Regression of Estrone-induced Mammary Tumors in the Rat

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SUMMARY

Mammary tumors induced in rats by prolonged stimulation with estrone regress if this source of estrogen is removed. Regression of this type of tumor is rapid and complete, and is accompanied by shrinkage of cells, increased nuclear pyknosis and a decrease in the number of dividing cells. The regression is permanent and is maintained as long as the animals are free of the exogenous source of estrogen.

INTRODUCTION

Induction of mammary carcinomas in rats by implantation of pellets of estrogen has been reported many times. The tumors so induced are responsive to a variety of hormonal manipulations, yet appear to retain their dependency upon the initiating stimulus (2–8). Total regression of estrogen-induced mammary tumors as a consequence of removal of the source of estrogen has been reported (1, 9). The present paper describes some of the histologic appearances of the estrone-induced mammary tumors which were regressing following removal of the estrone pellet.

MATERIALS AND METHODS

Mammary tumors were induced in a strain of hooded rats by implanting single pellets containing 8–10 mg of pure estrone into 21- to 40-day-old females. The pellet was inserted subcutaneously between the shoulder blades through a small incision in the skin. As described previously (1), the animals had been hysterectomized shortly after weaning, but the ovaries remained intact. The rats were maintained on a commercial cube diet and water ad libitum. Each week the animals were weighed and carefully examined for the appearance of mammary tumors. Growth of the tumors was estimated by caliper measurements (through the skin) of the greatest and least diameters. The tumors were allowed to progress to a diameter of approximately 3.0 cm to 4.0 cm, at which time all of the tumors were biopsied and the pellets removed from the test groups. The control group (i.e., nonregressing tumors) retained the estrone pellet throughout the period of study. The tumor regression was followed to completion, a period covering eight weeks from the time the pellet was removed. At two-week intervals animals from the control group and from the test groups were sacrificed and the tumors removed.

RESULTS

Histology and Growth Characteristics of Tumors after Removal of the Pellet

The growth characteristics and the general histologic features of the mammary carcinomas induced by estrone have been published already (1–3), but the general cytologic features of the tumors used in this study are shown in Figs. 1 to 3. Regression occurred in all of the epithelial tumors following removal of the estrone pellet, and a decrease in tumor size was marked even at one week, as is shown in Chart 1.

Regression was most rapid during the first two weeks, but continued, although more slowly, to complete extinction of the tumors. By six weeks tumors could be found only infrequently and were present as slightly thickened, yellowish areas in the mammary tissue (Fig. 7), while at 8 weeks the tumor had disappeared completely (Fig. 8). This regression was accompanied by a decrease in the number of mitotic figures and an increase in the number of cells showing pyknotic nuclei: This is shown in Chart 2 and Fig. 4. As shown in Chart 3, there was a generalized reduction in the size of all tumor cells, amounting to 13% of the initial, nonregressing size, by four weeks. Thereafter, the cell size remained fairly constant.

Tumors that had been regressing for two weeks often contained cystic spaces of variable size. In some tumors the spaces were filled with clear fluid, while in others the spaces were contained only by thin, delicate, acellular walls.
Chart 1. Regression of mammary tumors after removal of the estrone pellet. The tumor size is expressed as a percentage of the tumor area at the time the pellet was removed. △—△, control tumors; +—+, 2 weeks' regression; ○—○, 4 weeks' regression; •—•, 6 weeks' regression; ●—●, 8 weeks regression.

Chart 2. Change in the number of pyknotic and mitotic nuclei in regressing and nonregressing tumor. The data, shown for the regressing tumors, is the composite data from all regressing tumors. X—X, pyknotic cells in regressing tumor; ×—×, pyknotic cells in nonregressing tumor; •—•, mitotic cells in nonregressing tumor; ○—○, mitotic cells in regressing tumor.
Chart 3. Decrease in nuclear size in regressing mammary tumors. The chart shows data for two and four weeks' regression only: nuclear and cell sizes remained approximately constant after four weeks. The absolute values shown are the mean diameters at two and four weeks, ± the S. E.

became very prominent and contained a thin secretion, as shown in Fig. 5. As regression progressed, areas of leukocytic infiltration and necrosis could be found (Fig. 6). The interstitial tissue became less cellular, and there was a relative increase in the amount of fibrous tissue (Fig. 7). Ultimately, the previously tumorous mammary glands were shown to be relatively normal breast tissue containing a few cystic spaces, with a slight increase in the amount of fibrous tissue (Fig. 8).

Once the tumors have regressed, and in the absence of the external source of estrogen, the mammary tumors do not reappear even after prolonged intervals. Chart 4 shows that no mammary carcinomas reappeared during a period of forty weeks following removal of the estrone pellet, but that they rapidly reappeared when treatment with estrone was resumed. Nearly all of the tumors reappeared, and most appeared in their original site. Of 109 tumors present at the time the pellets were removed, 104 (95.4%) reappeared in the same site. Three tumors failed to reappear, and two tumors appeared in mammary glands not known previously to have been the sites of tumor.

