

## MORPHOLOGY OF RAT PROSTATIC LOBES AND SEMINAL VESICLES AFTER LONG-TERM ESTROGEN TREATMENT

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The growth of the prostatic lobes and seminal vesicles of castrated rats was studied morphologically after long-term treatment with estradiol benzoate. Estradiol promoted slightly the growth of the prostatic lobes but more markedly the seminal vesicles, although it had catabolic effects as reflected in low body and levator ani weights. Histologically there was proliferation of the fibromuscular stroma. In the coagulating glands and seminal vesicles there was squamous metaplasia of the epithelium. The histological study also revealed features of special interest, i.e. mucoid epithelium of the coagulating glands. Electron-microscopical and histochemical studies of the mucoid epithelium showed that it had similar characteristics as goblet cells of the intestine of the rat.

Key words: Estradiol; prostatic lobes; seminal vesicles; morphology.

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In previous short-term experiments when estradiol was administered in daily doses during 10 up to 21 days it was found to stimulate growth of accessory reproductive glands of castrated male rats. In coagulating glands and seminal vesicles there was an increase in the amount of fibrous and muscular tissue. In ventral and dorsolateral prostate, estradiol induced epithelial growth and secretory activity (21, 24).

Considering the extensive use of estrogen in the treatment of prostatic carcinoma in man it would be of interest to know the effects of long-term estrogen-treatment on the prostatic lobes and the seminal vesicles.

This paper deals with a quantitative and qualitative morphological study of the ventral and dorsolateral prostate, coagulating glands and seminal vesicles of castrated male rats treated with varying doses of estradiol for 45 weeks.

### MATERIAL AND METHODS

The investigation was performed on fifty rats of the Sprague-Dawley strain supplied by Anticimex AB, Stockholm. When weighing  $42.4 \pm 2.8$  g (mean and standard deviation) the rats were castrated by the transscrotal route, the epididymis being removed with the testis. Surgery was performed under ether anaesthesia.

Thirty days after castration the rats were divided into groups and given injections for 45 weeks according to the following scheme:

C: 7 control rats given 0.1 ml arachis oil  
E<sub>0.001</sub>: 14 rats given 0.001 mg estradiol benzoate  
E<sub>0.010</sub>: 14 rats given 0.010 mg estradiol benzoate  
E<sub>0.100</sub>: 15 rats given 0.100 mg estradiol benzoate.

Estradiol benzoate was given in subcutaneous injections of 0.1 ml at 8.30 a.m. three times a week. For this, Ovex® (Leo) 1 mg/ml was diluted with arachis oil. Rats not given estradiol benzoate had arachis oil instead.

The care of the rats, the dissection and weighing of organs and the preparation of tissues for histological examination were done as previously described (22). In

TABLE 1. Mean Body Weights and Mean Weights of Levator Ani Muscle, Pituitary Gland, Adrenal Glands and Thymus of Castrated Rats Injected with Various Doses of Estradiol Benzoate Three Times Weekly for 45 Weeks and of Castrated Control Rats

Groups	Number of rats	Body weight (g)		Levator ani (mg)	Pituitary gland (mg)	Adrenal glands (mg)	Thymus (mg)
		At start of treatment	At autopsy				
C	7	247 ± 13	568 ± 26	102.5 ± 25.6	16.8 ± 1.3	48.8 ± 8.0	380 ± 84
E <sub>0.001</sub>	14	248 ± 10	478 ± 13	76.6 ± 17.3	18.8 ± 3.0	54.6 ± 9.3	241 ± 73
E <sub>0.010</sub>	14	248 ± 10	363 ± 16	52.9 ± 12.5	25.5 ± 5.0	67.0 ± 8.6	127 ± 42
E <sub>0.100</sub>	13	248 ± 11	286 ± 11	46.6 ± 11.4	68.9 ± 23.9	54.9 ± 6.5	95 ± 28

addition to previously used histochemical stainings, the pituitary glands were stained according to Pearse, and the following methods were applied on the coagulating glands: PAS-staining, alcian blue at pH 2.5 and 1.0, alcian blue/PAS at pH 2.5 and high iron diamine (HID). For electron microscopy, small pieces from the coagulating glands of two rats were immediately immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and 4 °C for 4 h, washed in cold buffer, postfixed with 1% OsO<sub>4</sub> for 1 h, dehydrated in ethanol, embedded in Epon 812 and cut in an LKB Ultratome III ultramicrotome. One-micron thick sections were stained with toluidine blue; thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope.

