EFFECT OF RECURRING PREGNANCY ON MAMMARY GLAND GROWTH IN MICE

HIROSHI WADA AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

SUMMARY

It was shown by the increase of total desoxyribosenucleic acid (DNA) that mammary gland growth continues during the latter half of pregnancy. This observation was rather unexpected, since morphological study of lobule-alveolar growth can not distinguish between additional cell growth and the initiation of milk secretion. These data indicate for the first time that cell multiplication continues throughout pregnancy.

The effect of recurring pregnancy on mammary gland growth was studied, employing albino mice bred successively without nursing periods. Maximum growth of the mammary glands was attained in triparous animals. A slight decline in total growth was observed in quadriparous animals. Their age at this time was estimated to be equivalent to the age of cows which begin to decline in yearly milk and fat yields. It was also confirmed that increased mammary gland growth in multiparous animals was more closely related to hormonal stimuli associated with recurring pregnancy and lactation than to increasing body weight. Primiparous mice after 3 mo. of involution had mammary glands containing DNA equivalent to mice pregnant for six days. No variation in average litter size was observed during four consecutive pregnancies.

Milk and fat secretion in dairy cattle has been shown to increase with advancing age up to about 8 yr. The age-production relation has been used extensively to formulate age-correction factors for converting milk and fat production records to their mature equivalent. Two obvious factors are involved in the increase in milk secretion: (a) the increase in body weight up to 8 yr. (7, 9) and (b) recurring pregnancy and lactation. In the analysis of these data it was suggested that recurring pregnancy (and factors associated with pregnancy and lactation) influenced the increase in lactation with age far more than did the increase in body weight. Confirmation of this relation has been provided by an analysis of milk records of Swedish dairy cattle (5). It was shown that milk yield per lactation depends not only on age up to maturity but also on the number of lactations. To reach maximum yield the cows must, on the average, have had at least three pregnancies.

The most obvious factor in recurring pregnancy is growth of the mammary gland. While it has been inferred during the last 30 yr. that additional growth of the mammary gland was stimulated with each pregnancy up to the time of maximum milk yield, no quantitative method of determination of mammary gland growth has been available to prove that additional growth occurred.

Received for publication February 7, 1959.

1 Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1969. Approved by the Director.

2 Research scholar, Ministry of Education of Japanese Government, and Medical Fellow of Population Council. This investigation was supported in part by a grant from the American Cancer Society.
During the past few years, a quantitative method of determining the growth of the mammary gland has been developed (2, 6, 13). It has been shown that the desoxyribosenucleic acid (DNA) content of each mammary gland cell is constant during pregnancy (3). Thus, as the cells multiply, the amount of DNA in the glands increases. A chemical method of increased sensitivity for determination of DNA recently has been described (11). With this method available, it seemed desirable to determine (1) the extent of gland growth (as measured by total DNA) during stages of the first pregnancy, and (2) whether gland growth was extended by two, three, or four successive pregnancies.

EXPERIMENTAL PROCEDURE

Albino mice fed a standard laboratory feed were bred when weighing about 28–30 g. Pregnancy was dated from the morning a vaginal plug was observed. The multiparous mice were remated immediately after parturition, following the removal of the young. Not all mice conceived immediately, so there was a variable period before the second and successive pregnancies were initiated. At the time indicated in Table 1, the animals were sacrificed, skinned, and three-fourths of the mammary glands of each mouse taken for DNA determination. The remaining one-fourth of the glands was prepared for whole-mount examination, to observe the type of gland development.

The gland tissue for DNA determination was frozen, the fat extracted with hot alcohol and ether and ground to a fine powder. Then, 30 mg. of the gland was extracted twice with 5 ml. of hot 5% trichloroacetic acid to remove the DNA, which was then determined by the Webb-Levy method (11).

