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Stereological Analysis of the Dog Prostate (Analytical Model)*

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Summary. A stereological model which provides information on the structure of the normal dog prostate has been developed. The model consists of the morphologically defined space and membrane components which are used to describe the specific components of the protein and enzyme synthesising glandular cells. Comparisons of the dog, rat and normal human prostate in regard to quantitative light and electron microscopic analysis are presented.

Key words: Dog prostate, Stereological analysis, Sterological procedure.

Abbreviations: P = Prostate, IT = Interacinar (= stromal) tissue, AP = Acinar parenchyma, AL = Acinar lumina, AC = Acinar glandular cell, N = Nucleus, CYT = Cytoplasm, GS = Ground substance, MI = Mitochondria, RER = Rough endoplasmic reticulum, GA = Golgi apparatus, SG = Secretory granules, F = Fat droplets, V_V = Volume density, P_T = Total number of points of the test system, P = Number of counted points, m = Mean, s.e. = Standard error.

INTRODUCTION

Prostatic hyperplasia occurs commonly in only 2 species, man and dog (6, 7). Although there are histological differences between the two, the prostatic hyperplasia in man and dog have many features in common. Therefore, the dog prostate should be a good animal model for studying the pathogenesis of benign prostatic hyperplasia (10). Although a considerable amount of biochemical data on the dog prostate is available (3, 4, 9), morphological information has been restricted mainly to subjective descriptive findings. In recent years there have been advances in stereological methods which have made quantification of morphological findings possible (1, 8, 11). The purpose of this paper is to report the development of a method of analytical stereological analysis of the dog prostate, its glandular cells and its cell compartments.

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MATERIAL AND METHODS

Animals

Five male adult, sex mature, 7-11 months old Beagle dogs (weighing 9600 ± 1200 g) fed under rigorously standardised experimental conditions were used. The prostate was rapidly removed from the animal and weighed after the fat was carefully removed. Blocks were taken from the middle portion of the prostate and prepared for light and electron microscopy.

Light Microscopy

The blocks (taken in the way that the block represents a transverse section of the whole prostate) were fixed in phosphate-buffered formalin (pH 7.4) and embedded in paraffin.

Electron Microscopy

Tissue blocks, 0,5 mm on one side, were fixed in 1.3% phosphate buffered osmium (340 mOsm)

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at pH 7.4 for 2 h at 4°C. Tissue blocks were dehydrated in increasing alcohol concentrations and propylene oxide and embedded in Epon 812. Ultra-thin sections were cut with the Reichert ultramicrotome OMU 2 (interference colour: silver). After double staining with uranyl acetate and lead citrate they were examined with the Zeiss electron microscope EM 9A.

STEREOLOGICAL PROCEDURES

Stereological data are expressed as densities which relate a volume, a surface or a number to a unit volume. These densities, which are relative measurements, refer as basic values primarily to a unit volume which corresponds to the test volume of the test lattice of 1 of the 4 sampling levels.

The following reference volumes were used: Prostatic tissue, glandular cell, glandular cell cytoplasm, glandular cell organelles.

Volume densities were determined according to Weibel (11):

$$V_{Vi} = \frac{P_i}{P_T}$$

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

Stereological Methods

The model for the dog prostate is outlined in Fig. 1. It shows how the prostate (P) was divided into morphologically defined components which have been quantified by applying stereological methods. Essentially the model has 2 major divisions:
- the interacinar (= stromal) tissue (IT), including connective tissue, blood vessels, nerves and smooth muscle cells and
- the acinar parenchyma (AP), including the lumina of the acini (AL) and the glandular epithelial cells (AC).

The latter were divided into the nuclei and the various cytoplasmic compartments.

