Cancer of the Cervix of the Uterus in Hybrid Mice Following Long-Continued Administration of Estrogen

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The growth stimulating action of estrogen on the female genital tissues first focused attention upon the possibility of inducing cancer of the cervix of the uterus by long-continued administration of this hormone (2).

These experiments were first undertaken in the monkey. Atypical hyperplastic lesions were obtained which were considered "precancerous" (26, 27). Continued experiments in monkeys have so far failed to induce definite cervical cancer (3). In guinea pigs long-continued injections of estrogen, although producing fibromyomas and glandular cystic hyperplasias of the uterus (25), have failed to produce cervical cancer.

Carcinoma of the uterine cervix does not appear spontaneously in mice. In this respect it differs from mammary cancer. Unlike mammary cancer (31), it has not been induced by local application of carcinogens. After long-continued treatment with estrogen, however, cervical lesions in mice have been reported by several investigators (10, 13, 14, 24, 28, 29, 32). In these earlier reports the incidence was less than 15 per cent of the treated animals which lived beyond certain definite ages (usually a year or 15 months).

One reason why the incidence of cancer of the cervix uteri has been so low in mice treated with estrogens is that mammary cancer appears at an earlier age than does cervical cancer, and consequently animals may die of the former; i.e., many of them do not live long enough for the cervical cancer to develop. As previously reported (14), it was necessary to remove mammary tumors twice before the appearance of an advanced, transplantable carcinoma of the uterine cervix.

In our experience other mice from strains less susceptible to mammary tumors have not tolerated prolonged treatment with estrogens at high levels (13).

In mammary cancer in mice (a) hereditary constitution (22) (b) estrogenic stimulation (21, 23), and (c) maternal transmission (milk factor) (8), are all important factors in carcinogenesis. As female mice age and the mammary glands atrophy, multiple hyperplastic nodules are present (19) which provide several potential loci of origin of mammary cancer. The milk factor plays an important part in determining the presence of these hyperplastic nodules (11). Ovarian hormones or estrogenic stimulation are necessary for the growth of the tissues of the mammary glands. (1, 15, 20, 23, 33).

The present paper reports an incidence of cervical lesions and carcinoma of 50 per cent in one group of 20 mice, and an incidence of 62 per cent in a second group of 24 mice, which survived estrogenic treatment for more than one year. Previous work has been limited to inbred strains. This report involves hybrids between two strains inbred in regard to mammary cancer.

This high incidence focuses attention on estrogenic hormone as a very important, rather than an incidental factor in cancer of the uterine cervix.

ANIMALS AND TREATMENT

Two strains of mice were chosen, one of which, C57, is definitely resistant to mammary cancer. The other, CBA, has an incidence of about 70 per cent of spontaneous mammary tumors in "forced bred" animals, but these tumors appear relatively late in life. As previously reported, C57 mice have a high incidence of pituitary tumors (18). C57 animals have poor tolerance for estrogenic treatment, while CBA mice tolerate this treatment well. Therefore these two strains were hybridized in the hope that the F1 generation would tolerate estrogenic treatment well and that the incidence of hypophysial and mammary tumors might be determined.

In order to study possible influence of maternal transmission (milk factor) two crosses were made as follows:

1. Between C57 females × CBA males: This group was designated as CC2, and 53 of the F1 generation animals of both sexes were injected. Twenty-four females survived estrogenic treatment for more than one year.

2. Between CBA females × C57 males: This consti-
tuted group CC1, and 52 of the F1 generation of these hybrid animals were placed on experiment. Six of these mice died before one year of age, 5 of them of mammary cancer. Twenty females survived estrogenic treatment for more than one year.

Cancer of the cervix was not found in animals of either group sacrificed before one year of age. One large, transplantable carcinoma of the cervix of the uterus in a mouse 364 days of age had been reported previously (14).