Mammary tumor induction is accompanied by an increase in size of the pituitary or development of frank pituitary adenoma (2, 4, 10). Chart 5 presents some evidence that there is a concomitant decrease in the size of the pituitary tumors after removal of the estrone pellet. The pituitary does not return to normal, however, and even many months after pellet removal, the pituitary gland remains greatly enlarged. This is shown in Chart 5.

**DISCUSSION**

Removal of the estrone pellet resulted in complete regression of the mammary carcinomas that had been induced by the presence of the pellet. In many of the tumors, the behavior and histologic appearance during regression was similar to that of the dimethylbenz(a)anthracene (DMBA)-induced mammary tumors regressing as a result of hypophysectomy or ovariectomy. In these tumors Daniel and Pritchard (4) noted the same flattening of tumor epithelium that we have noted in the regressing estrone-induced tumor. Decreased mitotic activity was a noteworthy change in the regression of both types of tumor, and, as has been reported earlier (1, 4), either type of tumor could be reactivated by administration of estrogen. Spontaneous regression of the DMBA-induced tumor, as described by Young and Cowan (11), differs from that induced by hypophysectomy or ovariectomy and from regression of the estrone-induced tumor following removal of the pellet. No flattening of the tumor epithelium occurred, and mitotic activity was undiminished, while the tumors remained static or actually decreased in size. Spontaneous regression of the estrone-induced mammary tumors, i.e., regression of tumors in the presence of an estrone pellet, has never been seen.
Chart 4. Maintenance of tumor regression in absence of estrogen stimulation. A, rapid resumption of tumor growth following reinsertion of estrone pellets after four weeks of total regression. B, animals from which estrone pellets were removed remained free of mammary tumors over a period of 44 weeks. Rapid resurgence of tumors accompanies reinstitution of estrogen, even after many months. † pellets removed; ‡, pellets inserted.

Chart 5. Decrease in size of the pituitary during the eight-week removal of the estrone pellet. Cross-hatched bars represent pituitary weight from animals bearing pellets, open bars those of depelleted animals. The cross-hatched area represents range of weight of pituitary from normal animals.
The pituitary appears to be closely implicated in the genesis of mammary tumors by prolonged stimulation by estrone. Mammary tumors will not develop in the absence of a pituitary (2), and hypophysectomy results in complete and total regression of established estrone-induced mammary tumors (1, 2). The association of pituitary and mammary gland in mammary tumor development is further strengthened by the finding that the greatly enlarged pituitary tumors undergo a diminution in size after removal of the estrone pellet. The pituitary does not appear to return to normal, however, and this probably explains the rapidity with which the mammary tumors reappear after reinsertion of the estrone pellet.

ACKNOWLEDGMENTS

We would like to acknowledge the technical assistance of Mrs. E. Fraser and to thank Mr. Barry Forbes for preparation of graphs and photography.

REFERENCES


Figs. 1–3. Histologic types of mammary carcinoma evoked by estrone.
Fig. 1. Encephaloid type showing little tendency to acinar formation. H & E, × 325.
Fig. 2. Higher power of a portion of Fig. 1. showing cellular detail, mitotic figures, and absence of pyknosis. H & E, × 650.
Fig. 3. Papillary type of mammary carcinoma with clefts or spaces surrounded by plump cells. There is no evidence of secretion. Mitotic figures are noticeable and the nuclei show no evidence of pyknosis. H & E, × 325.

Figs. 4–8. Regressing mammary tumors.
Fig. 4. A cellular portion of a regressing tumor two weeks after removal of the estrone pellet. Cells with pyknotic nuclei are abundant and there is a generalized decrease in cell size. H & E, × 325.
Fig. 5. A portion of a tumor four weeks after removal of the estrone pellet. Large cystic spaces are present which contain a thin fluid. The lining cells are flattened and pyknotic cells are prominent. H & E, × 150.
Fig. 6. Another portion of the same tumor as Fig. 4 showing nuclear pyknosis and areas of leukocytic infiltration at the arrows. H & E, × 325.
Fig. 7. Tumor six weeks after removal of the pellet. The original biopsy showed this tumor to have had a histologic structure similar to that shown in Fig. 1. Only a firm nodule was present in the breast, showing a cluster of glandular elements in an area of increased connective tissue. H & E, × 125.
Fig. 8. Mammary carcinoma, papillary type, eight weeks after removal of the estrone pellet. A diffusely thickened area remained, consisting of loose fibrous tissue with small cystic spaces. H & E, × 125.
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