Experimental observations. As controls, a group of unmated mice with extensive duct development were used. The average total DNA of the group was 1,704 µg. (Table 1). A certain number of mice, when sacrificed at six and 12 days after coitus, were found not to be pregnant. As coitus stimulates the maintenance of the corpora lutea, i.e., pseudopregnancy (8), the mammary glands of these animals were included in the study. It will be noted that gland growth occurred (DNA, 2,269 µg. per animal) at six days, but was not further increased at 12 days. Since this value is based upon a limited number of animals, the DNA may be increased as a more representative sample is obtained. In the first pregnancy, progressive growth of the mammary gland was observed at six, 12, and 18 days, as indicated by increasing DNA content. The marked increase in DNA from the 12th to the 18th day was unexpected since, on the basis of the morphological observations, it had been assumed that the major part of lobule-alveolar growth occurred during the first half of pregnancy. It was observed, also, that growth of the glands of pregnant animals was greater than that of pseudopregnant animals at the same stages. While further study will be required to confirm these observations, they suggest the possibility that the pregnant state (fetal membranes?) contribute hormones which cause greater mammary gland growth than the ovarian hormones (corpora lutea of pseudopregnancy) alone.

Considering the extent of mammary gland growth produced at the end of the first pregnancy (mean total DNA) as a base, it will be noted during the second pregnancy that there was 28.70% additional growth of mammary gland tissue.
TABLE 1
Effect of recurring pregnancy on the mammary gland growth in mice

<table>
<thead>
<tr>
<th>Reproductive state</th>
<th>Stage of gestation</th>
<th>No. of mice</th>
<th>D.F.F.T.* Wt. (mg.) Mean ± S.E.</th>
<th>D.F.F.T. Wt. Wet wt. of glands (%) Mean ± S.E.</th>
<th>DNA (μg.) Mean ± S.E.</th>
<th>Total DNA (μg.) Mean ± S.E.</th>
<th>Initial body wt. (g.) Mean ± S.E.</th>
<th>Total DNA (μg.) Initial body wt. (g.) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin</td>
<td></td>
<td>7</td>
<td>44.0 ± 2.5</td>
<td>4.0 ± 0.7</td>
<td>38.8 ± 3.5</td>
<td>1,704 ± 180</td>
<td>30.8 ± 0.7</td>
<td>54.4 ± 5.1</td>
</tr>
<tr>
<td>Nullipara</td>
<td>6 Days after sterile coitus</td>
<td>15</td>
<td>52.5 ± 2.1</td>
<td>5.7 ± 0.4</td>
<td>42.9 ± 0.5</td>
<td>2,269 ± 126</td>
<td>31.6 ± 0.8</td>
<td>71.5 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>12 Days after sterile coitus</td>
<td>5</td>
<td>54.8 ± 3.1</td>
<td>4.3 ± 0.8</td>
<td>39.8 ± 1.4</td>
<td>2,202 ± 159</td>
<td>32.8 ± 1.1</td>
<td>77.8 ± 8.2</td>
</tr>
<tr>
<td>Primipara</td>
<td>6 Days of pregnancy</td>
<td>10</td>
<td>63.2 ± 2.4</td>
<td>6.9 ± 0.5</td>
<td>46.3 ± 2.0</td>
<td>2,883 ± 153</td>
<td>31.4 ± 1.0</td>
<td>90.4 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>12 Days of pregnancy</td>
<td>10</td>
<td>80.1 ± 4.6</td>
<td>6.3 ± 0.3</td>
<td>51.4 ± 2.8</td>
<td>4,118 ± 319</td>
<td>32.8 ± 1.0</td>
<td>125.3 ± 9.1</td>
</tr>
<tr>
<td></td>
<td>18 Days of pregnancy</td>
<td>10</td>
<td>121.8 ± 8.6</td>
<td>8.4 ± 0.4</td>
<td>49.4 ± 1.6</td>
<td>5,965 ± 353</td>
<td>32.0 ± 1.3</td>
<td>185.8 ± 7.0</td>
</tr>
<tr>
<td>Bipara</td>
<td>18 Days of pregnancy</td>
<td>10</td>
<td>154.0 ± 5.9</td>
<td>9.8 ± 0.5</td>
<td>49.3 ± 1.9</td>
<td>7,677 ± 438</td>
<td>33.4 ± 1.2</td>
<td>285.6 ± 20.2</td>
</tr>
<tr>
<td>Tripara</td>
<td>18 Days of pregnancy</td>
<td>10</td>
<td>165.9 ± 10.9</td>
<td>9.3 ± 0.3</td>
<td>45.4 ± 0.8</td>
<td>7,750 ± 497</td>
<td>36.1 ± 1.4</td>
<td>215.0 ± 9.2</td>
</tr>
<tr>
<td>Quadipara</td>
<td>18 Days of pregnancy</td>
<td>10</td>
<td>146.0 ± 8.6</td>
<td>9.2 ± 0.2</td>
<td>46.5 ± 1.2</td>
<td>6,982 ± 366</td>
<td>36.9 ± 0.9</td>
<td>188.5 ± 6.3</td>
</tr>
<tr>
<td>Primipara</td>
<td>Sterile over 3 mo. after first parturition</td>
<td>7</td>
<td>69.3 ± 8.0</td>
<td>6.5 ± 0.9</td>
<td>40.2 ± 3.9</td>
<td>2,720 ± 286</td>
<td>32.2 ± 1.7</td>
<td>83.8 ± 7.9</td>
</tr>
</tbody>
</table>