The stereological analysis was performed at 4 magnification levels since the cellular components have a broad range of size and frequency:

Level I: Primary magnification 1:150 (light microscopy)
Level II: Primary magnification 1:300 (light microscopy)
Level III: Primary magnification 1:1300 (light microscopy)
Level IV: Primary magnification 1:4100 (electron microscopy)

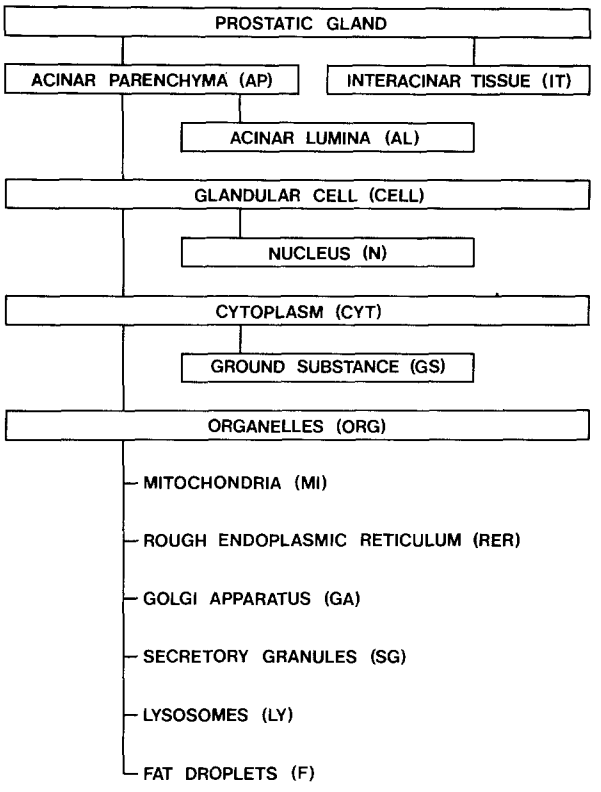

Fig. 1. Stereological model of the dog prostate

Stereological Calculations

Level I and II (150 x, 300 x)

Counted:

Test points on:
P_{IT} (multipurpose lattice, P_T = 100, level I)
P_{AC} (multipurpose lattice, P_T = 100, level II)
P_{AL} (multipurpose lattice, P_T = 100, level III)

Calculated:

$$P_P = P_{AC} + P_{AL} + P_{IT}$$

$$P_{AP} = P_{AC} + P_{AL}$$

Volume densities of AC (glandular cell), AL (acinar lumina) or IT (interacinar stromal tissue) in the reference space prostate (P) and acinar parenchyma (AP) respectively:

e.g. $$V_{VAL,\,P} = \frac{P_{AL}}{P_P}$$

and $$V_{VAC,\,P} = \frac{P_{AC}}{P_P}$$

and finally $$V_{VAL,\,AP} = \frac{P_{AL}}{P_{AP}}$$

$$V_{VAC,\,AP} = \frac{P_{AC}}{P_{AP}}$$

Level III (1300 x)

Counted:

P_N

P_{IT} $\quad P_T = 100$

P_{AL}

Calculated:

$P_{AC} = P_T - (P_{IT} + P_{AL})$

Volume density of nuclei (N) in the reference space "acinar cell" (AC):

$$V_{VN,AC} = \frac{P_N}{P_{AC}} = \frac{P_N}{P_T - (P_{IT} + P_{AL})}$$

Level IV (4100 x)

Counted:

Coarse test points ($P_T = 121$)

$P_N + P_{IT} + P_{AL}$, and P_{RER}

Fine test points ($P_T = 1089$)

P_{SG}, P_{GA}, P_{MI}, P_{LY}, P_F, and P_{GS}

Calculated:

$P_{CYT} = P_T - (P_N + P_{IT} + P_{AL})$

$$P_{CYT} = P_{RER} + \frac{1}{9}(P_{SG} + P_{GA} + P_{MI} + P_{LY} + P_F + P_{GS})$$

Since the ratio of fine to heavy test points equals 9:1, the number of points over the corresponding compartments has to be divided by 9.

Volume of e.g. rough endoplasmic reticulum (RER) in cytoplasm (CYT):

$$V_{VRER,CYT} = \frac{P_{RER}}{P_{CYT}}$$

or

$$V_{VMI,CYT} = \frac{P_{MI}}{9 \cdot P_{CYT}}$$

The test system applied to the 4 sampling levels were the following:

Level I (150 x): Multipurpose test lattice
Level II (300 x): (Weibel, 1969, $P_T = 100$)
Level III (1300 x):
Level IV (4100 x): Double square lattice system (Weibel, 1969, 1:9, 121:1089; where 1:9 signifies the ratio of coarse to fine points and 121:1089 the number of coarse to fine points).