Body and organ weights are expressed as mean values ± standard deviation. Student's t-test was used for testing differences between the means.

## RESULTS

### *Food Consumption, Body and Levator Ani Weight*

The average daily food intake for a control rat was about 22 g. Estradiol administration was associated with a dose-related decrease in food consumption. After the largest dose of estradiol the daily food intake was only about 15 g.

All animals gained weight during the experimental period. Estradiol administration led to a dose-related depression of body growth and levator ani weight (Table 1).

Two of the rats in group E<sub>0.100</sub> weighed more than other rats in the group. At the end of the experiment these rats were in bad condition with paretic hindlegs. At autopsy they were found to have particularly large pituitary glands (see below).

### *Weight of the Pituitary Gland (Table 1)*

In rats treated with 0.010 mg estradiol (group E<sub>0.010</sub>) the pituitary glands were significantly larger than in the controls ( $P < 0.001$ ). Rats having the largest dose of estradiol (group E<sub>0.100</sub>) had still heavier pituitary glands ( $P < 0.001$ ). Two of the rats in group E<sub>0.100</sub> had pituitary glands which weighed several times more (202 and 228 mg respectively) than those of other rats given the same treatment. These two rats are considered separately and their data are not included in the tables.

### *Weights of Adrenal Glands and Thymus (Table 1)*

Rats given 0.010 mg estradiol had heavier adrenals than control rats ( $P < 0.001$ ) and rats given other doses of estradiol ( $P < 0.05$ ). The adrenals of

TABLE 2. Mean Weights of Ventral and Dorso-lateral Prostate, Coagulating Glands and Seminal Vesicles of Castrated Rats Injected with Various Doses of Estradiol Benzoate Three Times Weekly for 45 Weeks and of Castrated Control Rats

Groups	Number of rats	Ventral prostate (mg)	Dorsolateral prostate (mg)	Coagulating glands (mg)	Seminal vesicles (mg)
C	7	7.1 ± 1.0	10.0 ± 3.3	3.1 ± 0.3	9.9 ± 1.4
E <sub>0.001</sub>	14	10.4 ± 3.5	15.5 ± 4.4	9.8 ± 4.2	64.0 ± 11.0
E <sub>0.010</sub>	14	10.9 ± 3.8	20.7 ± 4.4	23.0 ± 5.7	147.5 ± 15.7
E <sub>0.100</sub>	13	11.9 ± 1.5	18.3 ± 5.3	24.2 ± 5.7	211.4 ± 52.9

the two rats with extremely large pituitaries weighed 64 and 66 mg respectively. After estradiol the thymus weight decreased in a dose-dependent way.

*Weights of Ventral and Dorso-lateral Prostate, Coagulating Glands and Seminal Vesicles* (Table 2)

The weights of the prostatic lobes and seminal vesicles increased after estradiol administration. The maximum weight of the ventral prostate was reached already after the lowest dose (group E<sub>0.001</sub>) of the dorso-lateral prostate and coagulating glands after the intermediate dose (group E<sub>0.010</sub>) and of the seminal vesicles after the highest dose of estradiol (group E<sub>0.100</sub>).

The two rats with extremely large pituitary glands had heavier dorso-lateral prostatic lobes (33 and 38 mg respectively) than any of the other rats.

*Histological Examination of Pituitary Glands and Accessory Reproductive Organs*

*Pituitary gland.* In the anterior pituitary of untreated castrated rats, acidophil, basophil, chromophobe and also some amphophil cells were seen. Many of the basophil cells had a large vacuole in their cytoplasm.

The number of basophils diminished after

estradiol in doses of 0.001 mg and still more after doses of 0.010 mg. After the latter treatment the glands contained very few basophils and were in the main composed of varying proportions of chromophobes and acidophils. Noduli of acidophils and chromophobes were found in one gland from group E<sub>0.001</sub> and in another from group E<sub>0.010</sub>.

Pituitary glands of rats given 0.100 mg estradiol had round, rather well-demarcated but non-encapsulated noduli composed of acidophils. In some of the glands there were also noduli composed of chromophobic cells. Most of the two particularly enlarged pituitary glands mentioned above were composed of acidophilic partly infarcted and haemorrhagic noduli.

