Studies on Squamous Metaplasia in Rat Bladder

II. Effects of Estradiol and Estradiol plus Hexestrol*†

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SUMMARY

The effects of estrogens were studied with and without foreign body (rough glass beads and paraffin pellets) on the metaplasia of the bladder of rats on stock maintenance diet and on a vitamin A-deficient diet.

Estradiol increased the degree of metaplasia in the bladder of rats when combined with vitamin A deficiency and/or foreign body stimulation. Estradiol affected bladder epithelium already made squamous more effectively than it did the normal transitional uroepithelium. A high dose of hexestrol, when added to estradiol, showed no enhancement of the degree of metaplasia by estradiol benzoate in the bladder of the rat.

The combination of vitamin A deficiency, foreign body in situ, and estrogen administration was an effective means of obtaining keratinizing squamous metaplasia in the urinary bladder for studies of its developmental and reversal changes.

In a previous presentation (4) the relation of different forms of foreign-body irritation and of vitamin A deficiency to squamous metaplasia in the bladders of rats was reported. It is also known that estrogens will cause squamous metaplasia. The metaplasia following estrogen administration has been described in various organs of the genito-urinary system in different species (20). It has been found in the uterus (2, 17, 19), and the metaplastic effect of estrogens in the male has also been described for the prostate (14).

In this paper we are concerned with the effects of estradiol and the combination of estradiol and the synthetic estrogen hexestrol with vitamin A deficiency and irritative foreign body stimulation on metaplasia in the bladder.

MATERIALS AND METHODS

Young rats of the Sprague-Dawley strain were used. Some of the rats were fed regular stock Rockland rat diet (complete) and the others were fed a vitamin A-deficient diet and given a small vitamin A supplement to prevent early mortality (4). The same procedure described earlier for insertion of foreign bodies in the bladder was used.

The animals were divided into the following groups (the number of rats surviving with tissue for study and the total number in each group initially are given following each group):

I. Stock diet + estradiol (6 survivals/11 rats)
II. Stock diet, estradiol + hexestrol (4/9)
III. Stock diet, rough glass bead + estradiol (2/4)
IV. Stock diet, paraffin pellet + estradiol (3/7)
V. Stock diet, paraffin pellet, estradiol + hexestrol (6/16)
VI. Vitamin A-deficient diet + estradiol (11/15)
VII. Vitamin A-deficient diet, estradiol + hexestrol (18/25)
VIII. Vitamin A-deficient diet, rough glass bead + estradiol (13/19)
IX. Vitamin A-deficient diet, rough glass bead, estradiol + hexestrol (10/17)
X. Vitamin A-deficient diet, paraffin pellet + estradiol (4/8)
XI. Vitamin A-deficient diet, paraffin pellet, estradiol + hexestrol (6/16)

Injections of estrogenic agents were begun 1–2 weeks after the implantation of foreign bodies, unless otherwise noted, in Groups III, IV, V, VIII, IX, X, and XI. In groups without foreign bodies (I, II, VI, and VII), the estrogen treatment was begun 3–4 weeks after Groups VI and VII developed vitamin A deficiency. The dose in oil of estradiol benzoate (Progynon benzoate, Schering...
Corp.) was 0.05 mg. 3 times a week. The dose of meso-3,4-di-(p-hydroxyphenyl)-in hexane (Hexestrol, Wm. S. Merrell Co.) was 0.5 mg. 5 times a week. Injections were continued until sacrifice or death of the animals (see Charts 1 and 2). The bladders were fixed in neutral calcium formol and stained with hematoxylin and cosin, periodic acid-Schiff (PAS), and Alcian Blue (4).

The changes in the bladders have been classified as before (4): 1+, having loss of the PAS-positive material in the superficial layer; 2+, featuring parakeratosis; 3+, showing mild epidermidalization with no granular layer; and 4+, showing marked whitish thickening, often with opaque keratinized papillary projections and the keratin layer with stratum granulosum histologically.