Morphological Criteria

The different tissue and cell compartments were evaluated with the light microscope or on electron micrographs according to the following conventions: The interacinar (= stromal) tissue (IT) includes connective tissue, collagen fibres, blood vessels, fibrocytes, smooth muscle cells and nerves. The acinar parenchyma (AP) consists mainly of glandular cells (Fig. 2). The acinar glandular cell is a tall columnar cell. The basal portion of the cell contains abundant rough surface endoplasmic reticulum and the nucleus. The highly developed Golgi apparatus as well as the rough endoplasmic reticulum are distributed mostly in the supranuclear region of the cytoplasm. Mitochondria, multivesicular bodies are distributed throughout the cytoplasm. In the supranuclear and apical region a great amount of secretory granules can be detected (Fig. 3).

Sampling

The different cellular components cannot be evaluated at a single stage of magnification due to the differences in shape, size and frequency of the cell organelles. Therefore, sampling was done at 4 magnification levels (multi-stage sampling) in order to establish an adequate relationship between the size of the components and the points of the test screen.

Level I and II (150 x, 300 x)

For the stereological measurements 3 paraffin-embedded HE-stained sections were selected from each dog prostatic gland specimen. For each section 20-50 randomised test areas were analysed.

Level III and IV (1300 x, 4100 x)

At least 5 tissue blocks for each dog prostatic gland specimen were sectioned. The areas to be evaluated were selected according to systematic sampling procedures by photographing at level III and IV in regular steps in predetermined corners of the meshes of the supporting copper grid. A total of 40 micrographs for each dog was evaluated at level III and IV.

Statistics

Stereological calculations and statistics were performed on a Hewlett & Packard 9815 A 001 microcomputer with a multipurpose programme (Schmassmann, 1979, unpublished).

For each dog the mean (m), the median, mid-range, X min., X max., the 95% limit of confidence, variance and the standard deviation (s.d.) as well as the standard error (s.e.) of

