

# Lectin histochemistry of normal and neoplastic canine testicle

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balance by a combination of complementary actions. Although in man and rat the ultrastructural appearance and distribution of atrial specific granules have been well described,<sup>2</sup> less morphological data are available for other mammalian species. In order to better characterize the ultrastructural features of normal atrial myoendocrine cardiac cells and to obtain morphological data on the morphology and the distribution of atrial specific granules during normal condition in cattle, pig and rabbit, 19 samples (5 from cattle, 8 from pig and 4 from rabbit) were taken from the right and left auricular appendages and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series and embedded in an Epon-Araldite mixture. Thin sections were stained with uranyl citrate and lead citrate and photographed with a Zeiss EM 109 with transfiber-optic photography.

The general morphology of atrial myoendocrine cells did not differ from that of ventricular myocardium. However, atrial specific granules were readily found in almost all auricular myoendocrine cells. Electron microscopic observation revealed that the peculiar location of atrial specific granules in all samples was the sarcoplasmic cone adjacent to the nuclear poles (Fig. 1). However, not infrequently they were scattered between myofibrils in close relationship with mit-

ochondria and in proximity to sarcolemma and the T-tubule system. Mature granules, also referred als A-granules, contained a highly osmiophilic and electron-dense material surrounded by a membrane (Fig. 2). Frequently, between the dense core and the membrane a fine electron-lucent halo could be shown. The diameter of mature granules ranged from 200 to 300 nm in all species tested.

In some cells smaller progranules could be observed within cisternae of Golgi apparatus in close relationship with mature granules (Fig. 2).

No noticeable differences between the three species tested were detected.

The ultrastructural features of atrial specific granules in cattle, pig and rabbit described in this paper will be useful to compare the morphological picture of several pathological states of the endocrine heart.

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**LECTIN HISTOCHEMISTRY OF NORMAL AND NEOPLASTIC CANINE TESTICLE**

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In male reproductive system of different mammalian species, lectin histochemistry has been used to localize specific domains on spermatogenic cells during differentiation and maturation, and on Sertoli cells<sup>1,2</sup>. Moreover, in human neoplastic pathology, lectins have been employed to investigate the nature, the origin of several germ cell tumors<sup>3</sup>, and to distinguish different types of seminoma<sup>4,5</sup>. In order to characterize saccharide components of complex glycoconjugates in different tumours of the canine testicle and in an attempt to follow the fate of different cell types after neoplastic transformation, we have undertaken a study by means of 12 different biotinylated lectins (PNA, Con-A, DBA, SBA, GS-I, LCA, LEA, PWM, RCA-I, RCA-II, WGA and UEA-I) and the avidin-biotin-peroxidase method (ABC) on four normal canine testicles, five seminomas, five Sertoli cell- and five Leydig cell-tumours. All samples were selected from the files of our department. PNA, DBA and SBA were also incubated after neuraminidase treatment (PNA-N, DBA-N, SBA-N).

In normal canine testis, biotinylated lectins were divided into four groups according to their binding pattern with spermatogenic cells: group 1 (Con-A, LCA, LEA, RCA-II and WGA), reacting with all spermatogenic cells; group 2 (PNA-N and RCA-I), binding to spermatocytes, spermatids, and spermatozoa; group 3 (GS-I, PNA, PWM, SBA and SBA-N), staining spermatids and spermatozoa; group 4 (DBA, DBA-N and UEA-I) which did not stain any spermatogenic cells.

Sertoli and Leydig cells were stained only with six out of the 12 lectins used (Con-A, WGA, LEA, RCA-II, LCA and RCA-I). All the

positive lectins belong to the first and the second group. Eight lectins bound to the tumours tested (Table 1).

Six lectins (Con-A, WGA, LEA, RCA-I, PNA-N and SBA-N) bound to neoplastic cells like in normal conditions. While Con-A, WGA, LEA and RCA-I bound to all neoplasms tested, PNA-N and SBA-N stained only the neoplasms arising from the spermatogenic line. It is noteworthy that none of the lectins which selectively reacted with

Table 1. Distribution of lectin binding sites on normal and neoplastic spermatogenic, Sertoli and Leydig cells.