RESULTS

The mucosal changes are tabulated, with the groups subdivided by time periods of up to 7 weeks, 8—30 weeks, and 30—40+ weeks after the beginning of estrogen treatment, in Table 1.

A high early mortality prevailed in Groups I, II, III, IV, V, X, and XI. In Group I, on estradiol only, three animals were examined after 10 days, 36 weeks on estradiol, and one after 38 weeks. The bladders of the males showed 9+ changes. A single surviving female showed a 4+ change after 36 weeks of estradiol. All showed prominent hyperplasia of the epithelium, one with active mitosis.

In Group V, three males and three females were examined between 25 and 39 weeks after implantation of the paraffin pellet and 23—37 weeks of estrogen plus hexestrol administration. Three (two males and one female) of the six showed only 1+ modification of the bladder mucosa after such prolonged treatment with the combined estrogens, and three rats showed some minimal metaplastic alteration but less than 1+ change. Four of six showed epithelial hyperplasia, with one male showing some papillary hyperplastic mucosa.

In Group VI there were five males and six females on vitamin A-deficient diet plus estradiol.

Two females showed 3—4+ change after 10 weeks of vitamin deficiency and 13 and 24 weeks of estradiol (Fig. 5), respectively. Another female showed 2+ to 3+ change after 13 weeks of vitamin deficiency and 8 weeks of estradiol (Fig. 1). A fourth female showed 2+ change after 16 weeks of vitamin depletion and 12 weeks of estradiol. The remaining two females showed a 1+ grade of metaplasia after 17½ weeks. Three male rats on estradiol for 1½ weeks, 3 weeks, and 5½ weeks, respectively, showed a 1+ change. One of the two remaining male rats received estradiol for 2 weeks and showed minimal, i.e., less than 1+, change, and the other showed 2+ to 3+ change after only 1½ weeks of estradiol.

In Group VII there were eight males and five females, vitamin-deficient, treated with both estradiol and hexestrol. Only one of four males on the vitamin-deficient diet for 11 weeks, and 6 weeks of administration of estradiol and hexestrol, showed a 2+ change, three showing minimal
<table>
<thead>
<tr>
<th>Time of Estrogen Administration</th>
<th>Group*</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
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<th>XI</th>
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<td>Total Initial No.</td>
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<tr>
<td>Total studied</td>
<td></td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>11</td>
<td>13</td>
<td>13</td>
<td>10</td>
<td>4</td>
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* Group

I, stock diet + estradiol.
II, stock diet, estradiol + hexestrol.
III, stock diet, rough glass bead + estradiol.
IV, stock diet, paraffin pellet + estradiol.
V, stock diet, paraffin pellet, estradiol + hexestrol.
VI, vitamin A-deficient diet + estradiol.
VII, vitamin A-deficient diet, estradiol + hexestrol.
VIII, vitamin A-deficient diet, rough glass bead + estradiol.
IX, vitamin A-deficient diet, rough glass bead, estradiol + hexestrol.
X, vitamin A-deficient diet, paraffin pellet + estradiol.
XI, vitamin A-deficient diet, paraffin pellet, estradiol + hexestrol.
changes (0—1+) (Fig. 6). One female, with 11 weeks of both estrogens, showed a 3+ to 4+ alteration in the bladder; another female showed a 3+ change after 7½ weeks of such administration. Both showed calculi in the bladder. Two males showed 1+ change after 9 weeks and 15½ weeks of combined estrogen, and two females showed this degree of change after 8½ and 11 weeks. The remainder of the group (two males and one female) showed very minimal changes after periods of both estrogens varying from 9 to 17 weeks. Keratinization was found in the rats with calculi and was not present in the other rats.

In Group VIII there were five males and eight females. This group was kept on a vitamin-deficient diet for an average of 3—4 weeks and then had a rough glass bead inserted; after periods averaging 4—5 weeks post-operatively, estradiol was administered. All animals, with one exception, showed a 4+ change in the bladder after periods ranging from ½ week to 11 weeks of such administration.

