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A Morphological Study of the Prostatic Lobes and the Seminal Vesicles of Castrated Rats Injected with Oestradiol and/or Insulin

L.-E. Tisell, H. Andersson, and L. Angervall

Department of Surgery II and Department of Pathology II, University of Gothenburg, Sweden

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Summary. The growth of the ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles was studied morphologically in castrated rats following 21 daily injections of oestradiol benzoate or protamine zinc insulin alone or in combination. Oestradiol benzoate was given in daily doses of 0.010 mg, protamine zinc insulin in daily doses of 10 IU. In the ventral and dorsolateral prostate oestradiol had an androgen-like action inducing epithelial growth and secretory activity in the epithelium. In the seminal vesicles and coagulating glands oestradiol induced an increased fibromuscular growth. Protamine zinc insulin induced growth and secretory activity in the dorsolateral prostate, while no such effect was observed in the other accessory reproductive glands. Protamine zinc insulin accentuated the androgen-like action of oestradiol benzoate on the epithelium of the dorsolateral prostate but did not influence on the increased fibromusular growth seen after oestradiol in the coagulating glands and the seminal vesicles.

Key words: Prostatic lobes, Seminal vesicles, Morphology, Oestradiol, Insulin, Castrated rats.

The hypothesis has been put forward that benign prostatic hyperplasia in elderly men is a consequence of an elevated oestrogen/androgen ratio (4, 11, 17). In addition "diabetogenic hyper-insulinism" has been discussed as a possible factor in the pathogenesis of prostatic hyperplasia and malignancy (10). Bourke and Griffin (2) reported a significantly raised incidence of diabetes mellitus in patients with benign prostatic hyperplasia.

Some experimental studies have been performed which describe the effects of oestradiol and insulin on growth of the prostate and seminal vesicle in the rat. Oestradiol administration to castrated adrenalectomised rats increases the weight of all the prostatic lobes and the seminal vesicles. Histological examination revealed increase in the amount of fibromuscular tissue especially in the coagulating glands and seminal vesicles without any signs of secretory activity in the epithelium (13). In castrated non-adrenalectomised rats oestradiol induced growth and secretory activity in epithelial cells of the ventral and dorsolateral prostate (14).

Other experiments have shown that testosterone stimulates the growth of the prostatic lobes and the seminal vesicles to a lesser extent in castrated alloxan-diabetic than in castrated non-diabetic rats (1). Protamine zinc insulin when given jointly with cortisone, can promote the growth of the prostatic lobes and the seminal vesicles through effects not mediated by the adrenal glands or the anterior pituitary (16).

The purpose of the present study was to investigate whether administration of insulin influences the effect of oestradiol on the growth of the ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles in castrated non-adrenalectomised rats. The accessory reproductive glands were studied morphologically in castrated rats given oestradiol and insulin alone or in combination.

MATERIAL AND METHODS

The investigation was performed on thirty-one rats of the Sprague-Dawley strain supplied by Anticimex AB, Stockholm. The rats were

castrated under ether anaesthesia when weighing 40 ± 0.5 g. Four weeks after castration the rats were divided into the following groups:

C : 8 untreated control rats

E : 8 rats receiving 0.010 mg oestradiol benzoate once daily

I : 10 rats receiving 10 IU protamine zinc insulin (Novo, 40 IU/ml) once daily

E - I : 5 rats* receiving both 0.010 mg oestradiol benzoate and 10 IU protamine zinc insulin.

The rats were given daily subcutaneous injections throughout the 21 days of the experimental period and were killed on the day of the last injection.

The oestradiol benzoate was given in daily injections of 0.1 ml. For this Ovex^R (Leo) 1 mg/ml was diluted with arachis oil. Oestradiol and insulin were given at 8.30 a. m., at separate sites on the lower part of the body.

