Effect of Pregnancy, Lactation, and Pituitary Isografts on the Genesis of Spontaneous Mammary Gland Tumors in the Mouse

J. E. Bruni and D. G. Montemurro

Department of Anatomy, University of Western Ontario Health Sciences Centre, London 72, Ontario, Canada

SUMMARY

Forced breeding of female (C3H/HeJ X DBA/2J) mice increased the incidence and reduced the latent period of development of spontaneous mammary tumors. Conversely, virgin mice were minimally sensitive to tumor development after a lengthy latent period. Ectopic pituitary isografts were potent stimuli to tumor development, increasing the incidence in virgin mice to forced breeding levels. Tumor incidence in mice nursing full and adjusted litters was not appreciably different from that in force-bred or pituitary-isografted mice, despite a significant reduction in the number of litters per mouse. Both nursing groups, however, did have a significantly higher tumor incidence and shorter latent period of development than did virgin mice. Although litter size had little effect on tumor incidence, with larger litters tumor incidence was slightly lower, albeit not significantly so, and latency of development was longer. The data of this study suggest that the prolactin-progestational state that ensues during lactation or pituitary transplantation may be equivalent in terms of effect on tumor incidence to the predominantly progestational condition of pregnancy.

INTRODUCTION

In both agent-free and agent-bearing mice, tumor incidence increases with the number of pregnancies (2, 19, 20). Among agent-free mice, mammary tumors occur regularly only in females that have been pregnant. Virgin females, on the other hand, develop few or no tumors (21, 22, 24).

In both agent-free (20, 21) and MC2-tREATED, agent-free (12) mice, pseudopregnancy ranked with forced breeding in causing the earliest and most rapid appearance of mammary tumors. Agent-free mice permitted to lactate showed a lower tumor incidence and greater latency of tumor development than mice from which the young were removed at birth (20, 21). Similar findings have been reported in MC-treated, agent-free mice (12, 13).

Suckling has been shown to result in release of prolactin (5, 6, 26) and somatotropin (4) from the adenohypophysis. Suckling also induces the release of ovarian progesterone (3). Progesterone, acting either directly or in synergism with prolactin, is necessary for the growth of carcinogen-induced mammary tumors during lactation (16).

Release of prolactin is also favored by procedures which remove hypothalamic regulation of the anterior pituitary (7, 17). Transplantation s.c. of hypophyses stimulates mammary gland growth (18) and increases mammary tumor incidence in agent-bearing (10, 27) and agent-free (1, 9) mice. The enhancement of mammary tumorigenesis in pituitary-implanted mice occurs through the combined action of prolactin, estrogens, and progesterone on the mammary glands (23).

The present investigation is concerned with the induction of spontaneous mammary gland tumors in mice maintained under different social conditions known to alter the hormonal status of the animals. A comparison with the results obtained from similar mice bearing hypothalamic lesions (2) would then be possible.

MATERIALS AND METHODS

Agent-bearing female mice of the C3D2F1 (C3H/HeJ X DBA/2J) hybrid strain (Jackson Memorial Laboratories, Bar Harbor, Maine) were used in this investigation. Virgin groups were housed 5 females per cage; in force-bred groups, 2 males were housed with 5 females. They were maintained in a light-controlled (12 hr/day) and temperature-controlled (72 ± 2°F) room, with water and Purina laboratory chow available ad libitum.

Seventy-five female mice, 101 to 160 days of age, were randomly assigned to 1 of the following groups and were treated accordingly: Group 1, no treatment, force bred; Group 2, no treatment, virgin; Group 3A, allowed to nurse full litters for 25 days postpartum; Group 3B, allowed to nurse an adjusted litter (containing 2 to 3 young) for 25 days. Litter size was adjusted in this manner on Day 1 postpartum, and this litter size was retained for all subsequent nursing periods; Group 4, received 2 intact (anterior and posterior lobes) hypophyseal isografts under the kidney capsule. All hosts retained their own in situ hypophysis and were kept virgin.

Pregnant mice were separated and housed individually until...
delivery. Litters of force-bred (Group 1) and nursing (Groups 3A and 3B) mice were removed immediately (at the latest, 24 hr postpartum), to preclude suckling, and at 25 days postpartum, respectively, at which time the females were returned to their original colony cage. The observation period, autopsy procedure, histological technique, vaginal smear preparation, method of tumor detection, and pregnancy determination were identical to those previously reported (2).