*Accessory reproductive organs.* The ventral and dorso-lateral prostate, the coagulating glands and the seminal vesicles of castrated control rats (group C) exhibited an immature appearance. The small acini were lined by cubic to low columnar epithelial cells and contained no or little secretion. Scattered basal cells could be seen beneath the epithelium. The acini were surrounded by immature smooth muscle cells with small nuclei. The fibrous stroma appeared abundant.

The ventral prostate and lateral part of the dorso-lateral prostate had after all doses of estradiol larger

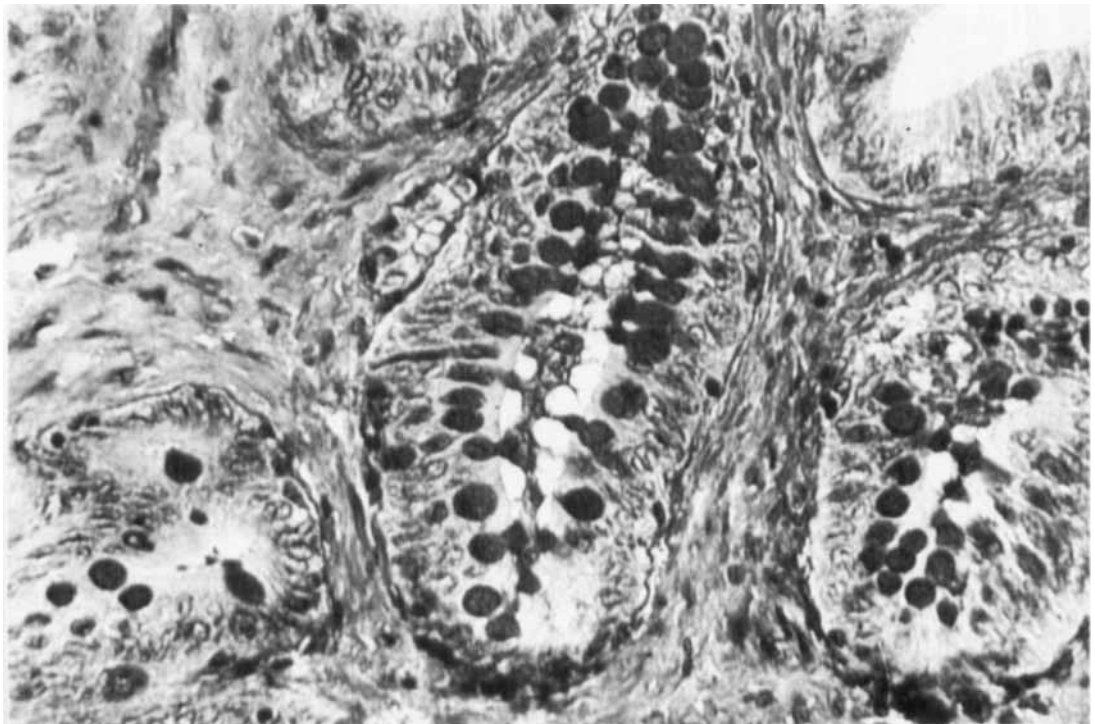


Fig. 1. Mucoid epithelial metaplasia in the coagulating glands of a rat treated with 0.010 mg estradiol. Mc Manus  $\times 450$ .

acini lined by higher epithelial cells than had the untreated controls. Supranuclear clear zones indicating secretory activity (13) were not observed. As in untreated rats there were scattered basal cells. Smooth muscle cells were better developed than in controls.

*The dorsal part of the dorso-lateral prostate* after estradiol administration had larger acini than had untreated controls. The epithelial cells, although mostly columnar, varied in size and shape and were often arranged in several irregular layers. The epithelial proliferation and cellular irregularities were most marked after the largest dose of estradiol (group E<sub>0.100</sub>). After the two higher doses of estradiol the concentrically arranged smooth-muscle layers were considerably broader than in group E<sub>0.001</sub>, and a few acini contained some PAS-positive material.

*The coagulating glands* of rats treated with 0.001 mg estradiol had larger acini with higher epithelium than in untreated controls. The acini were lined by one or several layers of epithelial cells that varied in size and shape. The acini contained slight secretion or none. The muscular layers were increased in width. In rats given 0.010 mg estradiol the epithelium showed marked changes. In many of the acini the epithelial cells were high columnar with

basally placed nuclei. The cytoplasm was PAS-positive. In several of the cells there were large droplets of PAS and Alcian blue positive secretion in the supranuclear cytoplasm as in goblet cells. There were acini with almost all cells having this appearance (Fig. 1). There were some papillary formations and in places the mucosa was folded in such a way as to remind one of the crypts of the colonic mucosa. In other acini a stratified squamous epithelium was seen. After estradiol doses of 0.100 mg only a few goblet-like cells were found. Squamous metaplasia was more frequent than after the lower dose of estradiol (Fig. 2). The amount of fibromuscular stroma was markedly increased both in group E<sub>0.010</sub> and group E<sub>0.100</sub>.