Group IX consisted of six males and four females. The rats were placed on a vitamin-deficient diet for 3—4 weeks and then had a rough glass bead inserted. In this group estradiol and hexestrol were administered beginning 2 weeks post-operatively. Only one male and one female were on estradiol and hexestrol for 10 weeks and 7½ weeks, respectively, and showed a 4+ change (Fig. 2) and a 1+ change. Four rats were carried on both drugs for 5—6 weeks. One of the two females showed a 4+ alteration, and the remaining female showed a 3+ change. Two males showed a 3+ change with some papillary areas of the mucosa in one showing a focal 4+ alteration (Figs. 3 and 8). The other rats were on the drugs for less than 3 weeks. One male at 3 weeks had a 3—4+ change, one female had a 2+ change, and two males showed a 1+ alteration.

In Group X there were only two males and two females that survived for study. These animals were placed on a vitamin-deficient diet for 4 weeks and then had a paraffin pellet inserted; the administration of estradiol began 1 week post-operatively. One male showed a 3+ change after 3 weeks of estradiol administration. The remaining three showed marked 4+ changes, one male after 1½ weeks of the drug (Fig. 9) and the two females after 18 weeks. The keratinization was marked and diffuse, and epidermoid cysts were present.

In Group XI, two males and four females survived. The conditions in this group were identical with those in Group X, except that estradiol and hexestrol were both administered 1 week post-operatively after a paraffin pellet was inserted in rats that had been depleted of vitamin A for 4 weeks. All six rats showed marked 4+ changes, one female as early as 7½ weeks after administration of the drugs, and the remaining female and two males after 16 weeks (Fig. 10). Maximal changes were present in all with invagination of epithelium and epidermoid cyst formation in two females and one male. In this series a high mortality prevailed early.

A separate group of rats was studied with vitamin A deficiency, with paraffin pellets in situ in the bladder, and with subcutaneous diethylstilbestrol pellets of 0.5 mg. for continuous slow absorption. In 30 such rats 24 showed 4+ metaplasia in periods varying from 3 to 8 weeks. These findings are not included in the tabulation because of the different form of the estrogen used.

Bladders stained with PAS and Alcian Blue showed an increase of staining in the stroma of the bladder when estrogen was given to animals with foreign bodies in situ, whether or not the animals were vitamin A deficient. In the bladders of the animals receiving hexestrol the staining was not as intense.

**DISCUSSION**

Vitamin A deficiency and the presence of a foreign body individually have been shown to yield keratinization in the bladder of rats; when the two treatments were combined, earlier and more marked metaplasia occurred (4).

Estradiol at the dose level employed enhanced the metaplasia produced by these means. However, it failed to yield metaplasia of the bladder epithelium of rats on the stock diet without a foreign body in situ, although others have found some such changes (20). The estrogens apparently are most effective on the epithelium of the bladder already altered by previous stimuli known to promote metaplasia, suggesting that squamous, rather than transitional, epithelium in the bladder is the more susceptible target tissue. When estradiol was administered to rats with implanted paraffin pellets on a stock diet (Group IV) keratinization appeared, while practically none was observed in such rats without estrogen (4). In vitamin A-deficient animals with foreign bodies in situ, estrogen also increased the metaplastic modification of the bladder epithelium (Groups VIII and X).

Hexestrol in the dosage used seemed to have a surprising effect of failing to increase the metaplasia of the other estrogen, estradiol, on the bladder epithelium. In some instances it seemed to diminish it. No valid comparison is permissible for the inhibiting effect of hexestrol in Groups IV and V because of the high mortality. In comparing Groups VI and VII some inhibiting effect of hexestrol was noted. However, in two of the rats...
in Group VII with a 3+ change, calculi were present. Hexestrol and estradiol together were less effective in producing metaplasia than estradiol alone, despite the discrepancy in over-all dosage in Group IX as compared with Group VIII. In Groups X and XI, a comparison of this differential effect cannot be made because, in addition to the small number of animals, the bladders were examined after a prolonged period, and the majority of results in both groups were all 4+.

