THE EFFECT OF CONCURRENT PREGNANCY ON LACTATION IN THE MOUSE

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The reasons why lactation is checked during pregnancy and initiates copiously at parturition have been studied and discussed by many workers (Benson *et al.*, 1959; Smith, 1959). Animals concurrently pregnant and lactating seem to serve to enlighten the hormonal mechanism in these respects. On the other hand, the examination of respiratory activity by using tissue slice has been of value in estimating the activity of mammary metabolism since the works of Folley and French (1949 a, b and 1950). And moreover, the nucleic acids have become increasingly important in the biochemical study of synthetic activity in the tissue; DNA has been used as a measure of the number of cells, since each type of cell appears to have a constant content of DNA. RNA is considered to be intimately concerned with protein synthesis (Brachet, 1955). Thus also in the study of mammary gland, nucleic acids content has been used as indices of growth and function since the work of Kirkham and Turner (1953).

The present experiment was attempted to throw some light on the relation between pregnancy and lactation, by examining the respiratory activity of mammary slices, nucleic acids content in mammary glands and litters' growth with non-pregnant and pregnant-lactating mice.

MATERIALS AND METHODS

Animals The Kasukabe mice which had been bred in the author's laboratory were used in their 1st lactation. All litters were reduced to 6 young per mother on the day of parturition (day 0 of lactation). A certain number of mother mice were mated at post-partum estrus and became pregnant concurrently with lactation (pregnant-lactating group). Litters were weighed daily to assess the lactational performance of the mothers.

The mice were killed by decapitation at the 14th day or 19th day of lactation. The inguinal mammary glands were quickly removed for determining the respiratory activity and the nucleic acids content.

Respiratory activity Oxygen consumption and R. Q. were measured manometrically with a Warburg apparatus by the same method as that described previously (Mizuno and Chikamune, 1958). Incubations were carried out in a Krebs-Ringer phosphate solution (pH 7.4) added with 0.3% glucose, and with a 100% O₂ gas phase for 1 hr. at 37.5°C. The oxygen consumption was expressed as μ l per 1 hr. on the basis of 100 mg initial wet weight.

Nucleic acids content The right and left inguinal mammary glands were separately weighed and

homogenized with ice-cold distilled water. The nucleic acids were extracted by the procedure of Schneider (1945), omitting the phospholipid extraction step. DNA was determined by the diphenylamine reaction of Dische (1930) as modified by Yagi (1951). RNA was estimated by the orcinol method described by Mejbaum (1939). The values of nucleic acids were presented as phosphorus content.

RESULTS AND DISCUSSION

It has been studied by several workers that the vigorous suckling stimulus or lactation would retard the implantation of fertilized ovum in rats and mice when animals were mated at post-partum estrus (Kirkham, 1916; Enzmann *et al.*, 1932; Yoshinaga *et al.*, 1955). However, in the present experiment, in most instances at autopsy on the 19th day of lactation, the fetuses were mature and in condition of a few days before parturition. Then under the present experimental condition, it seemed that the retardation of implantation of ovum might, if any, be very little or negligible.

Table I shows that there was no significant difference in the oxygen consump-

Table 1. Effect of pregnancy on mammary respiratory activity

	Day of lactation	No. of mice	Body wt. (g)	O ₂ consumption μ l/100 mg wet wt./hr.	R. Q.
Non-pregnant	14	6	31.3 $\pm$ 1.0	119.2 $\pm$ 10.8	1.64 $\pm$ 0.08
Pregnant	14	8	31.4 $\pm$ 1.2	95.9 $\pm$ 11.5	1.49 $\pm$ 0.08
Non-pregnant	19	6	30.1 $\pm$ 0.9	102.2 $\pm$ 14.5	1.38 $\pm$ 0.12
Pregnant	19	8	32.7 $\pm$ 1.4	108.7 $\pm$ 11.1	1.47 $\pm$ 0.08

Mean $\pm$ S.E.

tion and R.Q. between nonpregnant- and pregnant-lactating groups either at the 14th day or 19th day of lactation. Although significant correlation of respiratory activity of mammary tissue slices with lactational performance has never been investigated so far, it has been shown that respiratory activities are low during pregnancy and increase gradually with the advance of lactation (Folley and French, 1949b; Folley, 1956). Thus the result was against the expectation deduced from the work described above that the respiratory activity would be reduced if animal became pregnant concurrently with lactation.