The goblet cells were easily identified in the ultrastructural specimens. Characteristically, most of the cytoplasm was occupied by secretory granules. The nucleus was located at the base of the cell and frequently »squalloped« by the secretory granules. Prominent Golgi zones were often seen in the perinuclear area. At the luminal cytoplasmic membrane there were microvillous projections. Occasional goblet cells were seen to extrude their contents into the lumen, which contained secretory granules with intact limiting membranes (Fig. 3 and 4).



Fig. 2. Squamous epithelial metaplasia in the coagulating glands of a rat treated with 0.100 mg estradiol. Weigert-var Gieson  $\times$  280.

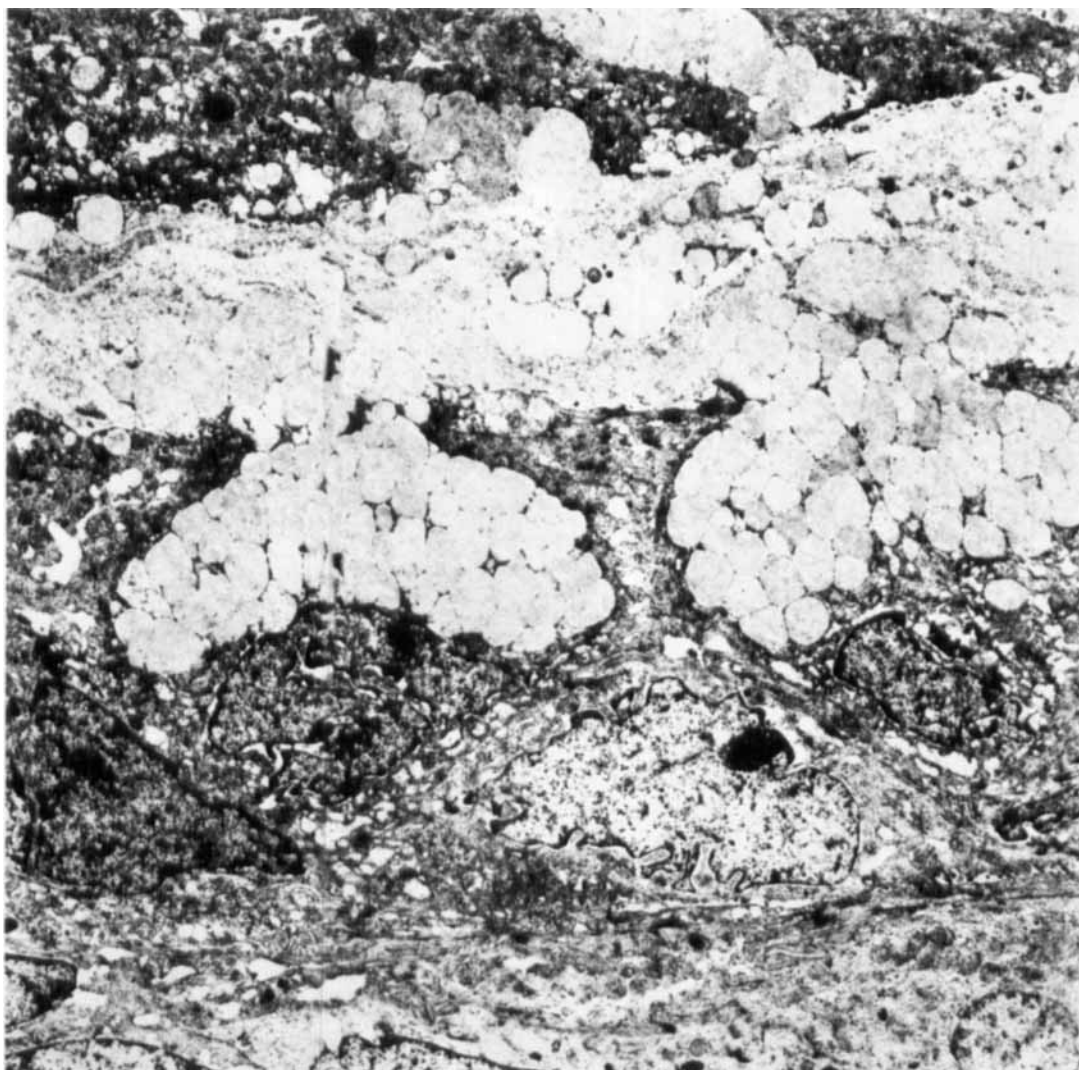


Fig. 3. Two prominent goblet cells in lining epithelium of a coagulating gland from a rat treated with 0.010 mg estradiol. EM  $\times$  4500.