Throughout the experiment all the rats were housed in temperature controlled and air-conditioned quarters (temperature 24° C and relative humidity 60%). An artificial lighting cycle with 12 h of light and 12 h of darkness was employed. The rats were given unlimited food and tap water. The commercial rat diet was supplied by Teknosan AB, Malmö. The daily food consumption was determined for the whole group and the food consumption was roughly estimated for each rat. The rats were weighed before castration, at the start of treatment and at autopsy. On each of the last three days of the experiment 0.05 ml of blood was taken from the tail vein for blood glucose determinations by the glucose oxidase method (12) using a commercial reagent. The rats were fed ad libitum at the time of sampling. At sacrifice the rats were exsanguinated through a large cardiac incision under ether anaesthesia.

The rats were taken in sequence from the different groups. The adrenal glands, the thymus, the ventral and dorsolateral prostate, the coagulating glands, the seminal vesicles and the levator ani muscle were dissected free, with the aid of a stereoscopic microscope. During the dissection the organs were moistened with physiological saline. The organs were blotted and weighed on an analytical balance to an accuracy of 0.01 mg.

Student's t-test was used for testing differences between means. For histological examination, the ventral prostate, the dorsolateral prostate, the coagulating glands and the seminal vesicles were fixed in Bouin's solution for two hours. After dehydration the organs were embedded in paraffin and cut into 4-5 μ sections which were stained with Weigert-van Gieson or with PAS (McManus) stains (6).

RESULTS

Food Consumption and Blood Glucose

During the period of injections the mean food consumption per 24 h per control rat (group C) was about 22 g. Oestradiol treatment (group E) decreased the food consumption to about 17 g per 24 h per rat. Insulin treated rats (group I) ate about 26 g per 24 h per rat. After the combined treatment (group E - I) the food consumption per 24 h per rat was the same as for the control rats (group C).

After administration of protamine zinc insulin alone (group I) or in combination with oestradiol benzoate (group E - I) there was a prolonged and marked hypoglycaemia (Table 1) followed by hyperglycaemia which was most marked after the combined treatment (group E - I).

Body Weight and Weight of the Levator ani Muscle (Table 2)

Body weight increased during the injection period in all the rats. At autopsy the insulin treated rats (group I) weighed somewhat more than the control rats (group C) ($P < 0.025$), while rats given oestradiol alone or in combination with insulin (groups E and E - I) weighed less than the controls (group C) ($P < 0.001$). The mean weight of the levator ani muscle of oestradiol treated rats (group E) was lower than in controls (group C) ($P < 0.001$). The weight of the levator ani muscle of the rats given both oestradiol and insulin (group E - I) did not differ significantly from that of the control rats (group C).

Weights of Adrenal Glands and Thymus (Table 2)

Rats treated with oestradiol (group E) had somewhat lighter adrenals than control rats (group C) ($P < 0.05$). Rats treated with insulin and oestradiol in combination (group E - I) had heavier adrenal glands than rats given oestradiol alone (group E) ($P < 0.005$). Insulin

* The number of rats in this group was initially ten, but five rats died during the experimental period.

Table 1. Blood glucose values from the last three days of the experiment

Groups	Number of rats	Blood glucose values in mg/100 ml		
		"Day 19" at 11 a.m.	"Day 20" at 3 p.m.	"Day 21" at 8 a.m.
C	8	122 ± 5[a]	134 ± 5[a]	115 ± 3[a]
E	8	113 ± 4	136 ± 9	105 ± 5
I	9	49 ± 3	63 ± 3	132 ± 16
E - I	5	44 ± 8	79 ± 11	156 ± 11

a Mean ± standard error of the mean

Table 2. Mean body weights and mean weights of ventral and dorsolateral prostate, coagulating glands, seminal vesicles, levator ani muscle and thymus in castrated rats injected with oestradiol benzoate and protamine zinc insulin alone or in combination and in castrated control rats

