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The Effect of Oestrogen on Stromal Growth of the Dog Prostate: A Quantitative Ultrastructural Study*

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Summary. The stromal tissue of the prostate in normal castrated dogs and castrated and oestrogen treated dogs were studied by quantitative morphological methods. Quantitative morphological (= stereological) procedures provide values of volume, surface and number of tissue and cell components. The stereological data show an activation of the smooth muscle cell of the stromal tissue in the oestrogen treated dog compared with the control group; related to the unit volume of smooth muscle cell cytoplasm, a threefold increase of the endoplasmic reticulum was observed in castrated and oestrogen treated dogs.

Key words: Dog prostate, Stereological analysis, Electron microscopy, Oestrogen effect.

Abbreviations: SMC = Smooth muscle cell, N = Nucleus, EX = Extracellular space,, CYT = Cytoplasm, MF = Myofilaments, R = Ribosomes, ER = Endoplasmic reticulum, G = Golgi apparatus, M = Mitochondria, VAC = Vacuoles, VES = Vesicles, LY = Lysosomes, V_V = Volume density, COMP = Cellular compartments.

INTRODUCTION

Benign prostatic hyperplasia (BPH) occurs commonly in only 2 species, man and dog (13, 23). Although there are histological differences between the two, they have many features in common. In light and electron microscopic stereological studies of the normal human prostate and of human BPH an increase in the amount of stromal tissue present was demonstrated (3); ultrastructural stereological studies showed an activation of the smooth muscle cells in human BPH (3). The present study was undertaken in order to perform an ultrastructural analysis of the smooth muscle cell of the dog prostate with respect to the influence of castration, and castration and 17 β-oestradiol treatment on stromal growth.

MATERIAL AND METHODS

Fifteen Beagle dogs weighing 6800 ± 1400 g, (m ± SD), maintained under standardised experimental conditions were studied (standard diet, water ad libitum, maintained in regular day and night rhythm). Five animals served as controls. Ten dogs were castrated and 5 of these animals were sacrificed at 3 weeks and the prostate removed. The other 5 castrated animals were treated twice a week with 2.5 mg oestradiol benzoate by intramuscular injection. After treatment for 3 weeks these animals, weighing 11,100 ± 1920 g (m ± SD), were sacrificed at the same time as the other 10 dogs (9 a.m.).

Electron Microscopy

Tissue blocks of 0.5 mm were fixed in 2% sodium phosphate buffered glutaraldehyde (pH 7.3, 0.1 M, 440 mOsm) for 4 h at 4°C and postfixed in 1.33% s-collidine buffered osmium tetroxide (pH 7.4, 580 mOsm). The specimens were dehydrated in increasing alcohol concentrations and propylene oxide and embedded in Epon. Ultrathin sections were cut with the Reichert ultramicrotome OMU2 (interference colour: silver). After double staining with uranyl-acetate and lead citrate they were examined with the Zeiss electron microscope EM9A.

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Stereological Procedure

The volume densities of the various subcellular organelles of the smooth muscle cell of the fibromuscular tissue were determined according to Weibel (24):

$$V_i = \frac{P_i}{P_T} \quad (1)$$

where i is the cellular component under consideration, P_i is the number of the test points in the test system associated with i and P_T is the total number of points of the test system.

For the stereology of the smooth muscle cell the reference systems 'smooth muscle cell' and 'smooth muscle cell cytoplasm' were introduced.

Sampling

We adopted the following sampling criteria: we recorded micrographs in strips covering the entire smooth muscle cell at a primary magnification of 4100 x. The determination of a representative sample size for the different parameters was performed by the approach proposed by Weibel (24). For each dog at least 3 tissue blocks containing stromal tissue were selected at random and processed for electron microscopy. From these blocks about 10 smooth muscle cells were recorded entirely in strips (Fig. 1), giving a total of 40 to 50 micrographs. Each group comprised 3 dogs, therefore we analysed at least 120 micrographs per group.

Test System Used for Counting Cellular Components The double square test system was used (1:9, 121:1089, where 1:9 signifies the ratio of 'heavy' and 'fine' test points, 121:1089 signifies the number of 'heavy' to 'fine' test points).

