The Role of the Ovary in Estrogen Production of Mammary Cancer in the Rat

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SUMMARY

The data from this experiment make it very evident that the ovary of the A X C rat furnishes something that is required for the earliest production of the greatest number of mammary cancers during continuous administration of diethylstilbestrol. It further appears that the substance furnished by the ovary is not progesterone. The tumors that do appear tend to grow rather more slowly than those in animals bearing ovaries.

INTRODUCTION

In our earliest reports of the estrogen induction of breast cancer in the A X C rat (2), most of the female rats treated with estrogen for prolonged periods of time died of uterine infections, frequently before the earliest tumor in that cancer in the A X C rat (2), most of the female rats treated was begun. In our studies the ovaries were left in situ. Cutts (1) reported without presenting data that oophorectomy in his hooded rats increased the latent period of tumor formation without influencing the number of tumors produced by estrone pellets.

We (3) reported that synergism exists between diethylstilbestrol and irradiation in the production of mammary cancer in the A X C rat. As we began to evaluate various factors in this synergism, we were able to report that progesterone exerts a substantial protective effect (4). It therefore became of great importance to evaluate the role of the presence of the ovary on synergism.

MATERIALS AND METHODS

Five groups each of 42 female A X C rats weighing between 40 and 50 g at weaning (29 to 31 days) were hysterectomized at 43 to 45 days to prevent the fatal estrogen-induced uterine infection. In the remaining animals the ovaries were removed at the time of hysterectomy, and these latter animals were divided into 3 groups, for a total of 5 groups. Group 1 was anesthetized with ether at 57 to 59 days but no pellet was implanted. (This group is not reported in Table 1 because no animals have developed mammary tumors although the youngest is older than 116 weeks.) Group 2 received a diethylstilbestrol-cholesterol pellet s.c. while under the ether anesthesia. Group 3 had the ovaries removed during hysterec-
tomy and a s.c. diethylstilbestrol-cholesterol pellet implanted subsequently. Group 4 was exactly the same as Group 3 but they were given, in addition, a s.c. injection of 2 mg of progesterone in sesame oil once weekly in order to induce a cycle. Group 5 was also identical to Group 3 but they received both diethylstilbestrol-cholesterol pellets and pure progester-
one pellets. In all groups with pellets, the pellets remained in place until the animals died or were sacrificed. In Groups 3, 4, and 5 vaginal smears were obtained for 10 days out of each month, and when evidence of deterioration of the progester-
one effect was seen in Group 5 an additional progesterone pellet was implanted. Although none of the animals in this study were irradiated, 48 hr after pellet implantation all the animals were anesthetized with sodium pentobarbital (Nembutal), 0.1 ml of solution containing 6.5 mg for every 20 g of body weight, given i.p., so that they would be comparable in all other regards to our rats that received irradiation. The animals were assigned to the appropriate treatment group by randomization methods with the use of a table of random numbers and suitable decks of cards. A total of 11 animals died of the multiple anesthetics, the manipulations, or infections between initiation of the study and completion of all the manipulations. The number of rats listed in Table 1, Column 1, are those that survived all these manipulations.

RESULTS

As can be seen from Chart 1, there was a substantial delay in the onset of the 1st tumors in all 3 groups without ovaries and a much smaller number of tumors was seen in these groups.

The tumors were all essentially the same as previously reported and were similar in all groups. The basic pattern is that of a poorly differentiated solid (medullary) infiltrating carcinoma with little desmoplastic reaction, showing a variable degree of glandular and papillary differentiation. The predominant solid carcinomata contain large central zones of necrosis within nodular aggregates of tumor cells. Lumen formation within some tumor nodules produces a cribriform pattern. In others there are irregular areas of duct and papillary formation lined by columnar cells with inspissated secretion within lumens.
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mammary chains at risk&lt;sup&gt;a&lt;/sup&gt;</th>
<th>First palpable tumor (wk)</th>
<th>Median time for 1st tumor (wk)</th>
<th>Av. tumor growth rates&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of tumors/chains at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DES-chol., ovaries intact</td>
<td>Initial</td>
<td>At 1st tumor seen</td>
<td>Rats with tumors</td>
<td>78</td>
<td>24</td>
<td>43.5</td>
</tr>
<tr>
<td>3</td>
<td>DES-chol.</td>
<td>38</td>
<td>38</td>
<td>21</td>
<td>76</td>
<td>35</td>
<td>51.5</td>
</tr>
<tr>
<td>4</td>
<td>DES-chol., progesterone, 2 mg/wk of injection</td>
<td>42</td>
<td>39</td>
<td>18</td>
<td>78</td>
<td>34</td>
<td>48.0</td>
</tr>
<tr>
<td>5</td>
<td>DES-chol., progesterone pellet</td>
<td>36</td>
<td>33</td>
<td>17</td>
<td>66</td>
<td>33</td>
<td>43.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> The 2 mammary chains in each animal were both exposed to circulating estrogen so both chains are considered at risk.

