Carcinomas of the Uterine Cervix and Vagina in Estrogen- and Androgen-Treated Hybrid Mice*

S. C. Pan, M.B.** and W. U. Gardner, Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication February 4, 1948)

Carcinomas of the uterine fundus occur more frequently among women showing unusual production of estrogenic hormone than in normal women (15). Although there is less evidence of a correlation of abnormal production of estrogens and tumors of the uterine cervix, at least one investigator has reported that deficiencies of vitamins, with possible coincident impairment of estrogen inactivation, was not unusual in women with precancerous lesions and early cancer of the cervix (2).

Invasive epithelial lesions (1, 6) 'precancerous' lesions (3, 10, 14) and carcinomas of the uterine cervix appear in estrogen-treated mice of all strains that have been adequately studied (5, 11, 12). Carcinomas of the cervix rarely occur in mice of most inbred strains, and none have been described that have arisen specifically from the cervix other than in mice from the PM stock, studied recently in this laboratory (9). Tumors of the genital tract of treated and control hybrid mice derived from crossing the PM stock1 with mice of the C3H strain are reported here.

MATERIALS AND METHODS

Reciprocal hybrid mice were used; group PC1 (C3H♀ X PM♂), and group PC2 (PM♀ X C3H♂).5 Fifty-seven female mice of the PC1 group were used, 14 received estrogens and 43 were untreated controls. Ten of the 14 treated mice lived for more than 200 days and are included in this study (Chart I). Of the 70 female mice of the PC2 group, 31 received steroid hormones and 39 were untreated. Twenty-five of the treated mice survived 200 days or more and are included in this study (Chart II).

All mice were maintained in an air conditioned laboratory and fed Purina fox chow and water ad libitum. Prior to weaning, fresh lettuce was placed in their cages once or twice weekly. After weaning, the young were divided into hormone-treated and control groups. Four or 5 females and 1 or 2 male mice were quartered in each cage occupied by the controls; young born in the cage were removed at birth so that pregnancies were repeated frequently. The hormone-treated mice received either (a) 16.6 µgm. or 25 µgm. of estradiol benzoate, (b) 0.25 mgm. of stilbestrol, (c) 16.6 µgm. of estradiol benzoate plus 1 mgm. of testosterone propionate2 weekly or one pellet weighing from 4.2 to 4.6 mgs. and composed of stilbestrol (one part) and cholesterol (3 parts). All injections were made subcutaneously and the pellets were implanted subcutaneously. Treatment was started when the mice were 25 to 65 days of age and continued until the animals died, had tumors or their general condition indicated the imminence of death. The period of treatment ranged from 174 to 594 days.

At necropsy all viscera and glands were examined and many tissues were preserved in Bouin's fluid or 10 per cent formalin. The uterine cervixes were usually sectioned serially and 2 of every 10 sections were mounted and stained in hematoxylin and triosin. The lesions were grouped in stages as described previously (1, 6).

OBSERVATIONS

Four of 10 mice of the PC1 group that tolerated the treatment more than 200 days had invasive cervical lesions or carcinomas, whereas in the treated mice of the PC2 group there were observed 9 carcinomas or invasive lesions, 8 arising from the uterine cervix and 1 from the vagina (Table I). All of the

---

*This investigation was aided by grants from The Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research.

**Anna Fuller Fund Fellow.

1Mice of the PM stock were derived from a pair of mice received from Dr. H. B. Andervont and were descendants of mice that he had received from Drs. Pybus and Miller. The mice of the C3H strain were derived from animals received from Dr. L. C. Strong.