Stereological Calculations

The electron micrographs were evaluated at a primary magnification of 4100 x as follows:

P_T = 121 (coarse lattice)	P_T = 1,089 (dense lattice)
Nuclei (N)	
Extracellular space (EX)	
Myofilaments (MF)	
	Mitochondria (M)
	Ribosomes (R) and rough endoplasmic reticulum (RER)
	Golgi apparatus (G)
	Vacuoles (VAC) and vesicles (VES)
	Lysosomes (LY)

The following calculations were performed: The total number of test points over the smooth muscle cell (P_{SMC}) can be expressed as follows:

$$P_{SMC} = P_T - P_{EX} = P_{CYT} + P_N \quad (2)$$

The number of test points over all cell compartments:

$$P_{COMP} = P_M + P_{RER} + P_R + P_G + P_{VAC} + P_{VES} + P_{LY} \quad (3)$$

The points over the cytoplasm of the smooth muscle cell can be deduced from equation (1):

$$P_{SMCYT} = P_T - (P_{EX} + P_N) \quad (4)$$

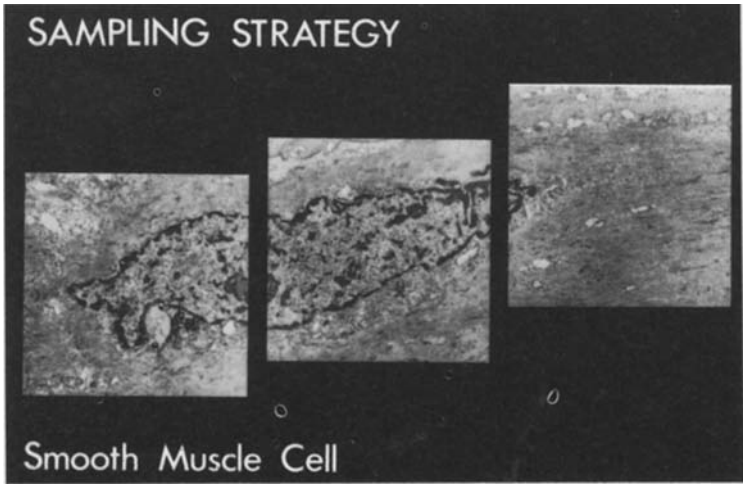

Fig. 1. Sampling procedure for the smooth muscle cell

Reference Space

In order to relate the volume density of these various subcellular organelles of the smooth muscle cell, the primary volume densities (see stereological calculations) are converted into volume densities related to the reference system 'smooth muscle cell' and 'smooth muscle cell cytoplasm'.

Smooth muscle cell (SMC):

$$\frac{P_{COMP}}{P_T - P_{EX}} = \frac{V_{VCOMP}}{V_{VSMC}} = \frac{V_{VCOMP}}{1 - V_{VEX}} = V_{VCOMP, SMC} \quad (5)$$

Smooth muscle cell cytoplasm (SMCYT):

$$\frac{P_{COMP}}{P_T - (P_{EX} + P_N)} = \frac{V_{VCOMP}}{V_{VSMCYT}} = \frac{V_{COMP}}{1 - (V_{VN} + V_{VEX})} = V_{VCOMP, SMCYT} \quad (6)$$

Statistics

Stereological calculations and statistics were performed on a Hewlett and Packard 9815A 001 microcomputer with a multipurpose programme (Schmassmann 1979, unpublished). For each dog the mean (m), the median, midrange, x min., x max., the 95% limit of confidence, variance and the standard deviation (SD) as well as the standard error (SE) of the mean were calculated for the different parameters. In a second step, the above mentioned statistical parameters were calculated for each group.

The group means of the different parameters were compared with the Student's t-test if normal distribution could be shown, otherwise with the Mann-Whitney test. A significant difference was ascertained if P was < 0.05. Normal distribution was stated by the ratio R/SD whereas R is the range.

