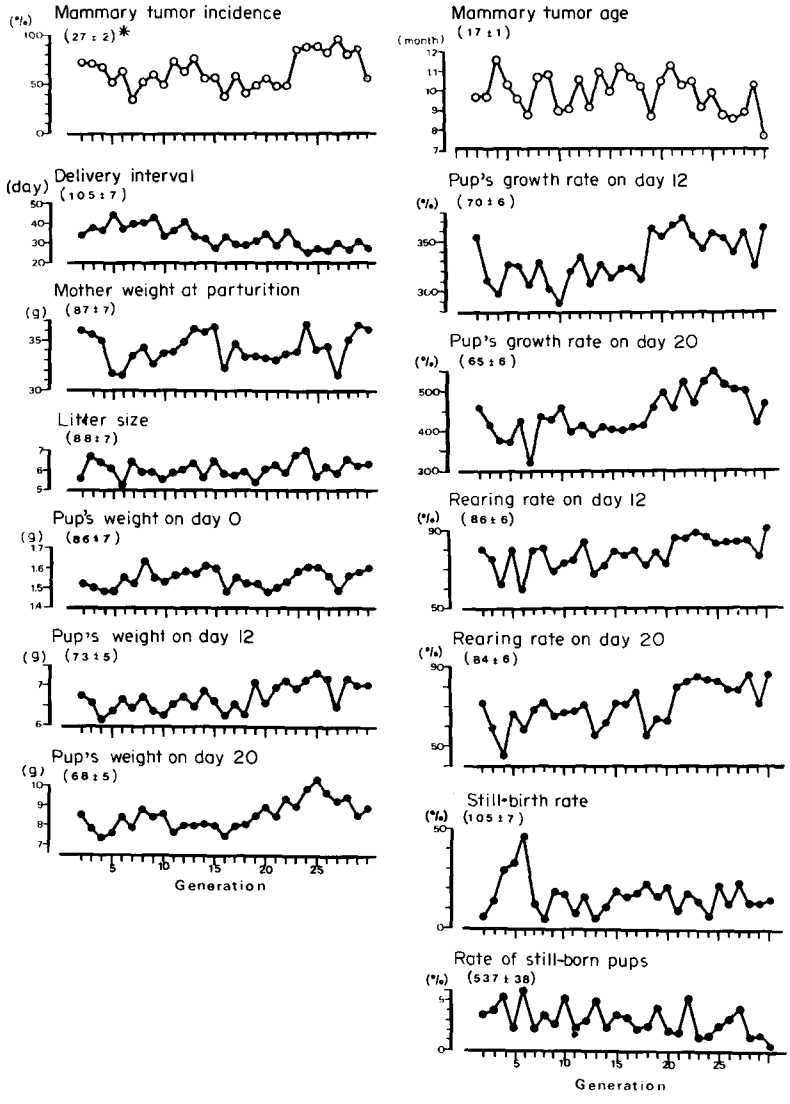

Fig. 1. General time trend of mammary tumorigenesis and reproductivity in SLN mice during the period between the 2nd and the 30th generations of inbreeding.
**Mean sample size at each generation with S.E.M.*

$$\textit{Mammary tumor incidence} = \frac{\textit{No. of mice with mammary tumors}}{\textit{Total No. of mice used}} \times 100$$

$$\textit{Pup's growth rate} =$$

$$\frac{\textit{Av. pup's wt. at} \text{ day } 12 \textit{ or } 20 - \textit{Av. pup's wt. at} \text{ day } 0}{\textit{Av. pup's wt. at} \text{ day } 0} \times 100$$

$$\textit{Rearing rate} = \frac{\textit{No. of pups at} \text{ day } 12 \textit{ or } 20}{\textit{No. of pups at} \text{ day } 0 \ (\textit{after reduced to } 8)} \times 100$$

$$\textit{Litter size: No. of live pups at} \text{ day } 0$$

$$\textit{Still-birth rate} = \frac{\textit{No. of still-birth}}{\textit{Total No. of birth}} \times 100$$

$$\textit{Rate of still-born pups} = \frac{\textit{No. of still-born pups}}{\textit{Total No. of pups born}} \times 100$$