2The estradiol benzoate and testosterone propionate used in this investigation were supplied by Dr. E. Schwenk of the Schering Corporation, and the stilbestrol was supplied by E. R. Squibb & Sons, New Brunswick, N. J. The stilbestrol-cholesterol pellets were made by a method similar to that described by T. R. Forbes (4).
TABLE 1: CERVICAL CARCINOMAS OR INVASIVE LESIONS AMONG HYBRID MICE (PM X C3H) THAT RECEIVED SEX HORMONES

<table>
<thead>
<tr>
<th>Group of mice</th>
<th>Mouse No.</th>
<th>Age at death, days</th>
<th>Level of treatment</th>
<th>Period of treatment, days</th>
<th>Location Stage</th>
<th>Morphology</th>
<th>Chronic</th>
<th>Vascular lesions</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>5</td>
<td>391</td>
<td>0.0166</td>
<td>0.830</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Hyaline change</td>
<td>Mammary tumor</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>282</td>
<td>1.0</td>
<td>0.830</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Endothelial change</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>349</td>
<td>0.747</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Endothelial proliferation</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>265</td>
<td>0.725</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Necrosis and leukocytic infiltration</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>396</td>
<td>0.880</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Endothelial prol. thrombosis</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>417</td>
<td>0.879</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Endothelial proliferation</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>474</td>
<td>1.013</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>End. prol. and necrosis</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>310</td>
<td>0.025</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>End. prol. and occlusion</td>
<td>Hydronephrosis</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>394</td>
<td>1.275</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>End. prol. and occlusion</td>
<td>Hydronephrosis</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>681</td>
<td>0.0166</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Fibrinoid degeneration and hemorrhage</td>
<td>Adenoma of lung</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>585</td>
<td>1.311</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Hyaline change</td>
<td>Mammary tumor</td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>612</td>
<td>1.378</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Hyaline change</td>
<td>Mammary tumor</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>658</td>
<td>1.411</td>
<td>1.0</td>
<td>Cervix</td>
<td>4</td>
<td>+</td>
<td>Hyaline and occlusion</td>
<td>Mammary tumor</td>
</tr>
</tbody>
</table>

* E B = estradiol benzoate  † T P = testosterone propionate  ‡ See text for elaboration  § Endothelial proliferation.

small lesions and apparently the carcinomas, except for one from the vagina, arose from the uterine cervical region with a predilection for the fornices of the vagina and posterior lip of the cervix. All lesions were composed of epidermoid cells that showed a lack of polarity, even in the small lesions, but that varied in the extent of invasion of adjacent tissue. Six among these 11 tumors were classified as stage 4 and considered to be carcinomas; they had extended beyond the cervix and vagina to adjacent tissues; 2 were grouped in stage 3 and had extended to the limits of the cervix; 1 in stage 2, and 2 in stage 1. Some of the carcinomas showed a cyst-like arrangement of the cells with cornified or mucoid material mixed with leukocytes in the cystic structure. Some of the tumors were composed of irregular sheets or solid strands of anaplastic or undifferentiated epithelial cells (Figs. 1, 2). Mitoses were frequently seen in these areas. In the larger tumors the entire uterine wall was invaded and the greater part of the genital tract was involved. Tumor cells invaded the lymphatics, blood vessel walls (Fig. 3) and the nerve sheaths (Fig. 4). The vaginal and cervical epithelia in the areas from which the smaller growths arose were thin, of low stratified epithelium showing no cornified layer. The epithelium of adjacent areas of the cervix and vagina was thicker and usually slightly hyperplastic and often showed mucoid degeneration. Marked hyalinization of the basement membrane of the nontumorous epithelium was observed in one animal. Marked mucoid or hyaline change of the stroma, and occasional hyperplasia of the fibrous tissue were noted in many of the neoplastic or cancerous, as well as in non-neoplastic portions of the genital tract. In the majority of the animals the musculature of the uterine wall was replaced partially by scar or hyaline tissue. Hyaline material also appeared along the walls of the lymphatics and blood vessels. In many instances both chronic and acute lesions of the parametrial and other arteries were encountered. In some areas the walls of the blood vessels were edematous; the endothelium was elevated and the media poorly defined; extreme proliferation of the intima, deposits of hyaline material or fibrinoid degeneration of the subendothelial tissue also occurred so that the lumina of some blood vessels were completely or partially occluded. Fibrous proliferation of the adventitial tissue was prominent and the cellular elements were infiltrated with mononuclear cells (Fig. 5). In one case hyalinized thrombotic material was mixed with the proliferating endothelial cells. Another artery in the same
region revealed necrosis and leukocytic infiltration of a part of its wall. Occasionally the walls of the blood vessels were impregnated with red blood cells.