* D.F.F.T. = Dry, fat-free tissue.

b Final body weight.

c Total DNA/final body weight.
(total DNA) and during the third pregnancy an additional further slight increase. Thus, the total additional mammary gland growth induced by two successive periods of pregnancy amounted to an increase of 29.59% over that induced by the first pregnancy. To the extent that additional epithelial cells present represent additional capacity to secrete milk by the tripara mice, it is assumed that their lactational capacity would thus be increased by about 30% as a result of two additional pregnancies. Since the average body weight of the mice at the beginning of the first pregnancy was 32.0 g. and of the tripara mice 36.1 g., the additional mammary gland growth induced was only slightly related to the increasing body weight. In other words, there is a marked increase in total DNA per gram increase in body weight in the bipara mice. It will be noted, also, that the weight ratio of dry fat-free tissue to wet tissue of the multiparous mice was greater than that of the primiparous mice. With only slightly higher nutritional maintenance requirement of the bipara and tripara animals, the increased lactational capacity of the animals with larger mammary glands would increase the nutritional efficiency for milk production of the latter groups.

The group of quadripara mice showed a 9.68% decline in average total DNA, in comparison with the tripara group. The decline in capacity for mammary gland growth is believed to represent a decline in capacity for the secretion of the hormones essential (directly and indirectly) for mammary gland growth. This compares with the gradual decline in yearly milk and fat production of dairy cattle after peak production is reached at 7 or 8 yr. Brody (1) reported that 1 mo. in the mouse was equivalent to 15.3 mo. in the cow. The quadripara mice in this experiment were in range of 5.5–6.5 mo. of age, averaging 6 mo. at the end of their pregnancies. This is equivalent to cows 85 to 100 mo. of age (see Brody's Figure 19.7). Thus, the quadripara mice were of an age comparable to cattle which begin to decline in yearly milk yield. It has been known that thyroid function has a relation to the age, and that features of the hypothyroid state are similar to some of the features of senescence. Recently, it has been reported that the thyroxine secretion rate of rats decreases with advancing age (12). Unpublished preliminary data also showed that thyroxine secretion rate per unit of body weight in aged mice is lower than in younger animals by radioactive replacement technique (10). Therefore, the decline of mammary gland growth in quadripara mice may be due to a decline of thyroxine secretion rate as well as pituitary activity and other physiological functions. In primipara mice which were not bred again for 3 mo. or more, there was observed involution of the mammary glands to an extent comparable to that observed at the sixth day of pregnancy. Even in animals at 25 days after parturition, without recurring pregnancy and nursing, there was marked involution of mammary alveolar tissue (not included in Table 1).

Ingram et al. (4) has recently shown that age of mice does not influence litter size up to the fourth; however, a decline in litter size occurs thereafter. The results of the present experiment show no direct relationship between the litter size and mammary gland growth, since average litter size in all groups was constant, ranging from 9.0 to 9.5.
ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Mary E. Powell.

REFERENCES