Previous experiments with estrogenic induction of mammary tumors (17) indicated the probable optimum dose at between 15 and 25 μg/m. In this experiment 16.6 μg/m. per week was used in the majority of animals. Some received as much as 50 μg/m. per week. Estradiol benzoate was the estrogen. Other cervical cancers have been obtained after injecting other estrogens; stilbestrol, triphenylethylene, estrone, and estradiol dipropionate, and also after combinations of estrogens with androgens and progesterone (12).

Treatment was begun at 28 to 56 days of age and continued at the same level throughout the animals' lives.

Similar numbers of untreated hybrid mice were maintained as controls. Since it had been shown that pregnancy definitely increased the incidence of mammary cancer, the controls were mated and the young removed immediately after birth. Since female mice ovulate the day after parturition, and males were always present, these control females were almost continually pregnant (7), at least during their early reproductive life.

The mice were all from Dr. L. C. Strong's colony, kept in air-conditioned environment and fed on Purina Fox Chow. It has previously been shown that mice kept on a diet of Fox Chow have a higher incidence of mammary cancer than those kept on one other diet (30). No data are available for estimating the possible effect of diet on cervical cancer.

RESULTS

The cervical cancers here reported are squamous cell carcinomas. They originated in the stratified epithelium of the lower cervical canal, external cervical os, or the fornices of the upper vagina. The most common site was the posterior lip of the cervix with its frenulum-like fold to the posterior wall of the vagina. The parts of the cervix and vagina involved by the lesion are shown in Fig. 1 for four of these tumors.

![Fig. 1.—Diagrams of coronal sections through the cervix uteri and upper vagina showing the dorsal half to indicate the extent of 4 of the lesions in animals 12 CC1, 15 CC1, 52 CC1, and 41 CC1 (see Figs. 9, 10).](image)

For purposes of classification the lesions have been divided into 4 stages (13), all of which are considered definitely pathological, as judged by histological criteria. Stages I and II are early invasive lesions, usually localized and limited to the mucosa, the posterior lip and external os being the most frequent locations. These lesions are frequently quite hyperplastic and may contain epithelial pearls (Figs. 1 and 2). Stage IV is an extreme lesion involving practically the whole cervical canal and the upper part of the vagina,

**DESCRIPTION OF FIGURES 2 TO 5**

**Fig. 2.—Stage II lesion in mouse 12 CC1, involving the posterior lip and the left side of the external os of the cervix, but not the adjacent vagina (see Fig. 1-left for the extent of the lesion).** The mouse was 508 days old and had received 1.33 mg/m. estradiol benzoate in 68 weeks, starting at 27 days of life. She also had a mammary adenocarcinoma in the 4th left gland and a pituitary tumor weighing 20.5 mg/m.

**Fig. 3.—Stage IV lesion in mouse 11 CC1. Photograph of a cervical carcinoma in a mouse treated for 528 days, total dose 1.25 mg/m. estradiol benzoate.** Besides this Stage IV squamous cell carcinoma, this mouse also had lymphatic leukemia.

**Fig. 4.—Stage III lesion in mouse 52 CC1, involving the posterior lip and the left side of the cervix and also the posterior wall of the upper vagina (See Fig. 1-right).** The section is through a lower level at the opening of the cervical canal. The frenulum-like fold connecting the posterior lip of the cervix with the posterior vagina is shown in the left center. This cervix was greatly enlarged, fibrous, and nodular. This mouse had a large tumor in the right ovary, and a pituitary gland weighing 10 mg/m. She had received 3.65 mg/m. estradiol benzoate in 73 weeks.

**Fig. 5.—Stage IV lesion in mouse 15 CC1. A squamous cell carcinoma involving the entire cervix and upper vaginal wall.** The cervix was enlarged, fibrous, and nodular. This mouse had received 1.2 mg/m. estradiol benzoate in 72 weeks; had also a mammary adenocarcinoma in the left 5th gland and a pituitary weighing 7 mg/m. (See Fig. 1-left.)
Fig. 6.—Stage IV lesion in mouse 53 CC. This tumor involved the whole right side, posterior lip, and part of the left side of the cervix and extended well down into the vagina. It was observed in the living animal and watched for several days before autopsy. The surface protruded into the vaginal canal and was necrotic. This animal had received 3.7 mgm. estradiol benzoate in 74 weeks, and had a pituitary tumor weighing 28 mgm.

