The Effect of Varying the Length of the Nursing Period on the Postpartum Growth of Chemically Induced Rat Mammary Tumors

George M. McCormick, II

Department of Pathology, University of Tennessee Medical Units, Memphis, Tennessee 38103

SUMMARY

Pregnancy stimulates the growth of rat mammary tumors induced by 7,12-dimethylbenz(a)anthracene, but tumors tend to regress during the subsequent lactation. Nursing by the young is necessary to maintain tumor growth. Tumor-bearing dams were allowed to nurse 6 pups for periods of 7, 14, or 21 days, and tumor growth was measured weekly. The mean of the two largest perpendicular surface axes was taken as an index of tumor size. No significant decrease in tumor size was seen during the first 7 days of nursing in any group. If nursing was terminated after 7 days, rapid reduction in tumor size ensued, with the majority of tumors showing significant reduction in size over the next 7 days. Nursing for 14 days maintained growth of more tumors than nursing for 7 days, while nursing for 21 days had the greatest effect on tumor growth maintenance. These data further strengthen the concept that maintenance of growth of chemically induced rat mammary tumors during the postpartum period depends on the continual presence of the nursing stimulus.

INTRODUCTION

The stimulatory effect of pregnancy on the growth of rat mammary tumors induced by DMBA seems to be well established (2–5). Tumor growth during the postpartum period is highly variable, however. Some tumors regress, some continue to grow rapidly, while others maintain a constant size (3). When litters are removed from tumor-bearing dams immediately after parturition, all tumors regress, demonstrating that continued tumor growth is dependent on the presence of the suckling stimulus (4, 5). Continued tumor growth is also dependent on the frequency or intensity of suckling, as more tumors continue to grow when 9 or 12 pups are nursed instead of 6 (4). The experiments to be reported here are concerned with the effect of the length of the nursing period on maintenance of tumor growth.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats were obtained from the dealer at 43 days of age. Sprague-Dawley male rats weighing 300 g were used for breeding. All rats were housed in stainless steel cages and given Wayne Lab Blox and tap water ad libitum. Room temperature was maintained at 78 ± 4°F.

At 50 days of age all females were stomach-tubed with a soft rubber catheter and fed 20 mg DMBA dissolved in 1 ml sesame oil. Eighteen days after DMBA feeding, groups of 5 females and 2 males were placed together and allowed to breed. Pregnant females were placed in individual cages to deliver, and were housed singly for the duration of the experiment.

On the 1st day postpartum, all litters were adjusted to 6 pups. Replacement of sick pups by healthy ones of a similar age from stock litters ensured a comparable suckling stimulus in all dams. Three experimental groups were utilized: Group I nursed their litters for 21 days, Group II nursed theirs for 14 days, and Group III had their litters removed after only 7 days.

Tumors were detected by palpation. Tumors grew laterally at a faster rate than they increased in thickness, although some increase in thickness was noted. Therefore, the 2 largest axes on the surface that were perpendicular to each other were measured weekly with calipers. The mean of these 2 axes in centimeters was taken as an index of tumor size. Because this method of measurement was inexact, tumors were not considered to have exhibited a significant decrease in size unless this mean value decreased to one-half the maximum value attained at parturition (5). All rats were observed for approximately 150 days and sacrificed with ether, and sections of all tumors were taken for histological study.

RESULTS

Histology. In general, the tumors produced could be classified as either adenocarcinomas or fibroadenomas. The former were composed of glandular or ductular arrangements of atypical epithelial cells, with minimal supporting stroma. Mitoses were present in fair numbers in some but absent in...
**Table 1**
Effect of length of nursing period on maintenance of postpartum tumor growth

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Adenocarcinomas</th>
<th>Fibroadenomas</th>
<th>Mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. 21-day nursing</td>
<td>9</td>
<td>3/9</td>
<td>2/3</td>
<td>1/1</td>
<td>6/13*</td>
</tr>
<tr>
<td>II. 14-day nursing</td>
<td>9</td>
<td>5/8</td>
<td>2/2</td>
<td>3/4</td>
<td>10/14</td>
</tr>
<tr>
<td>III. 7-day nursing</td>
<td>8</td>
<td>6/8</td>
<td>4/4</td>
<td>3/3</td>
<td>13/15*</td>
</tr>
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* Significantly different at the 1.0% level.

Nursing and Growth of DMBA-induced Mammary Tumors

Tumors were considered to have exhibited a significant reduction in size if the mean of their 2 largest perpendicular axes decreased to one-half the maximum value attained at parturition.

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Others. Most were fairly well differentiated, and papillary projections into large duct-like spaces were quite common. The fibroadenomas were composed of ducts, 1 cell layer thick, embedded in a dense, eosinophilic collagenous matrix. Some tumors seemed to be composed of equal portions of fibrous and epithelial elements and were therefore classified as "mixed." The glandular elements of such tumors appeared completely benign, and perhaps they should more properly be termed "adenofibromas."

While invasion of adjacent tissues such as muscle and/or fat by adenocarcinomas was common, no metastases to regional lymph nodes or distant organs were seen.