RESULTS

From the descriptive standpoint the smooth muscle cells are somewhat spindle shaped and contain an ellipsoid-like nucleus. Most of the or-

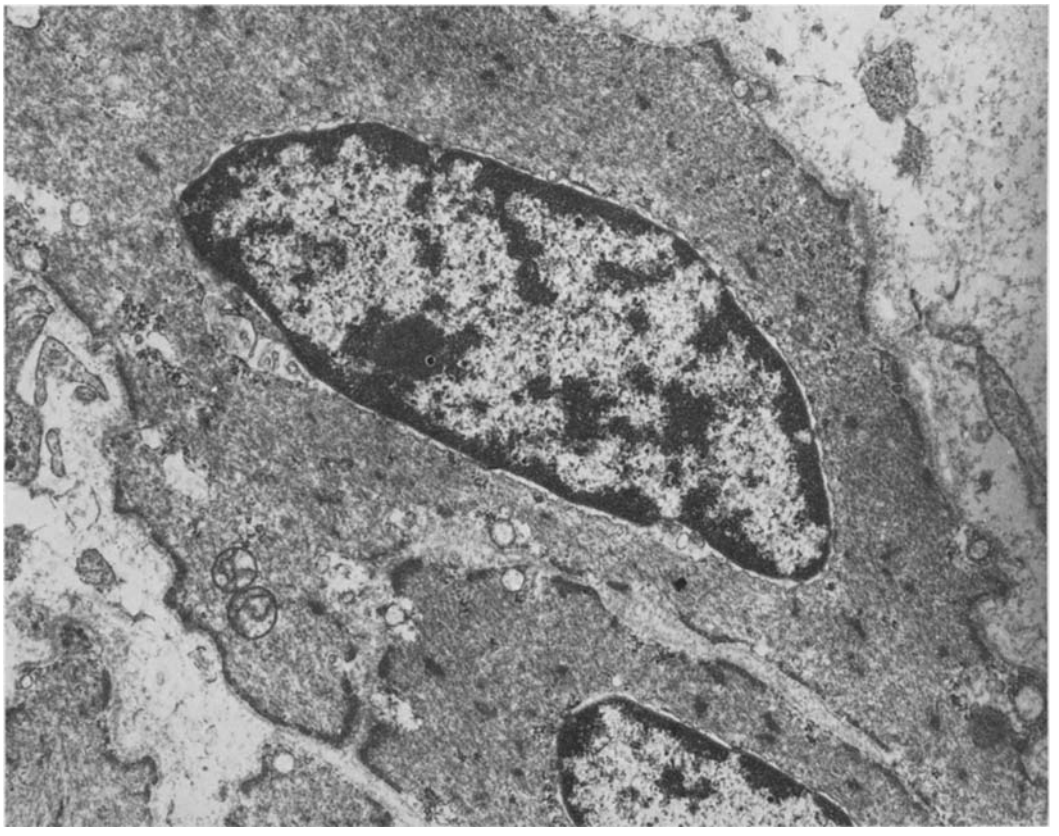

Fig. 2. Electron micrograph of a smooth muscle cell of a normal dog; note the great amount of myofilaments, beneath there are some profiles of rough endoplasmic reticulum and mitochondria. (Primary magnification: 4100 x)