Fig. 7.—Stage IV lesion in mouse 41 CC. A squamous cell carcinoma involving the entire cervix from the bifurcation of the uterine horns and including the upper portion of the vagina. The invasive nature of the small cords of epithelial cells is shown. The tumor was discovered at autopsy but not observed in the living animal. Fig. 1—right shows the extent of the lesion. This mouse had received 1.35 mgm. estradiol benzoate in 81 weeks. The animal had a pituitary tumor weighing 69 mgm.

Fig. 8.—Stage IV lesion in mouse 3 CC. This lesion was a squamous cell carcinoma observed in the living animal and followed for several days before autopsy. The tumor was over 2 cm. in diameter and involved the entire vaginal and cervical regions. The figure illustrates the invasion of the tumor at the lower (vaginal) border. This mouse had received 1.116 mgm. estradiol benzoate in 67 weeks, and had a pituitary weighing 8.5 mgm.
showing extensive areas of rapid hyperplasia and penetrating to the walls of the rectum or the bladder. These tumors may be 2 cm. or more in diameter. A few may grow so extensively as to fill the whole pelvis (Figs. 3, 6, 7, 8), or to protrude from the vagina. Stages II and III are intermediate (Figs. 2, 4).

Suntzeff, Burns, Moskop, and Loeb (11) have also made a subdivision into 4 stages. It seems to us that their stages 1 and 2 were earlier developments than our Stage I, and sometimes on the borderline of normal. We have purposely not included this type as a lesion. Our Stage I definitely includes lesions recognized as early invasive growths. Since they are localized to the genital tissues, and since their progressive growth cannot be followed, we do not consider them as necessarily malignant. The sequence of transitional changes from the earlier lesions to the malignant ones have indicated that they are probably early stages of the same process.

The incidence of lesions in these stages in the two groups of mice is indicated in Table II.

The age distribution is shown on the abscissa in Figures 9 and 10. The various stages of atypical growth are designated by the Roman numerals. Each Arabic number designates the animal in which the lesion occurred.

The highest incidence of cervical lesions appeared in the CC1 group, in which no mammary tumors developed. The lower incidence in the CC2 group was probably due to the 60 per cent incidence of mammary tumors which occur at an earlier age than cervical lesions.

Inevitably the question arises as to the malignancy of cervical lesions in these experimental animals. For instance, as late as 1938 Suntzeff, Burns, Moskop, and Loeb (32) still preferred to designate these lesions as "cancer-like" because of the difficulty of proof of malignancy. However, the report in 1938 of continued growth of cervical cancer when transplanted to other mice of the same strain, without continuance of estrogenic treatment of the hosts, demonstrated the autonomy of this cancer growth (14). This tumor also metastasized to the regional lymph nodes, thus providing very good evidence of the malignancy of induced cervical carcinoma. In the present series no metastases were noted but at least 3 cancers were lethal. Also those rated as Stage IV showed direct invasion of adjacent nongenital tissues.

The estrogen-treated CC1 animals (nursed by mothers of the C57 cancer resistant strain) had no mammary cancers, while the CC2 animals (nursed by the CBA mothers, of a cancer-susceptible strain) had a 62 per cent incidence of mammary cancer. This stresses the importance of the strain of mother used in the breeding of the experimental animals.
maternal transmission as a factor in mammary carcinogenesis (8). The lower incidence of cervical cancer in the CC3 hybrids is probably due to the early death of these mice with mammary tumors.

Maternal transmission (milk factor) does not seem to affect the incidence of carcinoma of the uterine cervix.