RESULTS

Forced breeding per se in female mice was characterized by an increased tumor incidence and a reduced latent period of development (Table 1). Virgin mice, on the other hand, developed significantly fewer tumors than did either force-bred mice (from 32 to 58 weeks) or those with ectopic pituitary isografts (from 38 to 58 weeks), and only after a lengthy latent period (369.4 ± 19.3 days).

Mice nursing adjusted litters did not differ appreciably in either tumor incidence or latent period of development from force-bred controls or mice with pituitary isografts. This was also observed to be the case with mice nursing full litters, with 1 exception. The latent period of tumor development was longer ($p < 0.05$) than that recorded for the mice in Group 1.

Although mice nursing full litters had a slightly lower tumor incidence and a longer latency of development than did mice nursing adjusted litters, these differences were not significant. Both of these groups, however, did have a significantly higher tumor incidence (from 38 to 56 weeks and from 36 to 58 weeks, respectively) and a shorter latent period of development than did virgin mice.

Most tumors were found in the 4th and 5th mammary glands, with no detectable difference in frequency of occurrence. These tumors were adenocarcinomas characteristic of agent-bearing mice (25).

Reproductive Activity

Forced-bred mice produced an average of $4.2 ± 0.4$ litters/mouse (Table 2). This number was not significantly different from the number of litters recorded in mice that nursed either full or adjusted litters, despite their longer survival time. In addition, both Groups 3A and 3B showed a significantly reduced interval between successive deliveries, compared with Group 1, when the constant 25-day period of postpartum lactation is eliminated from the calculation. The lesser number of days between successive deliveries and the significantly longer survival time of these mice account for the

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice with tumors</th>
<th>Latent period of tumor development (days postnatal)</th>
<th>Total no. of mice</th>
<th>Tumor incidence (%) at postnatal week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact force-bred</td>
<td>14</td>
<td>$227.5 ± 8.3^c$</td>
<td>15</td>
<td>0 20 87$d$ 93$e$ 93</td>
</tr>
<tr>
<td>2</td>
<td>Intact virgin</td>
<td>11</td>
<td>$364.9 ± 19.3^f$</td>
<td>15</td>
<td>0 0 78$g$ 20$h$ 73</td>
</tr>
<tr>
<td>3A</td>
<td>Nursing full litter</td>
<td>16</td>
<td>$263.3 ± 13.0^d$</td>
<td>20</td>
<td>0 15 55$i$ 75$k$ 80</td>
</tr>
<tr>
<td>3B</td>
<td>Nursing adjusted litter</td>
<td>10</td>
<td>$247.3 ± 8.9$</td>
<td>10</td>
<td>0 10 90$l$ 100$m$ 100</td>
</tr>
<tr>
<td>4</td>
<td>Ectopic pituitary isograft</td>
<td>14</td>
<td>$259.0 ± 9.9^n$</td>
<td>15</td>
<td>0 13 60$o$ 93$p$ 93</td>
</tr>
</tbody>
</table>

a Mean ± S.E. All other superscripts, with the exception of Superscript b, are substitute symbols for the figures used in the calculation of the probability ratio.

b Corresponds to time of pituitary transplantation.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Litters</th>
<th>Pups/mouse</th>
<th>Pups/litter</th>
<th>Days between deliveries</th>
<th>Litters at first tumor appearance</th>
<th>Survival time (days)</th>
<th>LFI$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact force-bred</td>
<td>14</td>
<td>4.2 ± 0.4</td>
<td>24.6 ± 2.4$^b$</td>
<td>6.0 ± 0.4$^c$</td>
<td>26.5 ± 1.0$d$</td>
<td>2.6 ± 0.3</td>
<td>137.1 ± 8.0$^e$</td>
<td>3.0 ± 0.1$f$</td>
</tr>
<tr>
<td>3A</td>
<td>Nursing full litter</td>
<td>16</td>
<td>4.3 ± 0.3</td>
<td>36.3 ± 2.4$^f$</td>
<td>8.7 ± 0.4$^h$</td>
<td>22.8 ± 0.7$i$</td>
<td>3.3 ± 0.3</td>
<td>214.7 ± 13.0$^j$</td>
<td>2.0 ± 0.1$k$</td>
</tr>
<tr>
<td>3B</td>
<td>Nursing adjusted litter</td>
<td>10</td>
<td>4.3 ± 0.3</td>
<td>35.8 ± 2.2$^l$</td>
<td>8.4 ± 0.2$m$</td>
<td>22.5 ± 0.7$n$</td>
<td>3.1 ± 0.2</td>
<td>206.3 ± 11.7$^o$</td>
<td>2.1 ± 0.1$^p$</td>
</tr>
</tbody>
</table>