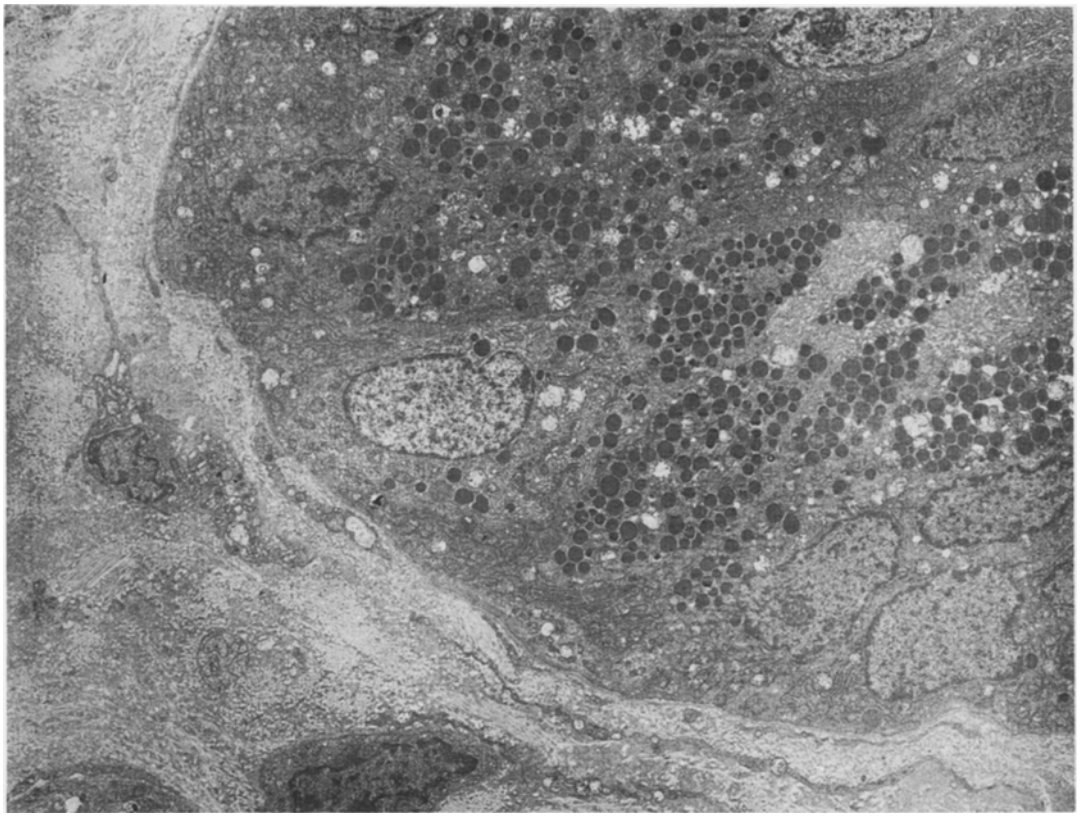

Fig. 2 Electron micrograph of glandular cells (x 1300)

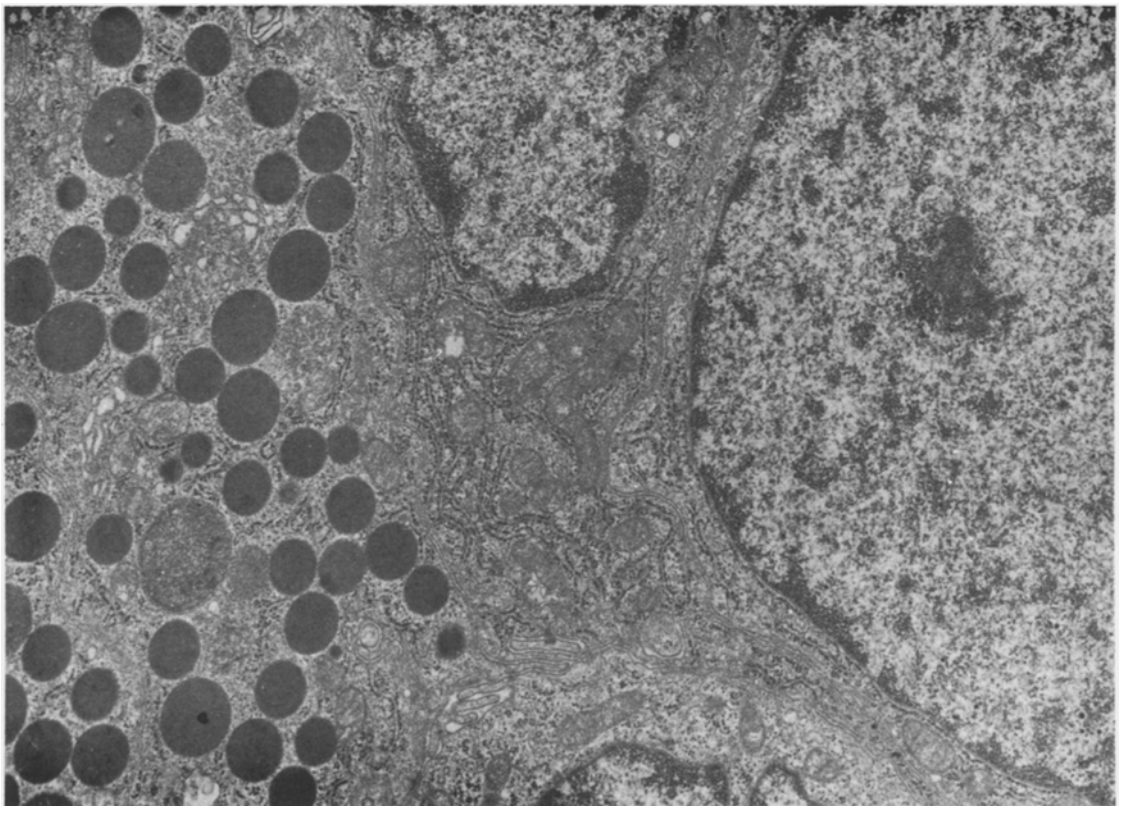

Fig. 3. Rough endoplasmic reticulum, Golgi apparatus, secretory granules, mitochondria (x 4100)