No cervical lesions appeared in either group before 400 days of age. None appeared in the CC1 group before 450 days of age (Figs. 9, 10). The conclusion is therefore drawn that with this hybrid stock chronic estrogenic stimulation at this level usually requires longer than 400 days for the appearance of cervical cancer. Some of the animals lived 650 or 700 days, nearly 2 years, without developing cervical cancer (Figs. 9, 10, Stage 0).

As previously noted (13), cervical cancer seems to develop in all strains of mice when treated with estrogens, regardless of their resistance or susceptibility to mammary cancer, if they will tolerate the estrogenic treatment.

In the animals under treatment with estrogen which did not develop cervical cancer the proliferation of vaginal epithelium usually continued, in many cases to a condition equivalent to that of estrus, even in very old animals. Other signs of atypical changes occurred in both vagina and uterus of these animals and also in those in which cervical carcinoma appeared (13, 32). The most striking of these changes was the hyaline transformation of the connective tissues. The walls of blood vessels of the uterus, cervix, and vagina, especially the small arteries, showed striking hyaline transformation, almost to the point of closure of the lumen in some cases. There was also extreme growth of the glands of the uterus proper. Sometimes they grew through the muscle layers and the tips appeared as distended vesicles under the peritoneum. Less frequent, but present in several animals, was metaplasia of the uterine glands and surface epithelium. This was usually associated with evidence of pyometra.

One of the most interesting findings in the old mice which survived prolonged treatment without cervical lesions, was the unevenness of growth of the vaginal epithelium. Patches of thick stratified and cornified epithelium were frequently interspersed with other thin areas devoid of cornification and sometimes infiltrated with leukocytes.

In many of the cervical lesions it was relatively easy to find penetrating down-growths of epithelium with high mitotic indices indicative of extreme hyperplasia. In cases where extreme cornification of the lesions occurred, intermediate stages showed primarily a down-growth of epithelial bulbs into the connective tissue, and secondarily, the continued proliferation of this epithelium leading to production of a central core of cornified material. Later continued growth often produced an extreme cornified core and frequently leukocytic infiltrations appeared here, just as they appear periodically after estrus in the normal vagina of rodents.

Frequently there were areas of almost complete erosion of the epithelium. Usually these contained some leukocytic or round cell infiltration. Frequently a definite zone of round cells separated the thickened basement membrane from the hyalinized connective tissue.

**DISCUSSION**

The growth of epithelium of the vagina and cervix of the uterus is entirely dependent upon hormonal factors in normal life. Therefore, knowledge of the fluctuation of the structure of these organs in normal rodents is necessary for an appreciation of the influence of estrogen in abnormal growths.

The structure of the vagina in the normal animal seems entirely dependent upon the level of estrogenic hormone (6). Before puberty the vaginal wall is composed of only 2 layers of epithelial cells (5). During the first estrus, which leads to puberty, the vaginal wall grows rapidly so that in the course of 2 or 3 days it becomes a thick stratified epithelium with a superficial cornified layer. After estrus passes, the superficial part of the wall is sloughed into the lumen and destroyed by leukocytic invasion. This leaves an epithelium between estrus periods of 5 or 6 layers in thickness.

A low stratified epithelium 2 to 4 layers of cells in thickness, extends through the external os well into the cervical canal to the point where the canal branches to enter the 2 uterine cornes. This is the point of transition between low stratified, cervical, and the columnar uterine epithelium.

As previously noted, the cervix of the mouse is different from that of women and monkeys in that it contains no mucous secreting cervical glands.

The most vulnerable part of the cervix is the posterior lip where the frenulum-like fold makes an attachment to the posterior vaginal wall. The early stages of cervical lesions which can be definitely localized occur most frequently here. Later stages extend both upward into the cervical canal and into the vaginal fornices as the tumors progressively invade extensive areas.

After the animal becomes pregnant, estrus cycles are postponed and the epithelium of the vagina, instead of becoming cornified as at estrus, develops a superficial stratum of mucous cells (6). This structure persists throughout pregnancy.