The effect of pregnancy on the nucleic acids content in mammary glands was shown in Table 2. There was no significant difference between right and left inguinal glands in wet weight, DNA, RNA and RNA/DNA, and then the comparisons between non-pregnant- and pregnant-lactating groups were made on right glands. It has been shown that even during non-pregnant-lactation total DNA continued to increase and reached maximum at the 14th day and thereafter decreased significantly to the 19th day, in mice (Chikamune and Mizuno, 1958; Brookreson and Turner, 1959; Mizuno, unpublished), although the histological observation by numerous workers indicated that the mammary growth was accomplished during the 1st two thirds of normal (non-lactating) pregnancy. In the present study, the comparison of nucleic acids content between 2 groups was

Table 2. Effect of pregnancy on nucleic acids content in mammary glands

	Day of lactation	No. of mice	Body wt. (g)	DNA*		RNA*	
				Right	Left	Right	Left
Non-pregnant	19	7	27.1 ±1.5	110.3 ±4.8	114.3 ±4.2	322.8 ±34.3	316.7 ±29.9
Pregnant	19	7	33.0 ±1.4	130.0 ±4.3	131.5 ±10.9	358.1 ±31.0	350.7 ±33.6

RNA/DNA		DNA Left/Right	Wet weight		Weight of		
Right	Left		Right	Left	Ovary	Adrenal	Uterus
			(mg)				
2.92	2.76	1.039	346	356	10.9	5.6	128.4
±0.28	±0.21	±0.019	±23	±20	±0.8	±0.7	±26.6
2.79	2.69	1.008	373	383	14.1	6.0	
±0.21	±0.20	±0.033	±38	±42	±1.1	±0.0	

Mean ± S.E.

* Expressed as DNA-P, RNA-P μ g

not carried out at the 14th day of lactation. At the 19th day of lactation, however, the total DNA content in the mammary glands from pregnant-lactating group was significantly higher than that from non-pregnant-lactating group ($P < 0.01$). This suggests that the cell division of mammary parenchymal tissues may be more stimulated by advancing pregnancy concurrent with lactation. Total RNA in mammary glands from pregnant-lactating group was also more than that from non-pregnant-lactating group. However, in RNA/DNA ratio there was no significant difference between 2 groups. These results may indicate that the synthetic activity of mammary gland was not changed but the mammary gland proliferation was actively stimulated by concurrent pregnancy.

The weight gain and rate of growth of litters were calculated over the periods of the 7th to 14th day and the 12th to 19th day of lactation (Table 3).

Table 3. Litter weight measurements

	From day 7 to 14 of lactation			From day 12 to 19 of lactation		
	No. of litters	Gain (g/litter)	Rate of growth	No. of litters	Gain (g/litter)	Rate of growth
Non-pregnant	32	11.5 ± 0.6	0.537 ± 0.023	16	15.5 ± 1.0	0.500 ± 0.032
Pregnant	32	11.7 ± 0.4	0.521 ± 0.023	14	16.6 ± 0.9	0.526 ± 0.029

Mean ± S.E.

It was deduced that the former period is the increasing phase and the latter is the declining phase of lactational function, from the previous experiment in which it was shown that total RNA and RNA/DNA ratio increased markedly after parturition and reached the peak at the 12th or 14th day of lactation and then began to decline in normal lactation in the mouse (Chikamune and Mizuno, 1958). The present result shows that there was no significant difference both in weight gain and rate of growth between 2 groups in either phase of lactation. Although the young begin to eat by themselves following their optical opening at the 12th

to 14th day, it is observed that they attach and suckle vigorously to their mother and that the appreciable amount of milk accumulates in the ligated glands 1 day after unilateral ligation of nipples. Therefore, it seems possible to take these litter weight measurements as an index of lactational performance of the mother. Thus, it is assumed from the results of litter growth measurement that there was no diminution of milk production by advancing pregnancy.

