The Effect of α-Estradiol-3-Benzoate on the Response of CHI Mice to 20-Methylcholanthrene

Albert Segaloff, M.S.

(From the Department of Anatomy, Wayne University, College of Medicine, Detroit, Mich.)

(Received for publication March 23, 1942)

The role of estrogenic substances in the production of neoplasms of the male and female genital tracts and of the mammary glands of both sexes has been discussed at length by many authors. It appears that the conclusions reached depend largely upon a number of variables; among these are the criteria of malignancy chosen, the specific estrogen, the route of administration, and the animals themselves.

However, it would appear from the excellent review of Gardner (1) that the estrogens really have some effect on the production of mammary, uterine, vaginal, and testicular neoplasms. Factors which modify this relation between estrogen and the induction of neoplasms include the heredity of the experimental animal, the type of estrogen substance used, and the duration of treatment.

In connection with the above, it is interesting to note that some carcinogenic hydrocarbons are believed to have estrogenic properties (4).

Although these considerations might indicate the existence of some relationship between the sex glands and reactions to carcinogenic hydrocarbons, such has not been the case. Indeed, Stewart (8) has pointed out that castration seems to have no significant effect on the carcinogenic action of 1,2,5,6-dibenzanthracene in C3H mice.

Fletcher (5) treated 2 rabbits for 13 months with folliculin and estrone and noted that one developed a carcinoma of the uterine adnomena and the other a carcinoma of the uterus. Gilmour (2) used mixed stock mice and painted 3,4-benzpyrene and estrone on the skin. The compounds were dissolved in chloroform. She concluded that estrone increased the susceptibility of the skin to the carcinogen and that it also hastened the appearance of the tumors. Perry and Ginzton (3) also employed stock mice. They dissolved estrone and 1,2,5,6-dibenzanthracene in benzene and painted this mixture, and 1,2,5,6-dibenzanthracene alone, on the skin. There was a higher incidence of tumors in the group receiving estrone and the carcinogen than in the group receiving the carcinogen alone.

Recently Smith, Wells, and D’Amour (7) have injected rats with 20-methylcholanthrene in paraffin and with estradiol benzoate in sesame oil. They found that neither castration nor the estradiol benzoate affected the tumor incidence or the latent period of induction.

The present work was undertaken because it seemed desirable to study the possible cocarcinogenic effect of estrogens in a pure strain of mice. Furthermore, it was believed that the results of Gilmour (2) and of Perry and Ginzton (3) might result in some measure of correlation between estradiol benzoate and the carcinogen.

As can be seen from Table I there was no essential difference in the latent period or in the incidence of tumors whether 20-methylcholanthrene was given alone or in combination with estradiol benzoate. All tumors arose subcutaneously in the region of the pellet.

The first occurred from 12 to 17 weeks after implantation of the pellets, when the mice were approximately 7 months old. The last tumor to appear was palpable in the 24th week after pellet implantation, when the mice were about 9 months of age. The average latent period for the entire group was 18 weeks.

Histologically all the growths except 2 mammary gland tumors in the untreated group were classed as carcinomas. The 2 mammary gland tumors in the untreated group were classed as adenomas.

1. Estradiol benzoate was generously supplied as progynon-B by Dr. Erwin Schwenk of the Schering Corporation.
2. The 20-methylcholanthrene was prepared by Dr. W. E. Bachmann and supplied through the courtesy of Dr. J. T. Bradbury of the University of Michigan, Ann Arbor.
3. I would like to thank Dr. Edgar H. Norris for his interest and for reviewing the microscopic classification of all the tumors.
sarcomas of grade II or III. Four from animals treated with estrogen and carcinogen and 4 from animals treated with carcinogen only were transplanted through 4 transplant generations. In the first group 110 animals were used for hosts and 100 in the second. These sarcomas all grew at about the same rate, took in the same proportion of animals (78 and 82 per cent), and retained their histological identity. Those transplanted were used when they had reached 1.5 cm. in their greatest diameter. In all other cases animals with present experiment the co- or anticarcinogenic effect of estradiol would be shown only if it were very great, since large amounts of carcinogen were employed. However, it should be noted that where a cocarcinogenic effect of estrogen on the skin of the mouse has been reported large concentrations of carcinogen have been employed also. Our studies, like those of Smith, Wells, and D'Amour (7), show no cocarcinogenic effect of estrogens on the connective tissue, subject, of course, to the conditions of the experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Total number</th>
<th>Number alive at 1st tumor</th>
<th>Number of tumors</th>
<th>Latent period, 1st tumor, weeks</th>
<th>Average latent period, weeks</th>
<th>Incidence * per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB, MC</td>
<td>M</td>
<td>25</td>
<td>17</td>
<td>14</td>
<td>15</td>
<td>17</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>6</td>
<td>16</td>
<td>19</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>37</td>
<td>25</td>
<td>20</td>
<td>15†</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>EB</td>
<td>M</td>
<td>25</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>37</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>MC</td>
<td>M</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>12</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>9</td>
<td>5</td>
<td>17</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>37</td>
<td>29</td>
<td>20</td>
<td>14†</td>
<td>18</td>
<td>69</td>
</tr>
<tr>
<td>None</td>
<td>M</td>
<td>25</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>37</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

EB = a-estradiol-3-benzoate in 0.05 cc. sesame oil once weekly.  
MC = Pellet of 20-methylcholanthrene (1.5 to 3.0 mgm.) subcutaneously.  
* Percentage of animals, alive at time of appearance of 1st tumor, which developed tumors before death.  
† Average of males and females.

tumors were allowed to live until they were dead or moribund. None of the growths metastasized, but practically all infiltrated through the body wall.

Two males, one treated with estradiol benzoate and a pellet of 20-methylcholanthrene and the other with only a pellet of 20-methylcholanthrene, developed what appeared in the gross to be neoplasms around the pellets, but on section they were classified as inflammatory reactions.

Characteristic estrogenic effects were seen in all animals which received estradiol benzoate. These included resorption of the pubic symphyses in the females, retention of urine and hydronephrosis, suppression of spermatogenesis, and pronounced stimulation of the female genital tract.

The cervix of all females were sectioned serially and studied. In no instance did they show changes which could be considered malignant.

DISCUSSION

From the results reported by Shear and his associates (6) it is possible that under the conditions of the

SUMMARY

a-Estradiol-3-benzoate had no effect on the incidence, degree of malignancy, or latent period of tumor induction by pellets of 20-methylcholanthrene in CHI mice.

The animals displayed the usual effects of hyperestrinism.

No malignant lesions of the cervix or upper vagina were observed in any of the animals.

I wish to thank Dr. Warren O. Nelson, Chairman of the Department of Anatomy, for encouragement and advice throughout this study and also for allowing me the use of his laboratory.

REFERENCES


The Effect of \( \alpha \)-Estradiol-3-Benzoeate on the Response of CHI Mice to 20-Methylcholanganthrene

Albert Segaloff