Most tumors showed considerable infiltration of mononuclear leukocytes and plasma cells and occasionally ulceration had occurred.

Cystic hyperplasia of the endometrium of the uterine horns was noted in many animals, and in some, the cysts penetrated the musculature and were in contact with the serosa. The stroma of the uterine horns revealed mucoid change in some cases and the blood vessels had walls thickened by intimal and adventitial proliferation and medial degeneration. Similar vascular lesions and extreme narrowing or even occlusion of the blood vessels occurred in some of the ovaries.

The adrenal glands revealed nothing unusual. Two mice had associated hydrenephrosis, 2 had mammary tumors and 1 had adenoma in the lung. Lymphosarcomas occurred frequently in mice of these groups; among 13 animals with cervical carcinomas, 4 had lymphosarcomas.

None of the 82 mice in the control groups had cervical or uterine carcinoma, although 5 had uterine fibroids or fibromyomas; 1 in the PC1 group at 508 days of age and 4 in PC2 group at an average age of 462 days. Grossly the fibromyomas varied from 0.8 to 1.5 cm. in diameter and were encapsulated. In places they replaced the walls of the uterine horns (Figs. 6, 7). The tumors were composed of irregularly interlacing bundles of muscle and fibrous tissues within a fibrous capsule. In some areas marked hyaline change was noted. The mucosa overlying the tumor was thinner than usual and had a reduced number of glands. The endometrium of the remaining uterine horn revealed slight atrophy or occasional cystic hyperplasia. The lumina of the cornua were narrower than usual. The blood vessel walls were thick and hyalinized, and in one animal marked cellular proliferation of the endothelium with impregnation of red blood cells in the subendothelial region was observed.

DISCUSSION

Earlier experiments have shown that uterine cervical and vaginal tumors occurred in estrogen-
treated mice and it has been reported that no hereditary tendency to develop carcinoma of the cervix had been observed. Among mice of the present groups the incidence of epidermoid carcinoma is higher than that in the untreated mice of the PM stock from which mice of these two groups descended. It is also slightly greater than that occurring in mice of other hybrid stocks and is comparable with that of the hybrid mice of CC₁ or CC₂ groups that survived treatment with estrogens for long periods (1). The duration of treatment was more important than the amount of the estrogenic chemicals within the range of dosage used. The average period of treatment preceding cervical neoplasm was shorter than in animals in other groups; no cervical lesions appeared in either CC₁ or CC₂ groups before 400 days of age. More than half of the tumors occurred within one year or less and one animal had a stage 3 lesion when it died at 272 days. Moreover about half of the tumors were classed as carcinomas and considered to be malignant as indicated by invasiveness. The average period of survival of mice in the two groups was 46 and 108 days shorter respectively than that of the control animals, none of which had cervical or vaginal carcinomas or invasive lesions. Over 40 per cent of the estrogen-treated mice died with lymphoid tumors at relatively early ages (8).

Mice of the group treated with stilbestrol-cholesterol pellets did not have uterine cervical cancer, apparently because of their shorter life span (Charts 1 and 2). Mice given the larger doses of estradiol benzoate (25 μg weekly) acquired no higher percentage of cervical cancer than did those given smaller amounts. Half of the mice that received both estrogen and androgen and that survived more than 500 days had malignant tumors. Administration of the androgen reduced the incidence of lymphoid tumors, a possible cause of the increased longevity and the high incidence of cervical cancer.
As reported previously the untreated mice of the PM stock had a number of malignant tumors of undifferentiated cell type, probably carcinoma. This type of tumor was lacking in the estrogen-treated mice of the PC₁ and PC₂ groups, and the reason has not been determined.