Table 1. Simple correlation coefficients between incidence or onset age of spontaneous mammary tumors and reproductive characteristics in SLN mice between the 2nd and the 30th generations of inbreeding

Reproductive characteristics		Mammary tumor incidence (%)	Onset age of mammary tumors (months)
Delivery intervals (days)		−0.396*	0.270
Breeding female weight at parturition (g)		0.313	−0.208
Litter size (day 0)		0.297	−0.037
Average pup's weight (g)	Day 0	0.289	−0.157
	Day 12	0.425†	−0.259
	Day 20	0.515†	−0.352
Pup's growth rate (%)	Day 12	0.212	−0.157
	Day 20	0.485†	−0.235
Rearing rate (%)	Day 12	0.184	−0.259
	Day 20	0.318	−0.257
Still-birth rate (%)		−0.095	0.117
Rate of still-born pups (%)		−0.102	−0.007

Significance of difference: *$P<0.05$, †$P<0.01$.

Table 2. Simple correlation coefficients between indices of lactational performance and litter size or rearing rate in SLN mice

		Pup's weight		Pup's growth rate	
		Day 12	Day 20	Day 12	Day 20
Litter size	Day 0	0.120	0.063	0.341	0.203
	Day 12	0.210	—	0.010	—
	Day 20	—	0.591*	—	0.536*
Rearing rate	Day 12	0.641*	—	0.682*	—
	Day 20	—	0.682*	—	0.625*

Significance of difference: *$P<0.01$.

Table 3. Partial correlation coefficient between mammary tumor incidence and delivery interval or indices of lactational performance in SLN mice

		Mammary tumor incidence (%)	Characteristics held constant
Delivery interval		−0.221	Pup's weight at day 12
		−0.155	Pup's weight at day 20
		−0.343	Pup's growth rate at day 12
		−0.172	Pup's growth rate at day 20
Pup's weight	Day 12	0.267	Delivery interval
	Day 20	0.391*	
Pup's growth rate	Day 12	0.052	
	Day 20	0.347	

Significance of difference: *$P<0.05$.

shorter the delivery interval, the more the risk for mammary tumors would increase. In this study, each female mouse was planned to be pregnant concurrently by always placing with the male in a cage. Therefore, the shorter delivery interval would reflect the increase in the concurrently pregnant state; during pregnancy, mammary gland DNA synthesis, a limiting factor for mammary tumorigenesis [2, 8], is maintained at high levels in mice [3]. This may be a cause of the negative relationship between delivery interval and mammary tumor incidence.

Mammary tumor incidence also had the significant simple correlations with pup's weights at days 12 and 20 and pup's growth rate at day 20 as the indices of lactational performance. Therefore, the present results suggest that lactational performance has an important role in mammary tumor incidence. This hypothesis is well accorded with our experience in maintaining this SLN strain; the offsprings of breeding females who develop no mammary tumors in their lifetimes are most suitable for the parents of the next generation, however, such females often have left few good offsprings primarily due to their poor lactational performance. This was also supported by the present result showing large dependence of litter size or rearing rate at day 12 or 20 on lactational performance.

The following are plausible as the causes of the positive relationship between mammary tumor incidence and lactational performance. Firstly, it is evident that lactational performance is highly paralleled to the amount of mammary parenchyma [9–11]. The higher the amount of mammary parenchyma, the more the frequency of malignant transformation of the cells would increase. Secondly, the amount of mammary tumor virus transmitted to the offsprings through milk is larger in the individuals with high lactational performance—high milk yield. Thirdly, mammary glands of individuals with high lactational performance are generally more sensitive to mammotropic hormones which participate the primary role in mammary tumor development in mice [12].