Groups	Number of rats	Body weight (g)		Ventral prostate (mg)	Dorsolateral glands (mg)	Coagulating glands (mg)	Seminal vesicles (mg)	Levator ani (mg)	Adrenal glands (mg)	Thymus (mg)
		At start of treatment	At autopsy							
C	8	215 ± 3	320 ± 6	9.4 ± 0.4	11.9 ± 0.8	3.9 ± 0.3	12.6 ± 0.9	90.0 ± 5.9	72.2 ± 2.4	986 ± 62
E	8	214 ± 3	246 ± 4	15.1 ± 0.7	27.0 ± 1.2	10.8 ± 0.5	54.7 ± 2.0	55.0 ± 3.8	64.8 ± 2.0	345 ± 23
I	9	216 ± 2	337 ± 3	10.8 ± 0.3	14.9 ± 0.4	3.8 ± 0.2	10.5 ± 0.2	89.5 ± 6.5	71.8 ± 2.3	1222 ± 69
E - I	5	217 ± 3	260 ± 5	18.0 ± 0.7	46.1 ± 7.7	13.5 ± 1.2	56.2 ± 3.5	78.0 ± 8.3	78.8 ± 3.0	280 ± 26

The figures given in Table 2 are mean weight ± standard error of mean

treatment somewhat increased the thymus weight ($P < 0.025$). In rats treated with oestradiol alone or in combination with insulin the weight of the thymus was markedly decreased (groups E and E - I).

Weights of Ventral and Dorsolateral Prostate, Coagulating Glands and Seminal Vesicles (Table 2)

After administration of oestradiol (group E) the mean weights of the ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles were higher than in the non-treated controls (group C) ($P < 0.001$).

Insulin somewhat increased the mean weights of the ventral and dorsolateral prostate ($P < 0.02$ and 0.005 respectively. The combined treatment of oestradiol and insulin (group E - I) resulted in a higher mean weight of the ventral prostate ($P < 0.02$), the dorsolateral prostate ($P < 0.01$) and the coagulating glands ($P < 0.05$) than treatment with oestradiol alone (group E).

Histological Examination of the Accessory Reproductive Organs

The ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles in untreated castrated rats exhibited an immature appearance histologically (group C). The small acini were surrounded by undeveloped smooth muscle cells. The epithelial cells were cubic to low columnar and the acini contained little or no secretion (Figs. 1 and 3).

In rats given protamine zinc insulin (group I) the histological examination revealed a slight stimulation of the growth of the dorsolateral prostate. In the dorsal part of the dorsolateral prostate the columnar epithelium formed some papillary formations. The acini were larger than in control rats and contained some PAS-positive secretion (Fig. 4). The acini of the lateral part were lined by columnar epithelial cells some of which had supranuclear clear zones indicating secretory activity (7, 8). The histological