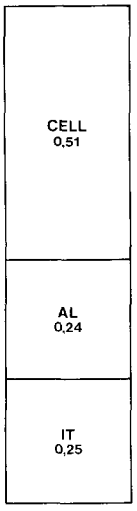

Fig. 4

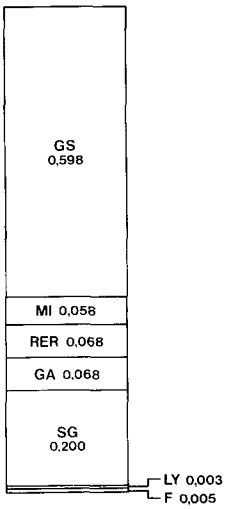

Fig. 5

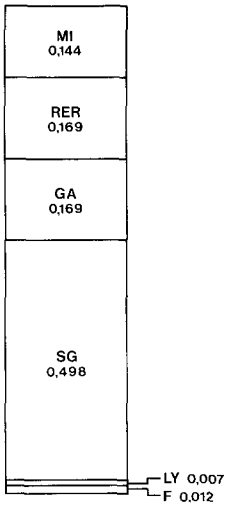

Fig. 6

Fig. 4. Tissue components of the dog prostate expressed as percentage of the total prostatic gland volume

Fig. 5. Volumes of the glandular cell compartments (cytoplasm, nucleus) expressed as a percentage of the total glandular cell cytoplasm volume

Fig. 6. Volumes of the glandular cell organelles expressed as percentage of the total glandular cell cytoplasm volume

the mean were calculated for the different parameters.

In a second step the above mentioned statistical parameters were calculated for the whole group.

RESULTS

Data for the whole prostatic gland, the acinar parenchyma, the acinar glandular cell and its various compartments are given in Tables 1 to 4 and Figs. 4 to 6. While in Table 1 the mean values of the parameters of the prostatic gland compartments are related to the unit volume of prostatic tissue (= 100%), Table 2 refers to the unit volume of glandular cell; Table 3 is related to the unit volume of glandular cell cytoplasm, whereas Table 4 refers to the unit volume of glandular cell organelles. Mean (m) and standard error (s.e.) are indicated in Tables 1 to 4.

Prostatic Gland

As shown in Fig. 4 and Table 1, the acinar parenchyma (defined as the sum of acinar lumina and glandular cells) contributed 75% to the whole prostatic gland. Whereas the acinar lumina represented 24% of the whole dog prostate, the glandular cell volume was calculated as 51%.

Table 1. Values per unit volume of prostatic gland tissue

Compartment	Parameter	Symbol	Unit	Mean	s.e.
Glandular cells	Volume	V_{VAC}	cm^3/cm^3	0.51	0.04
Acinar lumina	Volume	V_{VAL}	cm^3/cm^3	0.24	0.02
Interacinar tissue	Volume	V_{VIT}	cm^3/cm^3	0.25	0.05

Table 2. Values per unit volume of prostatic glandular cell

Compartment	Parameter	Symbol	Unit	Mean	s.e.
Cytoplasm	Volume	V_{VCYT}	cm^3/cm^3	0.81	0.11
Nucleus	Volume	V_{VN}	cm^3/cm^3	0.19	0.05

Table 3. Values per unit volume of prostatic glandular cell cytoplasm

Compartment	Parameter	Symbol	Unit	Mean	s.e.
Ground substance	Volume	V_{VGS}	cm^3/cm^3	0.59	0.07
Mitochondria	Volume	V_{VMI}	cm^3/cm^3	0.06	0.01
Rough endoplasmic reticulum	Volume	V_{VRER}	cm^3/cm^3	0.07	0.01
Golgi apparatus	Volume	V_{VGA}	cm^3/cm^3	0.07	0.02
Secretory granules	Volume	V_{VSG}	cm^3/cm^3	0.20	0.10
Lysosomes	Volume	V_{VLY}	cm^3/cm^3	0.01	0.001