Regression produced fairly characteristic changes in all tumors, with necrosis, liquefaction, cyst formation, and eventual fibrosis being commonly seen. Some tumors regressed to the extent that their size could not be measured. In such cases, histological sections of the involved area of the mammary gland revealed enough residual tumor that diagnosis could be made. The fact that such former tumor sites were quite fibrotic aided in localization of the area in question.

**Tumor Appearance and Growth During Pregnancy.** All rats in every group developed palpable mammary tumors during their pregnancy, and the majority of these tumors grew rapidly until parturition. Those which did not grow rapidly showed a steady increase in size, but at a somewhat slower rate. Of the 26 rats involved in these experiments, 16 had solitary tumors. The remaining 10 had multiple tumors. One rat produced 5 tumors, the maximum which was observed. However, as can be seen from Table 1, the number of tumors per rat (no. tumors in group/no. rats in group) was approximately the same for each group (13/9, 14/9, 15/8).

**Tumor Growth during Lactation.** Table 1 shows the effect of the nursing period on the number of tumors that showed significant regression. The values represent the number of tumors that had regressed by 21 days postpartum. In Group I, dams nursed their litters for the entire 21 days. At the end of this period, 6/13 of the tumors in this group had regressed. Animals in Group II nursed for the 1st 14 of the 21 days, and 10/14 of the tumors showed significant regression by Day 21 postpartum. In the final group (Group III), litters were nursed only for the 1st 7 days of the 21-day period, and 13/15 of the tumors had regressed by Day 21. The difference between Group I (6/13) and Group III (13/15) was significant at the 1.0% level ($\chi^2$).

In all instances in Table 1, the remainder of the tumors that did not exhibit significant regression increased in size. Therefore, there were no tumors with partial, but insignificant, regression.

As stated previously, there were rats bearing multiple tumors in all 3 groups. If such rats nursed for 14 or 21 days, there was no uniformity in behavior of their multiple tumors. Some individual tumors would grow, while others regressed in the same animal. When pups were nursed for 7 days, however, regression of all tumors occurred in rats bearing multiple tumors.

Tumor measurements were made throughout the 21-day postpartum period, regardless of the length of nursing. No tumors in any of the 3 groups regressed during the 1st 7 days. A rapid tumor regression was seen during the 1st 7 days after removal of the litter in rats nursing either 7 or 14 days. Also, in the group nursing for 21 days, the last 7 days of nursing saw 4 of the 13 tumors regress, although pups were still present and presumably nursing.

**Tumor Appearance after Pregnancy.** New tumors appeared in all groups following parturition, with the time of tumor appearance seemingly related to the length of the nursing period. Two rats in Group I (21-day nursing) had a single new adenocarcinoma each which appeared during the nursing period (Day 14 and 20 of nursing, respectively). In addition, a 3rd rat in this group developed a single adenocarcinoma 18 days following litter removal. No new tumors appeared during the 21-day postpartum period in either of the groups that nursed for the shorter time periods (7 or 14 days). However, 1 rat in Group II developed an adenocarcinoma and a mixed tumor, and 1 rat in Group III developed a single adenocarcinoma. In both instances the new tumors appeared in the interval between Day 21 postpartum and sacrifice (Day 150).

**DISCUSSION**

Several reports have described the stimulatory effect of pregnancy on the growth of rat mammary tumors induced by DMBA (2–5). Such rapid growth is usually transient and is terminated by parturition. Indeed, if pups are removed at birth, and nursing does not occur, all tumors will regress (4, 5). If nursing by the litter is allowed, however, some tumors will continue to grow rapidly, some will regress, while others will maintain a constant size (3). Continued tumor growth is also
dependent on the frequency or intensity of suckling, as more tumors continue to grow when 9 or 12 pups are nursed instead of 6 (4).

In the present studies, no significant tumor regression was seen during the 1st 7 days of nursing. If nursing was terminated at this point, rapid tumor regression ensued with the majority of the tumors regressing over the next 7 days, but even if litters were present and apparently nursing regularly (Groups I and II) a few tumors regressed by Day 14 postpartum. Moreover, in rats which were allowed to nurse for the full 21-day period, a significant number of tumors regressed during the last 7 days of nursing. This apparent failure in maintenance of tumor growth can probably be explained by the fact that pups begin to open their eyes around 13 to 15 days of age and will then start to eat rat chow. In doing so, their frequency of nursing decreases (1), and apparently the suckling-induced secretion of hormones necessary for the maintenance of tumor growth likewise declines.

These experiments again point up the fact that the rapid growth phase of chemically induced rat mammary tumors during pregnancy is difficult to maintain. Any diminution in the nursing stimulus, either by removing pups prematurely or even decreased nursing by maturing young, leads to rapid reduction in tumor size.

We have previously demonstrated that such maintenance of tumor growth during the postpartum period is dependent on the presence of the ovaries (5). Therefore, we have concluded that progesterone is necessary for maintenance of postpartum tumor growth (5). The present studies indicate that a continual secretion of progesterone, in response to the luteotropic action of suckling-released prolactin, is necessary to maintain the exuberant tumor growth of pregnancy.

New tumors arose in a few rats during the longest nursing period (21 days). Such data are inconclusive, but the observations suggest that the suckling-mediated release of progesterone may stimulate the growth of insipient neoplasms into palpable tumors, as well as maintaining the growth of established tumors.

REFERENCES
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