a LFI = [no. of litters/survival time (days)] × 100.
fact that the total number of their litters was not different from that of force-bred mice, despite the 25-day lactation period. However, when reproductive ability relative to survival time is expressed (LFI), a significant reduction in the total number of litters per unit time is observed in both nursing groups (Table 2).

The number of pups per mouse and pups per litter delivered by the mice in Groups 3A and 3B were significantly larger than that of Group 1 mice, although neither was appreciably different from weights recorded in virgin mice.

Endocrine Organ Weights

The mean weights of pituitaries removed from both virgin mice and mice nursing full litters were significantly larger than that of force-bred controls (Table 3).

The ovaries of only Group 3A were significantly larger than those of force-bred mice. There was no statistically significant difference between the ovarian weights of force-bred or virgin mice and those of any of the other groups. The mean weight of the ovaries of the virgin control mice was one of the largest recorded; many of these ovaries were cystic.

In mice nursing adjusted litters, adrenal weights were not significantly different from those of Group 1; however, they were larger than those recorded in mice nursing full litters ($p < 0.05$). The adrenal weights of Groups 3A and 3B were significantly larger than those recorded in mice nursing full litters ($p < 0.05$).

Histology

Ovaries. The ovaries of multiparous mice of Groups 1, 3A, and 3B resemble histologically those of cycling mice, with numerous mature follicles and well-developed corpora lutea. The ovaries of mice with ectopic pituitary isografts weighed $13.7 \pm 1.8$ mg and contained a predominance of large corpora lutea with some follicles at various stages of maturation from primary to mature.

Mammary Glands. Whole-mount preparations of the mammary glands of virgin control mice revealed moderate ductal development, the presence of many lateral buds along the main ducts, and small clusters of alveoli along the sides and ends of the ducts. Duct width was minimal, as was duct branching and the overall area covered by the mammary gland parenchyma. The mammary glands of normal cycling multiparous animals (Groups 1, 3A and 3B) show considerable variation in structure relative to the time of pregnancy. Mammary glands of mice with ectopic pituitary isografts were generally much larger than those of control mice and exhibited an extensive degree of ductal development and branching, as well as profuse alveolar development; in addition, ducts were distended.

Pituitary Isografts. All hypophyseal grafts appeared viable at autopsy. Histochemical examination of isografts confirmed the presence of a solid mass of cellular elements identifiable as adenohypophysial and intermediate lobe tissue and, in addition, confirmed that all isografts were well vascularized. Adenohypophysial tissue contained predominantly chromophobes and acidophils. Although a few thyrotropic and gonadotrophic basophils could be distinguished, generally they were conspicuously absent. Chromophobes in all preparations appeared hypertrophied, with an abundant, slightly basophilic cytoplasm, a large spherical vesicular nucleus, and a well-defined nucleolus. These cells were essentially indistinguishable from degranulated basophils.

Vaginal Smear Patterns

A quantitative analysis of the exfoliative vaginal cytology of mice from Groups 2 and 4 revealed that pseudopregnancy-like cycles, characterized by infrequent estrus and prolonged periods of diestrus, occurred shortly after pituitary transplantation. Before transplantation, all host mice appeared to have regular estrous cycles.

Among the virgin controls, 35% of the total smears taken were estrus and 64% diestrus. The percentage of estrous and diestrous smears occurring prior to pituitary transplantation in the mice of Group 4 were similar to those recorded in control virgin mice. During the postimplantation period, the proportion of estrus in Group 4 was reduced to 11%, whereas the proportion of diestrus increased to 88%. The estrus:diestrus ratio therefore decreased from 0.60 to 0.14.