It is generally accepted that synthetic estrogens like hexestrol (such as stilbestrol) induce keratinization of the vaginal and cervical epithelium in monkeys and in rats (3, 5, 6, 8, 9, 12-15, 17), and squamous metaplasia of the prostate (1, 10, 20). No reference to an antagonistic effect of stilbestrol on the metaplasia produced by estradiol or other natural estrogens could be found in the literature. Estrogens induce adrenal enlargement (18) and may increase glucocorticoid output. Such steroids may offset the metastatic effect of estrogens, much as glucocorticoids inhibit growth and healing processes. This failure to obtain a summation effect of hexestrol and estradiol may therefore represent a dosage phenomenon, for the dosage of hexestrol used was rather large, so that the amount of glucocorticoid produced may have been sufficient to offset the keratinizing influence of estrogens. Alternatively, the stimulated adrenal may put out amounts of progesterone (16, 18) sufficient to offset the influence of estrogens. In the vagina, at least, keratinization by estrogen is inhibited by progesterone (7).

The combination of vitamin A deficiency, foreign body in situ, and administration of estrogens is an effective means of obtaining keratinizing squamous metaplasia in the urinary bladder for a study of the finer morphological alterations.

REFERENCES


Fig. 1.—Group VI. Gross appearance of bladder of a female rat on vitamin A-deficient diet for 12 weeks and on estradiol for 8 weeks. Linear zones of elevated keratinization (2-3+ change) and opacity of the mucosa (k) are seen centrally.

Fig. 2.—Group IX. Gross appearance of bladder of a male rat on vitamin A-deficient diet for 15 weeks with rough glass bead for 12 weeks and estradiol plus stilbestrol for 10 weeks. The bladder shows folded thickened mucosa with opaque white keratinised crests (3+ change).

Fig. 3.—Group IX. Gross appearance of the bladder of a male rat on vitamin A-deficient diet for 10 weeks, with rough glass bead for 7 weeks and estradiol plus stilbestrol for 5 weeks. The metaplastic changes are limited to this papillary keratinized protruberant area only (3-4+ change). The remainder of the bladder surface (b) shows some cobble-stone granularity.
All sections stained with hematoxylin and eosin.

Fig. 5.—Group VI. Bladder of a female rat on vitamin A-deficient diet for 10 weeks with estradiol administration for 2½ weeks (3-4+ change). Most of the keratinized area lacks a granular zone, present at (g). X180.

Fig. 6.—Group VII. Bladder of a male rat on vitamin A-deficient diet for 11 weeks and estradiol plus stilbestrol administration for 6 weeks, showing no metaplastic changes. Epithelium cut tangentially at (t). X150.

Fig. 7.—Group VIII. Bladder of male rat on vitamin A-deficient diet for 12 weeks with rough glass bead in situ for 12 weeks and estradiol for 10 weeks (4+ change). Papillary, keratinized squamous epithelium with sharply delimited eleidin granular zone (g). Downgrowth of epithelium not included in photograph. X360.

Fig. 8.—Group IX. This is the microscopic section of the papillary formation in Figure 8. The keratin has been detached; a granular layer is not present; some inflammatory cells are seen in the underlying stroma (st). Some hyperplasia of basal layer is seen at upper margin of photograph (b). X150.

Fig. 9.—Group X. Bladder of a male rat on vitamin A-deficient diet for 19½ weeks with paraffin pellet in situ for 15 weeks and estradiol administration for 14 weeks (4+ changes). Numerous cysts, small cells and atrophy (a) of viable epithelium. X40.

Fig. 10.—Group XI. Bladder of a male rat on vitamin A-deficient diet for 21½ weeks with paraffin pellet in situ for 17 weeks and estradiol and stilbestrol for 16 weeks (4+ change). Wide basal, intermediate, and parakeratotic zones, with little keratin. (Compare with Fig. 9.) X40.
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