On the other hand, either delivery interval or any index of lactational performance was held constant, partial correlation between another characteristics and mammary tumor incidence was not statistically significant. These results indicate that it is difficult to determine which of these characteristics is more primary for mammary tumor incidence, while both participate in this process.

Although the present findings indicate the relationship between mammary tumor incidence and delivery interval or lactational performance in mice, values do not always represent the real correlations between these characteristics. The magnitude of the correlations was overestimated due to the autocorrelations of parameters; measurement in one generation more or less influences the measurement in the subsequent generation. However, the data collected in the present study do not permit to evaluate these quantitatively. This interesting subject surely warrants further study.

In the present study, mammary tumor incidence and characteristics concerning lactational performance showed the similar changes over generations. Lactational performance of SHN females selected for high mammary tumorigenesis is superior to that of SLN females selected for low mammary tumorigenesis [1]. Moreover, C3H/He mice, a high mammary tumor strain, had higher lactational performance than C57BL/6 mice, a low mammary tumor strain [12]. These observations would provide the hypothesis that mammary tumor incidence is associated with lactational performance both phenotypically and genetically. Further experiments designed to test specifically this hypothesis are needed.

There were no significant correlations between onset age of mammary tumors and any reproductive characteristics. This would be due to the fact that spontaneous mammary tumors in this strain are autonomous in growth; the progression of the established tumors is no longer responsive to mammotropic hormones which also largely control reproduction [13], whereas malignant transformation of mammary epithelial cells and their initial development are dependent upon these hormones [12].

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REFERENCES

1. H. Nagasawa, R. Yanai, H. Taniguchi, R. Tokuzen and W. Nakahara, Two-way selection of a stock of Swiss albino mice for mammary tumorigenesis: establishment of two new strains (SHN and SLN). *J. nat. Cancer Inst.* **57,** 425 (1976).

2. H. NAGASAWA, Mammary gland DNA synthesis as a limiting factor for mammary tumorigenesis (Forum). *IRCS med. Sci.* **5,** 495 (1977).
3. H. NAGASAWA and R. YANAI, Mammary nucleic acids and pituitary prolactin secretion during prolonged lactation in mice. *J. Endocr.* **70,** 389 (1976).
4. H. NAGASAWA, M. FUJIMOTO and K. KURETANI, Normal growth and reproduction of a high and a low mammary strains of mice. *Gann* **58,** 115 (1967).
5. G. W. SNEDECOR, *Statistical Methods*. 5th Ed., p. 161. Iowa State Univ. Press, Ames (1966).
6. G. W. SNEDECOR, *Statistical Methods*. 5th Ed., p. 395. Iowa State Univ. Press, Ames (1966).
7. S. WRIGHT, Systems of mating. V. General consideration. *Genetics* **189,** 167 (1921).
8. H. NAGASAWA, R. YANAI and H. TANIGUCHI, Importance of mammary gland DNA synthesis on carcinogen-induced mammary tumorigenesis in rats. *Cancer Res.* **36,** 2223 (1976).
9. R. C. MOON, Mammary gland cell content during various phases of lactation. *Amer. J. Physiol.* **203,** 939 (1962).
10. H. NAGASAWA, R. YANAI, M. KOSUGIYAMA and M. FUJIMOTO, Correlations between some characteristics as the indices of lactational performance of mouse. *Jap. J. zootech. Sci.* **40,** 61 (1969).
11. J. NAGAI and N. K. SARKAR, Relationship between milk yield and mammary gland development in mice. *J. Dairy Sci.* **61,** 733 (1978).
12. H. NAGASAWA and R. YANAI, Normal and abnormal growth of mammary glands. In *Physiology of Mammary Glands*. (Edited by A. Yokoyama, H. Mizuno and H. Nagasawa), p. 121. Japan Scientific Societies Press/University Park Press, Tokyo/Baltimore (1978).
13. H. NAGASAWA, R. TOKUZEN and W. NAKAHARA, Growth of mammary tumors in a high and a low mammary tumor strains of mice established from the same basal stock. *Gann* **67,** 913 (1978).