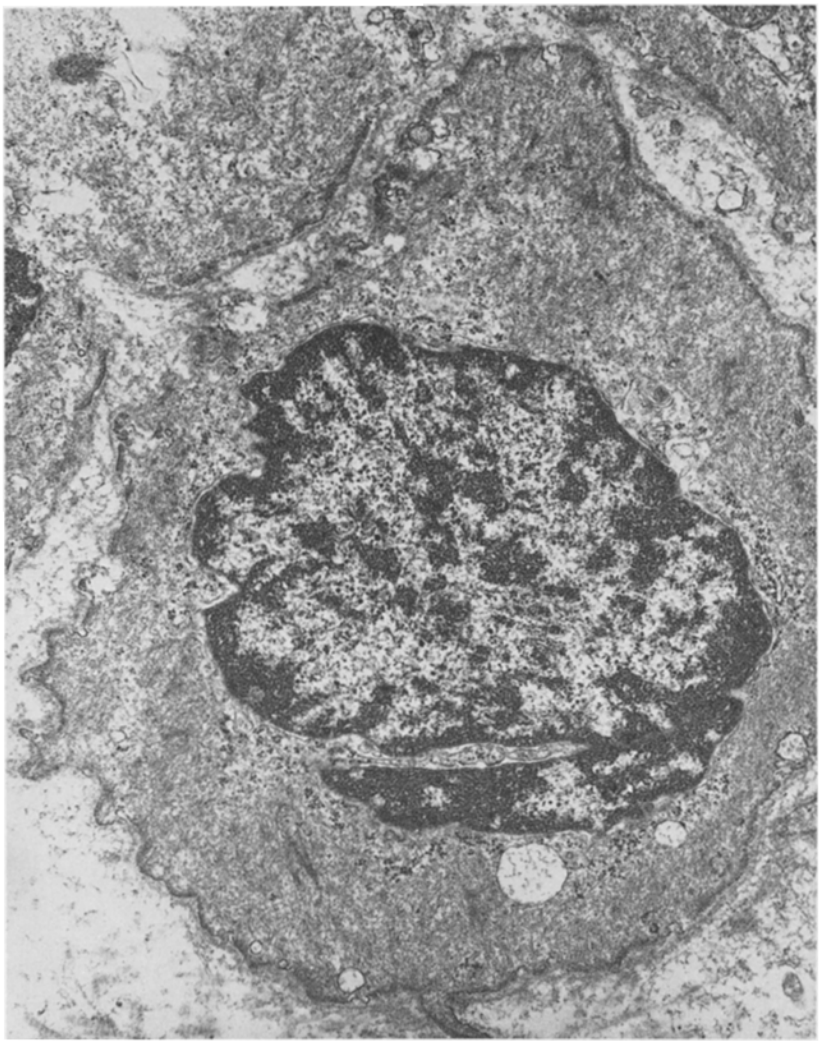

Fig. 3. Electron micrograph of a smooth muscle cell of a castrated dog; except for some profiles of vacuoles and perinuclear situated rough endoplasmic reticulum the cytoplasm is filled with myofilaments. (Primary magnification: 4100 x)

ganelles are located adjacent to the nucleus or in small clusters at the cell periphery (Fig. 2). Myofilaments occupy the largest portion of the cytoplasm and run parallel to the long axis of the cell. In the regions in which organelles such as mitochondria, endoplasmic reticulum and Golgi apparatus are present, the ground substance is pale and free of myofilaments. The rough endoplasmic reticulum consists of a few profiles of membranes with small numbers of attached ribosomes. Sometimes small Golgi apparatus and profiles of ovaloid mitochondira can be observed.

In the group of castrated dogs there was no difference in the ultrastructural findings (Fig. 3).

Contrary to these findings in the castrated and oestrogen-treated dogs the perinuclear zone is markedly increased. The rough endoplasmic reticulum with enlarged cisternae, studded with ribosomes is well developed (Fig. 4). The Golgi apparatus shows an enlargement and contains more vesical than cisternal elements. Many free ribosomes can be seen.

Stereological Data

Related to the unit volume of smooth muscle cell cytoplasm in the control group the volume fractions of the rough endoplasmic reticulum have been calculated to be 8% of the whole cytoplasm (mitochondria 2.7%, vacuoles and vesicles 1.2%). In the group of castrated dogs no statistically significant increase of the organelles could be observed; however, there was a small increase in the volumetric amount of mitochondria and rough endoplasmic reticulum. In the dogs treated with oestrogen there was a statistically significant increase of the volume fraction of endoplasmic reticulum (26%), vacuoles and vesicles (2%) (Table 1); in the castrated oestrogen-treated group there was a threefold increase in organelles of the smooth muscle cell (controls: 12%, castrated and oestrogen-treated group: 32%).

