BREEDING OF MICE WITH SPONTANEOUS ALVEOLAR
FORMATION IN THE MAMMARY GLAND
V. QUANTITY OF MAMMARY PARENCHYMA AT THE
VIRGINAL STAGE AND MILK PERFORMANCE

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It has been demonstrated that the quantity of the mammary parenchyma is one of the important factors which affect the lactation yield (Folley and Malpress, 1948; Naitô *et al.*, 1955; Moon, 1962). Brumby (1955) and Moon (1961) suggested that acceleration of the mammary development by growth hormone administration during the growth phase of the gland had a beneficial effect on the subsequent lactation. Further, Schmidt and Hansel (1961) reported the existence of positive correlation coefficient between total surface area and milk yield ($r=0.947$) in the goat.

Only mammary duct system develops at the virginal stage in mice generally, and lobule-alveolar proliferations occur after they are pregnant. However, the lobule-alveoli were formed spontaneously after about 50 days of age in some mice of the KP group (Nagai, 1962 and 1963). And the lobule-alveolar system of these mice, which were called SAF (spontaneous alveolar formation) positive ones, is supposed to be in the more advanced step of mammary development than that of the SAF negative ones at the virginal stage.

Thus, it is much interesting to compare the milk performance of these SAF positive mice with that of SAF negative ones.

The present experiment is conducted to examine whether the SAF positive animals are greater in the quantity of mammary parenchyma than the SAF negative ones in the KP group and the other at the virginal stage and further, to investigate whether the formers are superior in the milk performance to the latters.

MATERIALS AND METHODS

Animal

Animals used were the mice from the groups of KP and CFW which was known to show hardly SAF at the virginal stage.

Experiment 1: Relation between SAF and body weight and deoxyribonucleic acid (DNA) content of the mammary gland at the virginal stage

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After weaning on 20 to 25 days of age, 5 or 6 females were kept in a cage and weighed every 10 days. On day 70, their right third thoracic glands were autopsied under the ether anaesthesia. The degree of SAF were examined and classified as ++, +, ± and — by the same standard as described by Nagai (1962). On day 90 they were killed after weighing and the degrees of SAF were examined similarly with the left third thoracic glands. The fourth and fifth inguinal glands of both sides were defatted and dried with hot alcohol and ether and then, used for the measurement of DNA.

Experiment 2: Milk performance on day 12 in their first lactation

A few days after examination of SAF on day 70, mice in the KP group were mated. In the CFW group, SAF was not examined but mice were mated at the same age approximately as in the KP group. Only the individuals which delivered between 20 to 25 days after mating have been used, because some of them who delivered after this period may have experienced pseudopregnancy that can be one of the causes of mammary lobule-alveolar development.

The young were adjusted to 8 with the same sex ratio as possible on day 0—day of parturition. They were kept to be nursed till day 12 and weighed every four days. On day 12 the mothers were killed and their left third thoracic glands were used for nucleic acids determination and their fourth and fifth inguinal glands for measuring the respiratory activities.

The coefficient of growth (C.G.) and that of rearing (C.R.) were also calculated by the following formulas:

$$\text{C.G.} = (W_2 - W_1)/W_1 \times 100; \quad \text{C.R.} = (N_1 - N_2)/N_1 \times 100$$

where W_1 and W_2 were the average body weights of the young on days 0 and 12, and N_1 and N_2 were the number of the young on day 0 and that which died by day 12 respectively.

Nucleic acids determination

The defatted dry matter of the mammary gland was used. The nucleic acids were extracted by the procedure of Schneider (1945). DNA was determined by the diphenylamine reaction of Dische (1930) modified by Yagi (1951). Ribonucleic acid (RNA) was estimated by orcinol reaction method described by Mejbaum (1939). Their values were presented as P content and the correction of them with body weight was not made because there was no relation between them.

Manometric method

The mammary glands were dissected off and sliced in about 0.5 mm thick with microtome described by Stadie and Riggs (1944). The slices were washed repeatedly with Krebs-Ringer phosphate (K.R.P.) solution to remove the retained milk as possible and blotted on the filter paper. About 200mg of slices were incubated for 2 hrs. at 38°C in 4 ml of medium of K.R.P. solution with 0.3 % glucose as substrate. The reaction vessel was a 20 ml Warburg's ordinary flask with side arm and gas vent, containing 0.2 ml of 20 % KOH in the center well or 0.2 ml of 3 N-H₂SO₄ in the side arm. The gas phase was 100 % O₂. Oxygen consumption (QO₂) and respiratory quotient (R.Q.) were measured by the direct manometric method. The mean of the replicate determinations on different slices from the same tissue was taken as the representative value of the individual.

RESULTS

Relations between SAF and body weight and DNA content of the mammary gland at the virginal stage

The frequency of SAF and body weight on days 70 and 90 and DNA content

of the fourth and fifth inguinal mammary glands on day 90 are presented in Table 1.