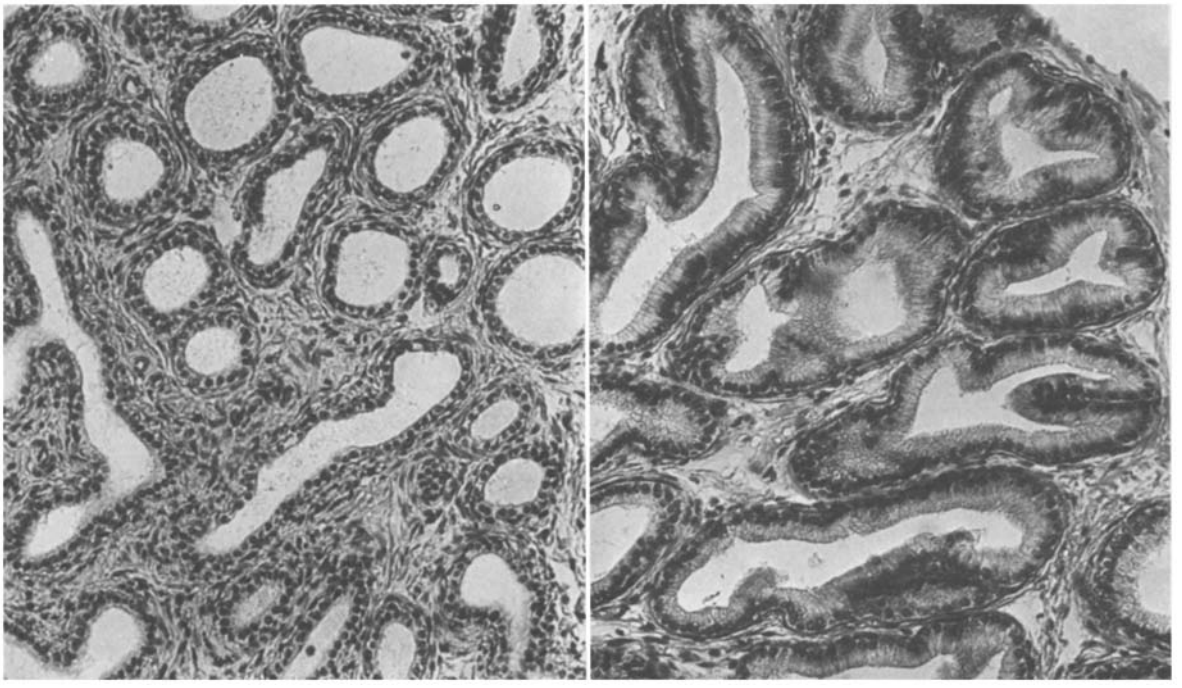

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Fig. 1. Section of undeveloped ventral prostate from an untreated castrated rat (group C). Weigert-van Gieson x 192

Fig. 2. Section of ventral prostate from a castrated rat subjected to 21 daily injections of 0.010 mg oestradiol (group E). Note the high epithelium with a supranuclear clear zone. Weigert-van Gieson x 192

appearance of the ventral prostate, the coagulating glands and the seminal vesicles after protamine zinc insulin administration was similar to that in the control rats.

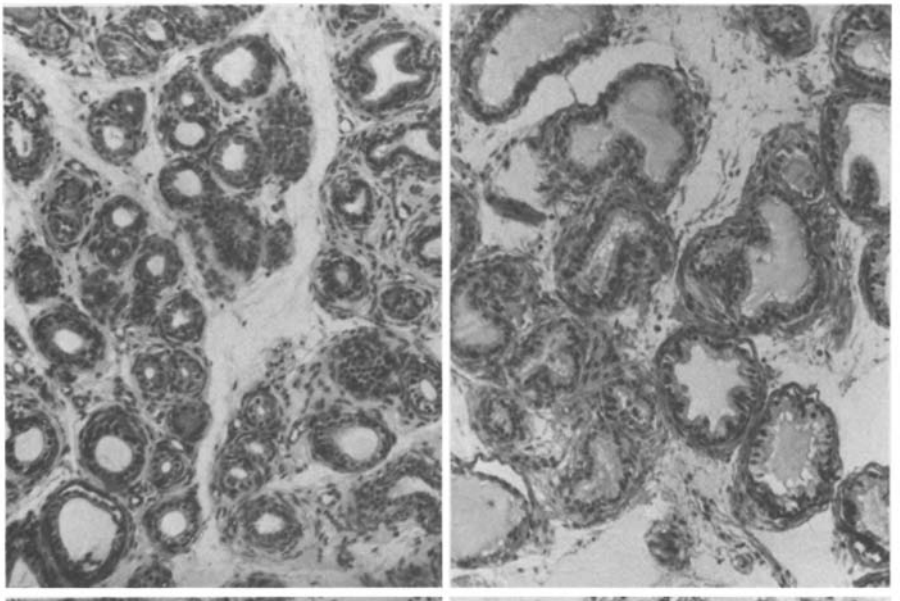
In rats injected with oestradiol benzoate (group E) the prostatic lobes and the seminal vesicles differed from that of the controls (group C). In the ventral prostate the acini were larger, contained more secretion and were lined with higher columnar epithelium than in the controls. Occasionally the epithelial cells of peripheral acini showed supranuclear clear zones (Fig. 2).