DISCUSSION

The stereological methods devised by Weibel (24) and Rohr et al. (16) make a stereological analysis

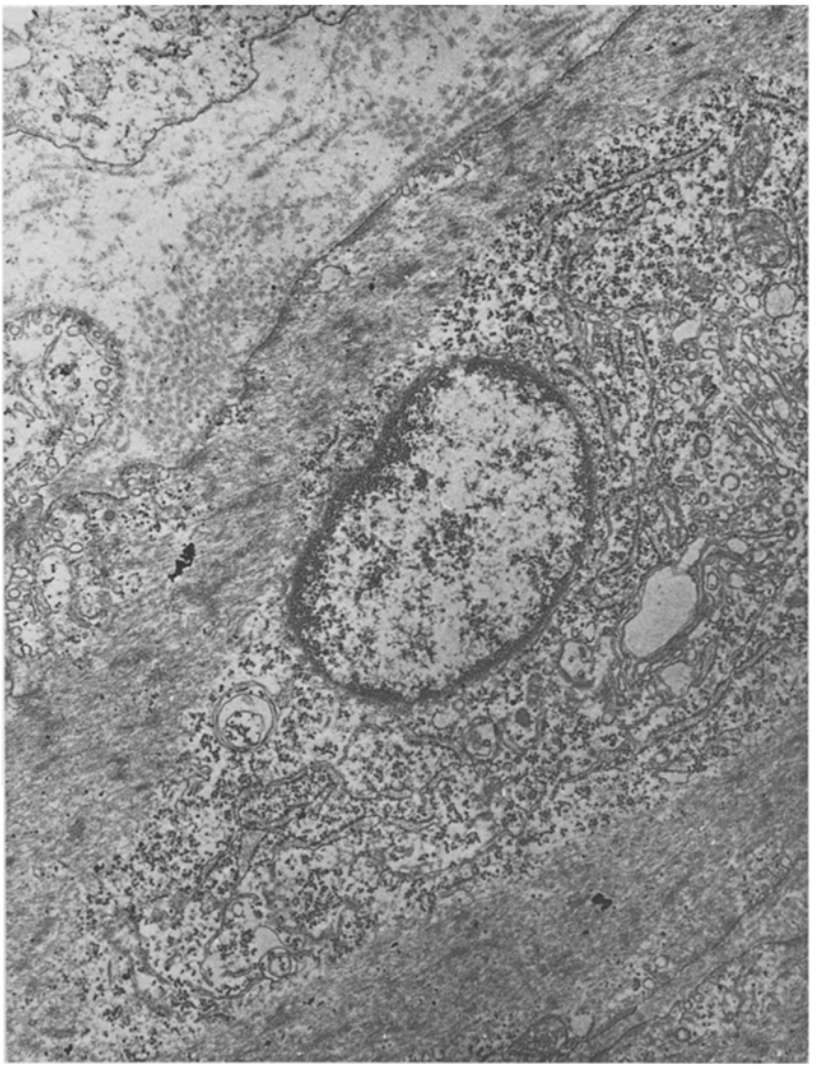

Fig. 4. Electron micrograph of a smooth muscle cell of a castrated and oestrogen treated dog; abundant rough endoplasmic reticulum, Golgi apparatus and mitochondria are seen. (Primary magnification: 4100 x)

of the various cell compartments at the ultrastructural level possible and allow a quantitative comparison between their sizes in normal and experimentally changed tissue. Stereological analyses of the rat prostatic lobe and of human prostatic tissue under normal and pathological conditions have been shown to be a successful approach to study prostatic function and disease (2, 3, 4). Up to now, little information on descriptive and quantitative morphological data of the stromal tissue of the normal and pathologically altered dog prostatic tissue is available.

In a previous study an attempt was made to characterise the normal human prostate and human benign prostatic hyperplasia by means of descriptive and quantitative light and electron microscopy (3). In benign prostatic hyperplasia a high volume density of the stromal tissue was demonstrated. 61% of the tissue of benign prostatic hyperplasia was made up of stromal tissue including nerves, vessels, lymphatics, collagen fibres, fibroblasts and smooth muscle cells. Glandular cells comprised only 12% of benign prostatic hyperplastic tissue. Comparing these data with the normal human prostate there was a significant increase of the stromal part in benign prostatic hyperplastic tissue. Comparing the stromal tissue of the normal human prostate and benign prostatic hyperplasia an activation of the smooth muscle cells of the stromal part in benign prostatic hyperplasia was shown.