On day 70, the frequencies of SAF were 31.1 % and 0 % in groups KP and CFW respectively.

In the KP group, the body weights of SAF positive mice were significantly larger than those of SAF negative ones both within and between litters ($P < 0.02$ or 0.05).

On day 90 the frequencies of SAF in the KP group elevated to 56.4 %. However, all animals in the CFW group remained negative.

With respect to body weight and inguinal mammary DNA content, the SAF positive mice significantly larger than the SAF negative ones in the group KP not only within but also between litters ($P < 0.01$ or 0.02). The mammary DNA of SAF positive mice in the KP group also was significantly larger than those in the CFW group ($P < 0.01$).

Milk performance on day 12 of lactation

The mammary gland weight, QO_2 , R.Q. and nucleic acids contents on day 12 of lactation are illustrated in Table 2.

Almost all of animals conceived by the first mating assigned. The body weights of mothers on day 0 and during lactation were not so different among groups.

The coefficient of growth and total QO_2 of SAF positive mice were fairly larger than those in negative ones in the KP group, and the CFW group was larger than the KP group in total RNA. However, these differences were not statistically significant. No remarkable differences were found in any other items. And it was suggested, in general, that the milk performances in all groups were similar on day 12 of lactation.

Some animals in the KP group showed the appearances of SAF which could hardly be judged as + or $\pm$ on day 70. This might be one of the causes of no differences between SAF positive and negative groups in all items. Thus, the comparison was performed between completely SAF positive (++ or +) and negative (—) animals by excluding the animals which showed the intermediate SAF on day 70. But the results were similar to those in Table 2 (Table 3). And the same tendency was evidenced even within litters in the KP group, although the numbers of litter were only 3 and the definite conclusion was not yet obtained (Table 4).

DISCUSSION

The body weights of SAF positive animals were always heavier than those of SAF negative ones on days 70 and 90 not only within but also between litters in the KP group.

It has been demonstrated that the SAF positive mice in the KP group have at least one long diestrus and that progesterone secreted from the active corpus luteum during the diestrus may be one of the important factors for SAF (Nagai, 1962; Kobayashi *et al.*, 1963; Nagasawa *et al.*, 1964). Dewar (1957a and b) report-

Table 1. SAF, body weight and mammary DNA at the virginal stage

Group	day 70					day 90					DNA of inguinal gland (μ g)		
	SAF		Body weight (g)			SAF		Body weight (g)			Between litter		Within litter
	Degree*	No. of anim.	Freq. (%)	Between litter	Within litter	Degree*	No. of anim.	Freq. (%)	Between litter	Within litter	Between litter	Within litter	Within litter
KP	$\oplus$	32	31.1	23.7 $\pm$ 0.3 (23) $\dagger$	23.7 $\pm$ 0.4 (7) $\dagger$	$\oplus$	57	56.4	24.8 $\pm$ 0.3 (37) $\dagger$	24.4 $\pm$ 0.4 (24) $\dagger$	70.1 $\pm$ 0.6 (14) $\dagger$	64.9 $\pm$ 6.0 (11) $\dagger$	
	$\ominus$	71		22.2 $\pm$ 0.3 (35)	23.0 $\pm$ 0.5 (17)	$\ominus$	44		23.1 $\pm$ 0.3 (29)	23.2 $\pm$ 0.3 (24)	43.2 $\pm$ 0.6 (12)	39.8 $\pm$ 5.9 (11)	
CFW	$\oplus$	0	0	—	—	$\oplus$	0	0	—	—	—	—	—
	$\ominus$	35		21.9 $\pm$ 0.7 (14)	—	$\ominus$	35		23.8 $\pm$ 0.7 (14)	—	53.0 $\pm$ 0.2 (8)	—	—

* $\dagger$, + and $\pm$ are designated as $\oplus$ and $\ominus$ respectively. $\dagger$ No. of litter examined

Table 2. Weight, respiratory activity and nucleic acids content of mammary gland on day 12 of lactation

Group	No. of anim.	No. of young rearing /litter	Coeffi. of growth (%)	Coeffi. of rearing (%)	Fresh wt. of 4th and 5th mammary glands (mg)	3rd mammary gland			Total QO ₂ (μ l)	R.Q.	DNA		RNA	
						Fresh wt. (mg)	Dry wt. (mg)	Dry wt. (%)			/mg dry wt. (μ g)	Total (μ g)	/DNA	Total (μ g)
KP⊕	9	7.5±0.7	95.8±2.1	261.4±9.5	1521±60	280±18	51±4	17.7±0.6	551.2±73.3	1.30±0.06	1.8±0.1	89.6±9.7	1.0±0.1	85.0±7.2
KP⊖	11	8.3±0.5	96.4±2.4	226.9±14.0	1503±106	283±16	51±4	18.1±0.6	424.5±44.7	1.28±0.22	1.7±0.1	87.7±6.3	1.0±0.1	81.2±7.8
CFW	6	9.0±1.1	93.2±5.0	272.3±16.5	1663±139	313±15	58±3	18.4±0.7	489.6±85.3	1.31±0.18	1.7±0.1	93.9±4.1	1.1±0.1	104.4±6.2