<sup>b</sup> Average tumor growth rate equals weekly sq cm increase in area of cross-section obtained by dividing cross-sectional area obtained at removal or death by the number of weeks during which the tumor has been palpable.

The solid areas consist of aggregates of large ovoid and polyhedral cells with nuclear crowding, moderate pleomorphism, and occasional gigantoform nuclei. The nuclei are hyperchromatic, with coarse chromatin clumps, prominent nuclear membranes, and 2 or 3 nucleoli.

The cells of the neoplasma are several times the diameter of the adjacent normal ductal and alveolar cells. Mitoses are frequent (often 3 or 4/high-power field) and occasional abnormal mitotic figures are seen.

The neoplasms usually show mixed patterns, solid and glandular. Metastases in regional lymph nodes show similar histological patterns.

Table 1 shows the comparative incidence in the same fashion that we had previously reported (1, 4). In addition we are reporting an index of the tumor growth rates for the various groups.

To date the animals in Group 1 have been followed for more than 2 years and no mammary tumors have been observed. They are also the only animals in this report that are still alive. Although the estrogen-treated animals all grew somewhat more slowly and leveled off at smaller sizes than the Group 1 animals, these differences are not substantial.

As noted above, the earliest tumor was seen in Group 2, which also had the greatest number of tumors in the largest number of animals. The numbers of tumors are significantly greater in this group when calculated in absolute numbers or by the mammary chains at risk.

The greatest delay in 1st tumor appearance as well as the median time of 1st tumor appearance was seen in Group 3. This group also had the smallest number of tumors and these tumors were the slowest growing of all those observed in this study.

The addition of weekly injections of progesterone (Group 4) increased the number of tumors slightly without any other significant effects.

On the other hand, in Group 5, where the progesterone was continuously present from the implanted pellets, we saw a median time for the 1st tumor that was the same as seen for animals with their ovaries. However, the 1st tumor appeared 9 weeks after the 1st tumor in Group 3 and the total number of tumors seen was still only one-third of those seen in animals bearing their ovaries.

The estrogen-treated animals in this study that did not develop multiple mammary tumors generally developed large pituitary tumors and died or were sacrificed when moribund. The pituitary tumors were chromophobic in appearance and were extremely hyperemic with large sinusoids.

It is apparent from the data presented in this study that the ovary plays an important role in the diethylstilbestrol production of mammary cancer in the A X C rat, the presence of the ovary being required for the highest tumor incidence at the earliest time. It is further apparent that progesterone is probably not the missing element when the ovaries are removed, since neither continuous nor cyclic administration of progesterone together with diethylstilbestrol is capable of replacing the ovarian effect.

DISCUSSION

This experiment makes it very evident that the ovary of the A X C rat furnishes something that is required for the earliest production of the greatest number of mammary cancers during the continuous administration of diethylstilbestrol. Since the...
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experiment also makes it appear that the substance furnished by the ovary is not progesterone, many further studies will be required to identify the substance.

The studies of Cutts (1) suggest the possibility that the missing agent may be relaxin. We hope to be able to test whether or not we can increase growth rate and incidence of tumors with the addition of relaxin and to see whether relaxin can increase a slower growth rate in animals that develop their tumors while oophorectomized.

It should also be recognized that the rat ovary may make androgens as well as 20α-hydroxy-4-pregnen-3-one and that these may contribute to the phenomenon and should also be tested.

REFERENCES

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