The mucus content of the goblet cells stained strongly positive with PAS, distinct blue with alcian blue at pH 2.5 and 1.0 and also blue with the combination PAS/alcian blue. With HID the mucus was stained grey-black. These staining results indicate the presence of acid, sulfated mucosubstances such as sulfomucins, known to be present in the goblet cells of the large intestine of the rat (9).

*The seminal vesicles* after estradiol treatment showed small lumina, without secretion, lined by irregular cuboidal epithelial cells arranged in one or two layers. In rats treated with 0.010 mg estradiol the main ducts were often lined by a stratified squamous epithelium. After the largest dose of estradiol (group E<sub>0.100</sub>), squamous metaplasia was seen here and there throughout the glands. In all

estradiol treated rats there was a dose-dependent increase in the amount of submucous connective tissue and in the width of the muscular layers. After the largest dose of estradiol the lumina were almost obliterated by the fibromuscular growth.

The histological appearance of the accessory reproductive glands of the two rats with very large pituitary glands (see above) did not differ from that of the other rats given the same doses of estradiol.

## DISCUSSION

In this study, estradiol treatment in varying doses for 45 weeks increased the weight of the ventral and dorso-lateral prostate to about the same extent as it did after ten or twenty-one days in our previous

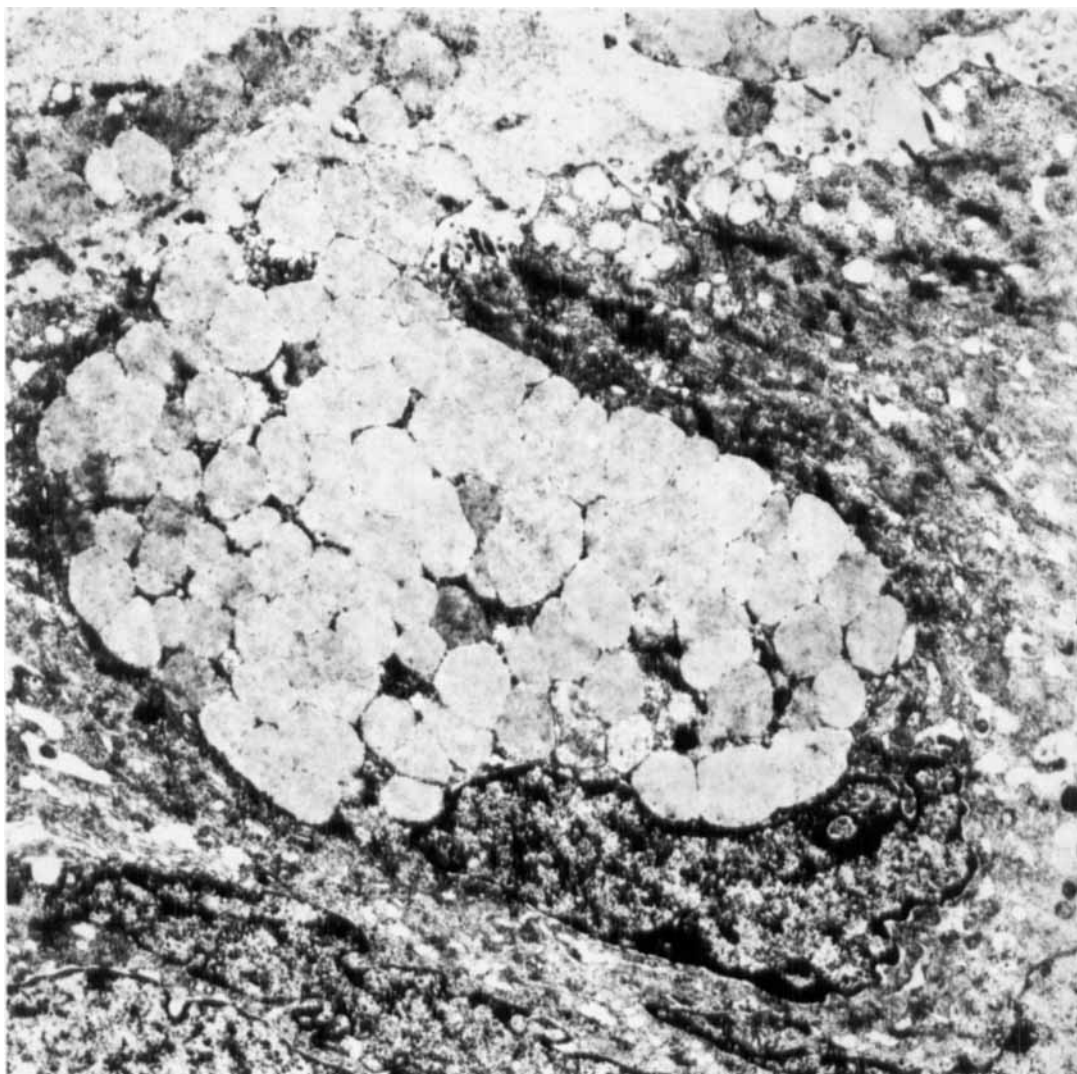


Fig. 4. Larger magnification of a goblet cell extruding its content into the duct lumen. EM  $\times 10,500$ .

experiments. In those studies, secretory activity of the epithelium was found in the ventral and dorso-lateral prostate after estrogen. This androgen-like action was supposed to be due to the combined effects of endogenous adrenal steroids and estradiol (21, 24). In the present experiment, secretory activity was not found in the epithelium and it had a similar appearance as in the estradiol-treated castrated adrenalectomized rats in our previous short-term experiments (20, 21). This indicates that after long-term estrogen administration endogenous adrenal steroids have small effects or none on the morphology of the ventral and dorso-lateral prostate.

In the present experiment the weight of the seminal vesicles was dose-dependent while the maximum weight of the coagulating glands was

reached already after the intermediary dose. These weights were considerably higher than those found in our previous short-term experiments (21, 24). The weight increase was mainly due to proliferation of connective tissue and smooth-muscle elements. High doses of estrogen induced squamous cell metaplasia in some of the epithelium of the seminal vesicles and coagulating glands. There are previous reports on such epithelial changes after estrogen in mice and rats (1, 7).