The acini of the dorsal part of the dorsolateral prostate in oestradiol treated rats were larger with higher epithelium and contained more secretion and exfoliated cells than in the controls. Furthermore the epithelial cells formed more papillary formations (Fig. 5). The acini of the lateral part of the dorsolateral prostate in the oestradiol treated rats contained secretion and were lined by a high folded columnar epithelium with a supranuclear clear zone.

The acini of the coagulating glands in oestradiol treated rats were lined by one or two layers of epithelial cells that varied both in size and shape. The acini did not contain more secretion than the acini of the untreated controls. The muscular layer was markedly increased in width. As compared to the control rats there was an increase in the amount of fibrous tissue between the epithelium and smooth muscle layers. The fibrous tissue between the muscle bundles of the muscular layers also seemed to be increased in amount.

After oestradiol administration the seminal vesicles showed small lumina lined by irregular cuboidal epithelial cells arranged in one or two layers. Compared to the control rats there was an increase in the amount of fibrous tissue between the epithelial and smooth muscle layers and in the core of the few papillary formations. The fibrous tissue between the muscle bundles also seemed to be increased in amount. The muscular layers were markedly increased in width and the muscle cells had a larger nucleus and more cytoplasm than in the control rats.

After oestradiol and insulin in combination (group E - I) the histological examination revealed a more pronounced epithelial growth and secretory activity in the dorsolateral prostate than after oestradiol alone (group E) (Fig. 6). The histological appearance of the ventral prostate, the coagulating glands and the seminal vesicles of rats given oestradiol and insulin in combination (group E - I) was similar to that of rats given oestradiol alone (group E).



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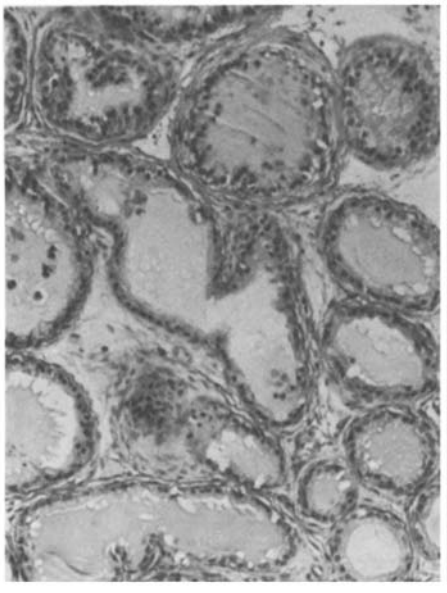

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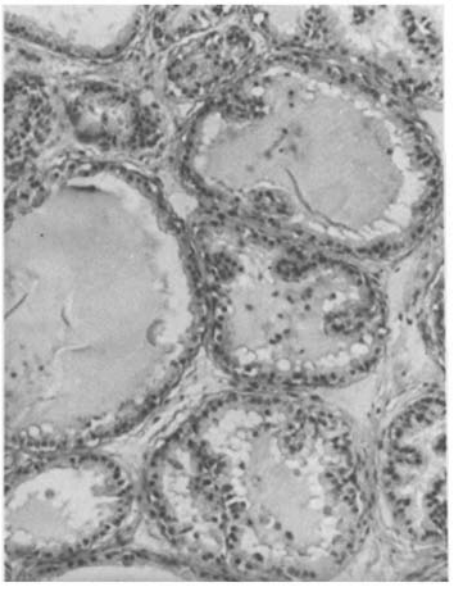

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Fig. 3. Section of undeveloped dorsal part of the dorsolateral prostate from an untreated castrated rat (group C). McManus x 120

Fig. 4. Section of dorsal part of dorsolateral prostate from a castrated rat subjected to 21 daily injections of 10 IU of protamine zinc insulin (group I). Note the rather high epithelium and the secretion in the acini. McManus x 120