In association with the neoplasia of the epithelium and the marked regressive lesions of the stroma, the chronic proliferative and obstructive vascular lesions in the uterine wall are of interest. Hyaline change and occlusion of the blood vessels have previously been observed in estrogen-treated mice in this laboratory (1, 6). Cellular proliferation and occlusion of the blood vessel walls were exceedingly prominent and involved the larger arteries in the parametrium and the vaginal and cervical walls. The so-called endarteritis obliterans type of lesions has been known to occur in various conditions and also in the genital tract during advanced age and is generally believed to be a result of disuse atrophy. Whether certain underlying hormonal imbalances or hereditary factors might have played a role and whether they were the causative or coincident findings in the tumorous animals is unknown.

The untreated mice of the PC groups did not have carcinomas of the genital tracts, in this respect being quite unlike the mice of the parental PM stock. The relatively high incidence of uterine fibromyomas was of interest because such tumors being quite unlike the mice of the parental PM stock. The relatively high incidence of uterine fibromyomas was of interest because such tumors were the causative or coincident findings in the tumorous animals is unknown.

The untreated mice of the PC groups did not have carcinomas of the genital tracts, in this respect being quite unlike the mice of the parental PM stock. The relatively high incidence of uterine fibromyomas was of interest because such tumors have not been noted so frequently in mice of other hybrid groups or strains in this laboratory. The appearance of fibromyomas in the old mice and their absence in the estrogen-treated mice, whereas cervical tumors did appear in the latter, indicates a dissimilar etiology of the two types of growth.

SUMMARY

Four of 10 estrogen-treated hybrid mice (C₃H♂ × PM♀) that tolerated the treatment more than 200 days had carcinomas of the uterine cervix or vagina. Nine cervical carcinomas or invasive epithelial lesions were observed among 25 treated hybrids (PM♀ × C₃H♂) that survived 200 days or more. All the 13 tumors were epidermoid carcinomas or invasive epithelial lesions that were considered to be early stages in tumor formation. None of the 82 mice in the control groups had carcinomas of the uterus or vagina although 5 had uterine fibromyomas. In association with the neoplastic change of the uterine and cervical tissue, chronic proliferative vascular lesions have been observed involving the parametrial and uterine vessels.

REFERENCES


DESCRIPTION OF FIGURES 1 TO 4

Fig. 1.—Photomicrograph showing junction of normal cervical epithelium (upper left) with the epithelium over the infiltrative lesion in 24PC.

Fig. 2.—A section through the vaginal fornix of a mouse (91PC; Table I). The irregular basement membrane and folded arrangement of the non-tumorous epithelium (upper part of photograph) is common in mice given testosterone propionate and estrogen. An anaplastic tumor is shown in the lower part of the photograph.

Fig. 3.—An extension of tumor cells perivascularly into the outer vaginal wall (Stage 3 tumor; 49PC; Table I).

Fig. 4.—Extension of the tumor beyond the cervical wall and proliferative and degenerative lesions of adjacent arteries (18PC; Table I).
Figs. 1-4
DESCRIPTION OF FIGURES 5 TO 7.

**Fig. 5.—** Obliterative arterial lesion in the parametria as a result of intimal proliferation. (One artery reveals marked intimal proliferation with narrowed lumen and the other artery has a completely obliterated lumen.)

**Fig. 6.—** Fibromyoma of the uterine horn of a mouse (46 PC.) in the control group. The tumor is well encapsulated. The endometrium shows cystic glands.

**Fig. 7.—** Photomicrograph of the same tumor revealing the interlacing bundles of fibrous tissue and the overlying endometrium.
Carcinomas of the Uterine Cervix and Vagina in Estrogen- and Androgen-Treated Hybrid Mice

S. C. Pan and W. U. Gardner

Cancer Res 1948;8:337-345.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/8/7/337.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.