Of particular interest is the occurrence of cells in the coagulating glands, which electron-microscopically and histochemically are similar to intestinal goblet cells. Goblet cells have not previously been observed in any of the prostatic lobes in the rat. Similar mucous epithelial cells have been found by *Chesterman et al.* in the outer prostatic gland, but

rarely in the coagulating glands of guinea pigs with age or after treatment with stilbestrol (2). They thought that the mucous epithelium might be associated with increased corticosteroid production. In our experiments the rats with goblet cells in the coagulating glands had heavier adrenals than the other rats. We found goblet cells only in the coagulating glands. This is another example of different response of the separate rat prostatic lobes to various hormones (4, 5, 18, 19, 20).

The fact that goblet cells can occur in the prostate of guinea pigs and rats is especially interesting when one considers that the glandular portion of the prostate arises from the epithelium of the cloacal endoderm (10).

Mucinous adenocarcinoma of the prostate has been observed in man (3, 6). Some of the cells of these tumours have the appearance of goblet cells (6). The human prostate is composed of three paired lobes (23). The medial prostatic lobes in man and the coagulating glands in rat are probably homologous (14, 23). At present, however, there are no data indicating the lobe origin of the rare mucinous prostatic carcinoma in man.

The growth-promoting effect of estradiol on the prostatic lobes and seminal vesicles was obtained in rats otherwise showing catabolic reactions to estradiol, as reflected in low body and levator ani weights. This shows that estradiol has specific growth-promoting effects on male accessory reproductive glands. In estrogen-treated rats there is a progressive increase of plasma prolactin related to the weight of the pituitaries (8, 11). Prolactin has been found to promote accessory reproductive gland growth in the rat (4, 12, 15, 16, 17, 25). Especially the growth of dorsolateral prostate is augmented by prolactin (4, 12, 16, 17). This may explain why our two rats with extremely enlarged pituitary glands had much heavier dorso-lateral prostatic lobes than any of the other rats. The growth of the other prostatic lobes and the seminal vesicles after estradiol administration might also have been due to a synergistic action of estradiol *per se* and an increased amount of prolactin.

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