DISCUSSION

The results of this investigation confirm the previous findings (12, 13, 19–21) that forced breeding in agent-bearing mice caused the earliest and most rapid appearance of

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Ovarian wt. (mg)</th>
<th>Pituitary wt. (mg)</th>
<th>Adrenal wt. (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact force-bred</td>
<td>12</td>
<td>14.0 ± 1.6</td>
<td>2.1 ± 0.2</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>Intact virgin</td>
<td>11</td>
<td>17.9 ± 1.5</td>
<td>2.6 ± 0.2</td>
<td>6.0 ± 0.7</td>
</tr>
<tr>
<td>3A</td>
<td>Nursing full litter</td>
<td>16</td>
<td>18.7 ± 1.4</td>
<td>2.8 ± 0.1</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>3B</td>
<td>Nursing adjusted litter</td>
<td>10</td>
<td>15.4 ± 1.1</td>
<td>2.5 ± 0.1</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>Ectopic pituitary</td>
<td>14</td>
<td>13.7 ± 1.8</td>
<td>2.4 ± 0.1</td>
<td>5.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values in Columns 4 to 6 are mean ± S.E. All superscripts are substitute symbols for the figures used in the calculation of the probability ratios. a/b = $p < 0.05$; b/c = $p < 0.01$; c/d = $p < 0.001$; d/e = $p < 0.01$; e/h = $p < 0.05$.
mammary tumors (Table 1). By comparison, however, virgin mice were least sensitive to tumor development. Mice bearing ectopic pituitary isografts showed the same tumor incidence as did force-bred mice, albeit after a significantly longer latent period of development.

In contrast to the observations of Mühlbock (20, 21) and Marchant (11, 12), mice permitted to lactate did not show a significantly lower tumor incidence or greater latency of development than did either pseudopregnant mice or those from which the young were removed at birth. However, the latency of tumor development in mice that nursed their entire litter was appreciably longer than that in force-bred mice (Table 1). Although litter size little affected tumor incidence, one might suggest that with larger litters tumor incidence was slightly lower, although not significantly so, and latency of tumor development was longer. This is the converse of the observation that, with larger litters, more DMBA-induced tumors per rat did not regress but, rather, continued to grow (14, 15).

Both nursing groups exhibited a significantly reduced LFI compared with that of force-bred mice (Table 2), despite the fact that tumor incidence was not appreciably different (Table 1). This suggests that the prolactin-progestational state that ensues during lactation or pituitary transplantation may be equivalent in terms of effect on tumor incidence to the predominantly progestational condition of pregnancy. Jull (8) observed that administration of estrone to virgin C3Hb mice increased their sensitivity to 1,2:5,6-dibenzanthracene-induced tumors, but pseudopregnancy was more effective, whereas lactation had no particularly inhibitory effect. The data of this study are compatible with these observations, as is also enhanced tumorigenesis associated with hyperestrogenization following anterior hypophalamic lesions (2).

Although it is obvious from the literature that the effects of different social conditions on tumorigenesis vary with mouse strain and induction mode, it is likely that the response is to a large extent mediated through the internal hormonal environment of the mouse. Marchant (12) suggested that high progesterone levels were associated with greatest sensitivity to MC-induced tumors in virgin mice. She also suggested that the enhanced sensitivity of pseudopregnant mice may be explained on this basis. Similarly, the predominantly progestational condition of pregnancy may also be responsible for the enhanced growth of DMBA-induced tumors observed in rats (14). Certainly, the ovarian and pituitary weights (Table 3), target organ histology, and vaginal smear cytology of the groups in question would be compatible with this suggestion. Suckling has been shown to result in release of prolactin (5, 6) from the adenohypophysis. In addition, suckling also results in the release of ovarian progesterone (3). McCormick and Moon (16) have demonstrated that ovarian progesterone, acting directly or in synergism with prolactin, is necessary for the growth of DMBA-induced mammary tumors during lactation. It would be possible, therefore, that this prolactin-progestational condition during lactation per se or combined with the complex hormonal alteration in pregnancy is responsible for enhanced tumor incidence. The data of this study, as well as a previous report (2), suggest that pregnancy and/or lactation, with their accompanying hormonal status, are more effective in this regard than are elevated endogenous levels of prolactin, progesterone, or estrogen alone, in the absence of pregnancy.

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