Table 4. Values per unit volume of prostatic glandular cell organelles

Compartment	Parameter	Symbol	Unit	Mean	s.e.
Mitochondria	Volume	V_{VMI}	cm^3/cm^3	0.16	0.03
Rough endoplasmic reticulum	Volume	V_{VRER}	cm^3/cm^3	0.17	0.03
Golgi apparatus	Volume	V_{VGA}	cm^3/cm^3	0.17	0.06
Secretory granules	Volume	V_{VSG}	cm^3/cm^3	0.49	0.25
Lysosomes	Volume	V_{VLY}	cm^3/cm^3	0.01	0.001

The stromal tissue accounted for 25% of the whole gland volume.

Glandular Cell

Fig. 5 and Table 2 show the percentage of cytoplasm and cell nuclei per unit volume of prostatic tissue. Related to the unit volume of glandular cell, the cytoplasm contributed 81% and the nucleus 19%.

Cell Compartments

As shown in Fig. 6 and Table 3, the rough endoplasmic reticulum as well as the Golgi apparatus represented 7% of the unit volume of cytoplasm. The compartment of secretory granules amounted to a volume density of 20%, whereas the volume density of the mitochondria was 6%. The volume density of lysosomes and fat droplets was found to be less than 1% of the cytoplasm. Related to the unit volume of glandular cell organelles (Fig. 5 and Table 4) the secretory granules represented 49% of the cell, the rough endoplasmic reticulum represented 17%.

DISCUSSION

Our data show that it is possible to apply the stereological procedures devised by Weibel (11) and Rohr et al. (8) to determine quantitative morphological data of the whole prostatic gland, its glandular cells and its various compartments. A comparison of our data with the literature is not possible since there are no other quantitative data available for the dog prostatic gland.

The light microscopic analysis shows clearly the glandular character of the dog prostate. The dog prostate (related to the unit volume of prostatic tissue = 100%) consists of 75% acinar parenchyma. A division of the acini into their 2 compartments (acinar lumina and glandular cells) indicates that the glandular cells comprise more than half the volume (51%) of the whole prostate. In comparing the light microscopic analysis of the rat ventral prostatic lobe with that of the dog prostate, the volume of glandular cells in the dog prostate is twice that of the rat (1). In the normal human prostate the volume density of the acinar parenchyma (= glandular part) was calculated to be 55% in the outer part and 48% in the inner part of the prostate (2). Regarding the stromal tissue, there is no difference between the dog and the

ventral prostatic lobe of the rat, but a striking difference in the volume of the stromal tissue in the normal human prostate (inner part: 55%, outer part: 45%) (2).

The great density of the secretory granules, rough endoplasmic reticulum and the Golgi apparatus indicates the main function of the glandular cell: protein and enzyme synthesis and secretion. In comparing these data again with the rat ventral prostatic lobe and the human prostate, in the rat a higher amount and in the normal human prostate a smaller amount of rough endoplasmic reticulum can be shown. Conversely, the amount of the secretory granules is higher in the dog than in the rat. The most striking characteristic of the glandular cells of the human prostate is the presence of large numbers of secretory vacuoles which contain polymorphic material (42% of the whole cytoplasm); conversely in the ventral lobe of the rat prostate the volume of the secretory droplets (counted together with the lysosomes) is low (3%).

Defining a relationship between morphological and biochemical data permits the determination from intact tissue of relative amounts of individual membrane systems within a total membrane component in a given homogenate. For example, a microsomal fraction of dog prostate tissue containing rough endoplasmic reticulum and Golgi apparatus in the normal gland would be expected to contain a volume fraction of about 50% rough endoplasmic reticulum and 50% Golgi apparatus. In extending this concept under some restrictions quantitative data also should be obtained from homogenates; thus a comparison of these stereological data of the homogenates with the biochemical data of the cell fractions should be possible

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