When the ovaries are removed from adults, the vagina returns to a 2- or 3-layered condition, indicating extreme atrophy. This is the condition typical of old age. Also younger animals in which spontaneous mammary cancers develop show precocious atrophic vaginal changes associated with ovarian atrophy (4).

Perry (28) and Perry and Ginzton (29) reported 3 cases of cervical carcinoma in a group of 27 mice (11 per cent) which had been painted on the back with estrone and dibenzo-18-estrane and which survived more than 6 months of this treatment.

In 1938, Suntzeff, Burns, Moskop, and Loeb (32) reported an incidence of 10.7 per cent; 25 mice out of a total of 234 showed invasive or "cancer-like" lesions.
Gardner and Allen (13) reported 27 cervical lesions in 183 mice, an incidence of 14.2 per cent. Also Gardner, Allen, Smith, and Strong (14) reported the transplantability of cervical carcinoma through 3 generations of transplants, indicating that this type of growth is malignant.

In comparing these animals treated for long periods of time, often extending through the second year of life, it is clear that estrogenic treatment at this level maintains extensive growth of the uterus and vagina beyond the time at which this usually declines, and often practically stops; i.e., the time equivalent to the menopause in women. Frequently, however, in old animals this growth may be uneven or spotty.

Other conditions present in these animals indicate the successful maintenance of a high level of estrogen. The symphysis pubis was resorbed and replaced by long pubic ligaments (13), and new osseous tissue had formed in the marrow cavities of the long bones (16).

Cervical carcinomas or invasive lesions appeared in the hybrid animals described above only after at least one year's treatment. Since the smaller lesions cannot be diagnosed until after death, there is no way of telling how long before autopsy they might have been present.

As has been reported (14), in one estrogen-treated mouse of the C3H strain, however, a malignant (transplantable, metastasizing) carcinoma occurred. This mouse at 45 days of age (about the time of puberty) was treated with estradiol benzoate, 16.6 mgm. being given every 3 weeks until the age of 266 days, by which time a total of 183 mgm. had been injected. Toward the end of this period a mammary tumor appeared and this was removed at 266 days of age. At this time the dose was tripled to 16.6 mgm. weekly. At 290 days of age a secondary mammary tumor was removed. At 359 days of age enlargement of the pelvis was noted and at 364 days a tumor protruded from the vagina. The mouse was sacrificed at 364 days of age, one day short of a year.

The tumor proved to be a large cervical carcinoma extending up to the bifurcation of the uterus. It surrounded the rectum, filled the pelvis, and infiltrated the muscles of the bladder. It was an extremely hyperplastic squamous cell carcinoma. The lesions and carcinomas of the uterine cervix have appeared in 15 of 24 mice (62 per cent) and 10 of 20 mice (50 per cent) which survived the treatment for more than one year. These two groups were hybrids of the C57 and CBA strains, inbred with regard to mammary cancer.

Genetic factors involved in mammary cancer seem to have little influence as determining factors in cancer of the cervix uteri.

The two groups mentioned above were formed to test the possible importance of maternal transmission (milk factor) in cervical cancer. Although important in mammary cancer, maternal transmission seemed to have little to do with the incidence of experimental carcinoma, except that a certain number of animals, nursed by mothers susceptible to mammary cancer, died of mammary cancer before the age at which cervical cancer appears.

The high incidence of cervical cancer in these experimental groups emphasizes that estrogen is a very important factor, not merely an incidental one, in cervical carcinogenesis.

**SUMMARY AND CONCLUSIONS**

After long treatment with high doses of estrogen, lesions and carcinomas of the uterine cervix have appeared in 15 of 24 mice (62 per cent) and 10 of 20 mice (50 per cent) which survived the treatment for more than one year. These two groups were hybrids of the C57 and CBA strains, inbred with regard to mammary cancer.

**REFERENCES**


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