Fig. 5. Section of dorsal part of the dorsolateral prostate from a castrated rat subjected to 21 daily injections of 0.01 mg oestradiol (group E). McManus x 120

Fig. 6. Section of dorsal part of dorsolateral prostate from a castrated rat subjected to 21 daily injections of 0.01 mg oestradiol and 10 IU of protamine zinc insulin. McManus x 120

DISCUSSION

In the present experiment with castrated rats the weights of the prostatic lobes and seminal vesicles were greater in the oestradiol treated than in the non-treated rats. Oestradiol administration resulted in an increase in epithelial growth and secretion in the dorsolateral prostate and to a smaller extent in the ventral prostate. Furthermore, oestradiol caused an increase in the amount of fibrous and smooth muscle tissue in the coagulating glands and seminal vesicles. The fact that oestradiol can induce epithelial growth and secretory activity in the ventral and dorsolateral prostates of castrated rats, although to a small extent, is interesting since this capacity is characteristic of androgens (3, 9). The effect of oestradiol described here is in agreement with previous findings and has been assumed to be the result of the combined action of oestradiol and endogenous adrenal steroids (14).

Administration of protamine zinc insulin to castrated rats caused growth and secretory activity in the dorsolateral prostate, while no definite effect was observed in the ventral prostate, the coagulating glands or the seminal vesicles. These results are contrary to those obtained in an earlier experiment in which protamine zinc insulin not only stimulated the growth of the dorsolateral prostate but also to some extent the growth of the other prostatic lobes and the seminal vesicles (15). In the earlier experiment protamine zinc insulin was given for fifteen days in increasing daily doses up to 10 IU and caused morphological signs of an increased secretion of adrenal steroids; the adrenals became enlarged and the thymus atrophic. The growth observed in the prostatic lobes and the seminal vesicles in our previous experiment was interpreted as the result of a combined action of insulin per se and increased amounts of adrenal steroids and hypophyseal growth hormone released by the insulin induced hypoglycaemia (16). In the present experiment, when protamine zinc insulin was given in a constant daily dose of 10 IU for twenty-one days, no morphological signs of an increased production of adrenal steroids were observed. This seems to be in accordance with the findings of Kraicer & Logothetopoulos (5) who demonstrated an adrenal cortical adaption in rats to repeated daily injections of a constant dose of protamine zinc insulin during 25 days. The failure of protamine zinc insulin injections to cause such an increase in adrenal cortical secretion, as to manifest itself morphologically in the weight of the adrenals and the thymus, might explain why the growth of the prostatic lobes and the seminal vesicles was less than in our previous experiment (15).

The addition of insulin to the oestradiol treatment could not further stimulate the growth of the fibromuscular tissue in the seminal vesicles and coagulating glands, nor could it promote the development of a secretory epithelium in these glands. The weights of the ventral prostate and coagulating glands were somewhat greater after the combined treatment than after oestradiol alone. However, in the absence of histological changes no definite conclusion can be drawn from these observations.

A more pronounced epithelial growth and secretory activity in the dorsolateral prostate was observed after treatment with oestradiol and protamine zinc insulin than after insulin or oestradiol alone. The effect of the combined treatment on the weight was greater than the addition of the individual effects of insulin and oestradiol. From earlier experiments it is known that the effects of these two hormones on the dorsolateral prostate are influenced by adrenal steroids (14, 15). After the combined treatment with oestradiol and insulin the adrenals were heavier than after oestradiol alone suggesting a higher production of adrenal steroids after the combined treatment. Therefore the growth observed in the dorsolateral prostate after the treatment with protamine zinc insulin and oestradiol very possibly is due to the combined action of oestradiol, insulin and endogenous adrenal steroids.

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L. -E Tisell, M. D.
Kirurgiska kliniken II
Sahlgrenska sjukhuset
S-413 45 Gothenburg
Sweden