Table 3. Comparison of milk performances of completely SAF positive and negative mice in the KP group

Group	No. of young anim. /litter	Coeffi- cient of rearing (%)	Coeffi- cient of growth (%)	Fresh wt. of 4th and 5th mammary glands (mg)	3rd mammary gland		Total Qo ₂ (μ l)	R.Q.	DNA		RNA			
					Fresh (mg)	Dry wt. (mg) (%)			/mg dry wt. (μ g)	Total (μ g)	/DNA (μ g)	Total (μ g)		
KP $\oplus$	6	8.2 $\pm$ 0.6	100.0 $\pm$ 0.0	265.1 $\pm$ 11.5	1558 $\pm$ 70	275 $\pm$ 23	51 $\pm$ 4.7	17.9 $\pm$ 1.0	591.1 $\pm$ 103.4	1.33 $\pm$ 0.06	1.6 $\pm$ 0.2	83.0 $\pm$ 11.4	1.1 $\pm$ 0.1	88.5 $\pm$ 7.4
KP $\ominus$	5	8.3 $\pm$ 0.5	95.8 $\pm$ 4.2	264.5 $\pm$ 12.9	1413 $\pm$ 82	288 $\pm$ 29	56 $\pm$ 5.0	19.4 $\pm$ 0.5	437.2 $\pm$ 91.4	1.17 $\pm$ 0.25	1.8 $\pm$ 0.2	87.0 $\pm$ 14.8	1.0 $\pm$ 0.1	89.4 $\pm$ 11.3

Table 4. Within litter comparison of milk performances of SAF positive and negative mice in the KP group

Litter	No. of the young/litter	Coefficient of rearing (%)	Coefficient of growth (%)	Fresh wt. of 4th and 5th mammary glands (mg)	3rd mammary gland		
					Fresh wt. (mg)	Dry wt. (mg)	Dry wt. (%)
A B C	$\oplus$ 7.0	$\oplus$ 100.0	$\oplus$ 231.5	$\oplus$ 1440	$\oplus$ 330	$\oplus$ 59	$\oplus$ 17.9
	$\ominus$ 7.7	$\ominus$ 100.0	$\ominus$ 278.6	$\ominus$ 1785	$\ominus$ 375	$\ominus$ 69	$\ominus$ 18.4
	$\ominus$ 8.0	$\ominus$ 100.0	$\ominus$ 278.6	$\ominus$ 1428	$\ominus$ 273	$\ominus$ 51	$\ominus$ 18.5

Litter	Total Qo ₂ (μ l)	R.Q.	DNA		RNA	
			/mg dry wt. (μ g)	Total (μ g)	/DNA	Total (μ g)
A B C	$\oplus$ 551.4	$\oplus$ 1.24	$\oplus$ 1.8	$\oplus$ 105.8	$\oplus$ 1.1	$\oplus$ 112.5
	$\ominus$ 550.2	$\ominus$ 0.97	$\ominus$ 1.8	$\ominus$ 107.1	$\ominus$ 1.0	$\ominus$ 99.2
	1086.7	370.4	1.53	1.22	1.5	1.4
	488.2	564.8	1.47	2.35	1.7	2.1
				86.3		68.3
					1.1	0.6
						94.8
						38.0

* $\oplus$, $\ominus$ and $\pm$, — are designated as $\oplus$ and $\ominus$ respectively.

ed that the mice increased their (extra-uterine) body weights by progesterone or by pregnancy. Then progesterone might be postulated to have some effect on the body weight gain as well as SAF in the SAF positive animals.

Further, endogenous growth hormone (GH) may also be one of the factors for SAF. The hormone, which is perhaps secreted more in the more vigorous animals, would promote the development of body on the one hand, and at the same time would play more effectively some role on SAF synergistically with progesterone on the other hand, because many authors reported that GH was required for mammary lobule-alveolar development (Flux, 1958; Lyons, 1958; Lyons *et al.*, 1958; Ahren, 1959; Cowie and Lyons, 1959; Nandi, 1959).

Recently, Ferguson and Wallace (1961) and Josinovich and McLaren (1962) discussed that human GH had the prolactin like properties structurally and biologically. If this discussion is applicable to GH of mice, it is also assumed that GH has the luteotrophic property as prolactin and is one of the causes of the long diestrus in the SAF positive animals, and progesterone which is secreted from the luteinized ovary acts in turn on SAF and body weight gain.