Meites and Sgouris (1953, 1954) have shown that the combined ovarian hormones optimal for mammary growth make refractory the mammary gland itself to prolactin, but if the dose of prolactin was sufficiently high, initiation of lactation could take place in the presence of estrogen and progesterone. On the other hand, since it has been shown that prolactin evokes mammary alveolar growth in addition to lactation (Lyons, 1942; Mizuno *et al.*, 1955), additional mammary growth during lactation may be attributable mainly to prolactin. In this experiment prolactin assay of pituitaries was not yet carried out. Although the hormonal balance in blood should be further studied in connection with the number of fetuses and of suckling young, it may be possible to conclude from these results that lactational function was not inhibited while additional mammary growth was stimulated by advancing pregnancy, and that the hormonal condition suitable for mammary growth can coexist with that for lactation and vice versa, at least in mice under the present experimental condition. However, these results does not necessarily contradict the well known phenomenon in the dairy cow, that the milk yield would begin to reduce markedly towards 6 months after animal became concurrently pregnant. It should be remembered that the peak of lactation in the cow comes at earlier stage of lactation period whereas it comes at later stage of lactation in the mouse.

SUMMARY

The effect of pregnancy on the established lactation was examined with simultaneously pregnant and lactating mice. The measurements of respiratory activity of mammary tissue slices, nucleic acids content in mammary glands and litters' growth rate were taken as indices of lactational functions. There was no significant differences in oxygen consumption and R.Q. between non-pregnant-lactating and pregnant-lactating groups either at the 14th or 19th day of lactation. Total DNA content was significantly higher in mammary glands of pregnant-lactating animals than non-pregnant-lactating animals. Total RNA content was also higher in pregnant-lactating group but there was no significant difference in RNA/DNA ratio between 2 groups. The litter gain and rate of growth over the periods of the 7th to 14th and of the 12th to 19th day of lactation were not significantly different between 2 groups. These results may indicate that advancing pregnancy did not exert an inhibitory effect on lactation and that the hormonal condition suitable for mammary growth can coexist with that for lactation and vice versa, at least in mice under the present experimental condition.

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REFERENCES

- Benson, G. K., A. T. Cowie, S. J. Folley and J. S. Tindal. *in* Recent Progress in the Endocrinology of Reproduction. Ed. by C. W. Lloyd, Acad. Press Inc., New York, p. 457 (1959).
- Brachet, J. *in* The Nucleic Acids. Vol. II. Ed. by E. Chargaff and J. N. Davidson, Acad. Press Inc., New York, p. 475 (1955).
- Brookreson, A. D. and C. W. Turner (1959). *Proc. Soc. Exptl. Biol. Med.* **102**, 744.
- Chikamune, T. and H. Mizuno (1958). *Japan. J. Zootech. Sci.* **29**, suppl. 1, 13. (In Japanese)
- Dische, Z. (1930). *Mikrochemie* **8**, 4.
- Enzmann, E. V., N. R. Saphir and G. Pincus (1932). *Anat. Record* **54**, 325.
- Folley, S. J. The Physiology and Biochemistry of Lactation. Oliver & Boyd, London (1956).
- Folley, S. J. and T. H. French (1949a). *Biochem. J.* **45**, 117.
- Folley, S. J. and T. H. French (1949b). *Biochem. J.* **45**, 270.
- Folley, S. J. and T. H. French (1950). *Biochem. J.* **46**, 465.
- Kirkham, W. R. (1916). *Anat. Record* **11**, 31.
- Kirkham, W. R. and C. W. Turner (1953). *Proc. Soc. Exptl. Biol. Med.* **48**, 590.
- Lyons, W. R. (1942). *Proc. Soc. Exptl. Biol. Med.* **51**, 308.
- Meites, J. and J. T. Sgouris (1953). *Endocrinology* **53**, 17.
- Meites, J. and J. T. Sgouris (1954). *Endocrinology* **55**, 530.
- Mejbaum, W. (1939). *Z. physiol. Chem.* **258**, 117.
- Mizuno, H. and T. Chikamune (1958). *Endocrinol. Japon.* **5**, 265.
- Mizuno, H., K. Iida and M. Naito (1955). *Endocrinol. Japon.* **2**, 163.
- Schneider, W. C. (1945). *J. Biol. Chem.* **161**, 239.
- Smith, V. R. Physiology of Lactation. Ames, Iowa State Univ. Press (1959).
- Yagi, Y. *in* Nucleic Acids and Nucleoproteins. Vol. I. Ed. by F. Egami, Kyoritsu Publish. Co., Tokyo, p. 132. (1951).
- Yoshinaga, K., K. Sekiguchi and T. Hoshi (1955). *Jap. J. Animal Reprod.* **1**, 107. (In Japanese)