A consistent action of oestrogen in the male accessory sex organs is the induction of fibromuscular growth (12). It was shown that oestrogens in small dosage in the dog can enhance dihydrotestosterone or androstendione-induced glandular cell growth to a four and five-fold increase respectively (18). As stated by Mawhinney and Neubauer (1978) positive interactions of oestrogens and androgens on the glandular part and oestrogen-induced fibromuscular tissue induction are responsible for keeping normal organ function (12). However, it remains unanswered whether oestrogens or androgens have selective

Table 1. Volume densities of the cellular compartments of the smooth muscle cell related to the unit volume of the smooth muscle cell cytoplasm

Parameters		V_{VM}	V_{VER+R}	V_{VG}	$V_{VVAC} + V_{VVES}$
Control dogs (n = 3)	$\bar{m}$	0.027	0.080	0.003	0.012
	median	0.027	0.078	0.003	0.012
	X min.	0.026	0.077	0.002	0.009
	X max.	0.027	0.086	0.003	0.014
	SE	-	0.003	-	0.001
	R/SD	1.732	1.824	1.732	1.987
Castrated dogs (n = 3)	$\bar{m}$	0.029	0.095	0.001	0.040
	median	0.030	0.101	0.001	0.040
	X min.	0.026	0.071	-	0.015
	X max.	0.032	0.112	0.003	0.066
	SE	0.002	0.012	0.001	0.015
	R/SD	1.964	1.932	1.964	2.000
Castrated and oestrogen treated dogs (n = 3)	$\bar{m}$	0.033	0.258	0.006	0.018
	median	0.031	0.262	0.005	0.019
	X min.	0.028	0.201	0.003	0.014
	X max.	0.040	0.311	0.010	0.020
	SE	0.004	0.032	0.002	0.002
	R/SD	1.922	1.996	1.941	1.867

or additive influences on the stromal and glandular part respectively, regarding the various cellular elements of both parts of the prostate.

Growth of the fibromuscular tissue due to oestrogen administration was shown in non-human primates (7, 25, 26) and in the canine prostate (8, 27). As shown on the rat ventral prostatic lobe the oestrogen-induced stromal growth is caused by an increased number of cells (20).

Ross and Klebanoff (19) demonstrated an activation of the smooth muscle cells of the uterus of the prepuberal rat following injection of 17-β-oestradiol. In young female virgin mice the injection of oestrogens for 3 days leads to an increase of the cell organelles in the smooth muscle cells of the uterus. These findings are considered as evidence of an increased synthetic activity of these cells. Autoradiographic electron microscopic techniques demonstrated that the activated smooth muscle cells of the uterus incorporate radiosulphate and tritiated proline (17). These autoradiographic findings indicate that under certain experimental conditions the smooth muscle cells can participate in synthesis of ground substance of connective tissue.

There is evidence that the male accessory sex organs of primates and humans contain high concentrations of endogenous oestrogens and oestrogen receptors (1, 5, 9, 10, 11, 14, 15, 21, 22); similarly in the canine prostate oestrogen receptors were analysed (6, 14).

Unfortunately up to now it has not been shown whether these endogenous oestrogens or oestrogen receptors are selected to the stromal or to the glandular tissue of the prostate. In the guinea pig seminal vesicle, where the muscle can be effectively separated from the epithelium, androgen and oestrogen receptors are found in both cell types (12); in the glandular part androgen receptors predominate, whereas oestrogen receptors are more numerous in the muscular part (12).

Our study shows a clear activation of the smooth muscle cells of the stromal part in the oestrogen-treated dog and we suggest that oestrogens can induce an activation of the smooth muscle cell and overgrowth of stromal tissue.

The stereological results were very similar to those obtained on the smooth muscle cells in human benign prostatic hyperplasia, showing smooth muscle cell activation.

These results support the suggestion that oestrogens play a role in the development of human BPH, which was shown to be primarily a stromal disease.

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