As SAF positive and negative animals were recognized to be hardly different in the degree of the mammary duct development, although the precise measurement was not performed, the difference in DNA content between both animals is supposed to be due mainly to the existence of the lobule-alveoli, and it is confirmed that the mammary gland of the SAF positive animals is in the more advanced phase than that of SAF negative ones quantitatively as well as qualitatively at the virginal stage.

There were no remarkable differences among groups in most of the items examined as the indices of the milk performance on day 12 of lactation, even in DNA content—the quantity of mammary parenchyma which had been suspected as one of the most probable causes of differentiating the SAF positive animals from the negative ones in the milk performance after delivery. From this result, it will be suggested that the predisposition of mammary proliferation is not so different among groups used in the present experiment, only the difference in the degree of actions of several factors on SAF in the developmental stages of body bringing about the difference in SAF.

Moreover, the difference in the degree of mammary proliferation at the virginal stage would no longer matter in the lactational phase, because it is well known that the mammary proliferation is much more remarkable during the pregnancy than at the virginal stage.

The present result suggests that there are some problems concerning the time of treatment and the predisposition of animals in the case of hormone treatments for the purpose of acceleration of mammary development of animals—the improvement of their lactational performance.

SUMMARY

The relations between spontaneous alveolar formation (SAF) and body weight and the quantity of mammary parenchyma at the virginal stage and the milk

performance on day 12 of lactation were investigated using the mice of groups KP (a strain which has been established on this SAF in the authors' department) and CFW, mainly by measuring the nucleic acids contents and respiratory activities of mammary tissues.

The frequencies of SAF on day 70 were 31.1 % and 0 %, and those on day 90 56.4 % and 0 % in groups KP and CFW respectively.

In body weights on days 70 and 90 and deoxyribonucleic acid (DNA) content of the inguinal mammary gland on day 90, the SAF positive animals were significantly larger than the SAF negative ones not only within but also between litters in the KP group. The mammary DNA contents of these animals were also larger than those in the CFW group.

The differences were hardly found among groups in most of items measured as the indices of milk performance. Further, the same tendency was obtained even within litters in the KP group.

The significance of these findings was discussed.

REFERENCES

- Ahren, K. (1959). *Acta Endocrinol.* **31**, 228.
 Brumby, P. J. (1955). *New Zealand J. Sci. Technol. A.* **38**, 152.
 Cowie, A. T. and W. R. Lyons (1959). *J. Endocrinol.* **19**, 29.
 Dewar, A. D. (1957a). *Ibid.* **15**, 211.
 Dewar, A. D. (1957b). *Ibid.* **15**, 230.
 Dische, Z. (1930). *Mikrochemie* **8**, 4.
 Ferguson, K. A. and A. L. C. Wallace (1961). *Nature* **190**, 632.
 Flux, D. S. (1958). *J. Endocrinol.* **17**, 300.
 Folley, S. J. and F. H. Malpress. The hormones I. Ed. by G. Pincus and K. V. Thimann, Academic Press, New York, p. 745 (1948).
 Josinovich, J. B. and J. A. McLaren (1962). *Endocrinology* **71**, 209.
 Kobayashi, H., J. Nagai and M. Naitō (1963). *Endocrinol. Japon.* **10**, 169.
 Lyons, W. R. (1958). *Proc. Roy. Soc. (London) B.* **149**, 303.
 Lyons, W. R., C. H. Li and R. E. Johnson (1958). *Recent Progr. in Hormone Research* **14**, 219.
 Moon, R. C. (1961). *Am. J. Physiol.* **201**, 259.
 Moon, R. C. (1962). *Ibid.* **203**, 939.
 Meibbaum, W. (1939). *Z. Physiol. Chem.* **258**, 117.
 Nagai, J. (1962). *Japan. J. Zotech. Sci.* **33**, 256.
 Nagai, J. (1963). *Jikken Dobutsu (Bull. Exptl. Anim.)* (In Japanese) (in press)
 Nagasawa, H., J. Nagai and M. Naitō (1964). *Endocrinol. Japon.* **11**, 25.
 Naitō, M., Y. Shōda and J. Nagai (1955). *Ibid.* **2**, 205.
 Nandi, S. (1959). *Univ. Calif. (Berkeley) Pubs. zool.* **65**, 1.
 Schmidt, G. H. and W. Hansel (1961). *J. Dairy Sci.* **44**, 2259.
 Schneider, W. C. (1945). *J. Biol. Chem.* **161**, 239.
 Stadie, W. C. and B. C. Riggs (1944). *Ibid.* **154**, 687.
 Yagi, Y. *Kakusan to Kakutanpakushitsu (Nucleic Acids and Nucleoproteins)*. Ed. by F. Egami, Kyoritsu, Tokyo p. 123 (1951). (In Japanese)