Lectin used	Spermatogenic Cells		Sertoli Cells		Leydig Cells	
	A*	B	A	B	B	
Con-A	1	5/5	4/4	5/5	4/4	5/5
WGA	1	5/5	4/4	5/5	4/4	5/5
LEA	1	5/5	4/4	5/5	4/4	5/5
RCA-I	2	5/5	4/4	5/5	4/4	5/5
RCA-II	1	5/5	4/4	0/5	4/4	5/5
LCA	1	5/5	4/4	0/5	4/4	5/5
PNA-N	2	5/5	0/4	0/5	0/4	0/5
SBA-N	3	2/5	0/4	0/5	0/4	0/5

A = normal; B = neoplastic

\* The number is referred to the group according to the binding pattern with spermatogenic cells.

Lectins which do not stain any type of tumor are omitted.

spermatogonia in normal testis stained any cells in seminomas. These data suggest that seminomas do not contain spermatogonia. Furthermore, except SBA-N, which reacted only with two seminomas, none of the lectins that bound to postmeiotic spermatogenic cells in the normal testis reacted with any cells in seminoma.

Finally, RCA-II and LCA did not stain Sertoli cell tumours even though they bind to Sertoli cells in normal conditions. These preliminary results suggest that lectin histochemistry may be useful for the differential diagnosis of neoplastic lesions of dog testicle. However, further studies on a higher number of cases and on tumours with

intermediate differentiation and/or in combined tumours with different degrees of intermingling of cells are needed to confirm our observations.

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### IMMUNOHISTOLOGICAL STUDY ON KIDNEYS FROM DOGS WITH SPONTANEOUS LEISHMANIASIS

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The canine leishmaniasis is an endemic disease in the Mediterranean area. In our department we receive numerous patients, that are, most of them, sacrificed.

From the decade of the 70's on we began the study on the renal lesions as this organ is one of the most affected by the disease, according to Millin et al. (1975), Castaño et al. (1981) and Poli et al. (1989).

Most of the authors (Poli et al., 1986; Castano et al., 1988; Pizarini (1989), say that the most frequent lesion observed is a membranoproliferative glomerulonephritis (MPGN).

We would like to study the pathogenesis of the process and analyze the possible relationship between the glomerular lesions and the deposits of IgG, IgA, IgM and C<sub>3</sub> in leishmaniasis.

### Material and methods

We used 16 dogs between 3 and 6 years old, positive 1/400 with IFI techniques.

Once the necropsies were carried out, the renal samples were fixed in 10% formalin and Bouin's solution and embedded in paraffin and methacrylate. The samples were stained with H-E, PAS, Silver methenamine and PAP methods. Anticanine IgG, IgA, IgM and C<sub>3</sub> were used. We carried positive and negative controls.

For the ultrastructural study the tissues were fixed in 3% Milloning-buffered glutaraldehyde, pH 7.3 and post-fixed in 1% osmium tetroxide, embedded in epon-araldite and stained with lead citrate and uranyl acetate.

### Results and discussion

We divided the different forms of glomerulonephritis into 4 types: Minimal changes, MPGN focal and segmental, MPGN type I and MPGN type II; they were represented in proportions of 12%, 24%, 42% and 22% respectively. (Fig. 1)

Kidneys of the dogs with minimal changes had very little morphological modifications, we only observed a small increase of the mesangial area. In this case we detected glomerular deposits of IgM and C<sub>3</sub> of medium degree; they were negative for IgA and IgM, like Churg and Sobin (1982) observed.

The MPGN focal and segmental was characterized by a partial increase of the mesangial area and hypertrophy of the basement

membranes. In the immunohistological studies we observed intramembranous deposits of IgG and C<sub>3</sub>. In the chronic process IgM

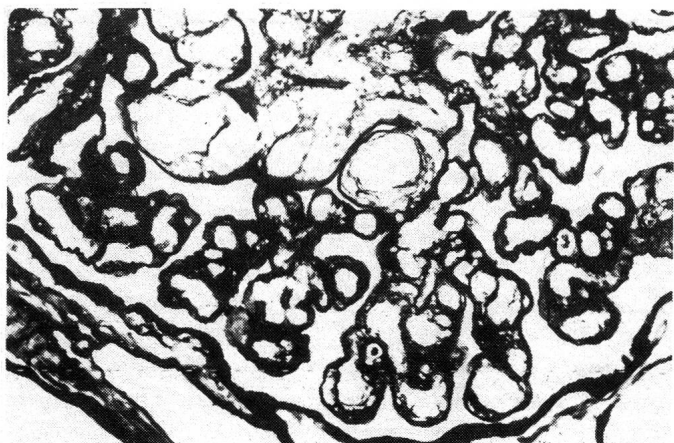


Fig. 1: MPGN type I. Double contour of basement membrane. Silver Methenamine. 40X.

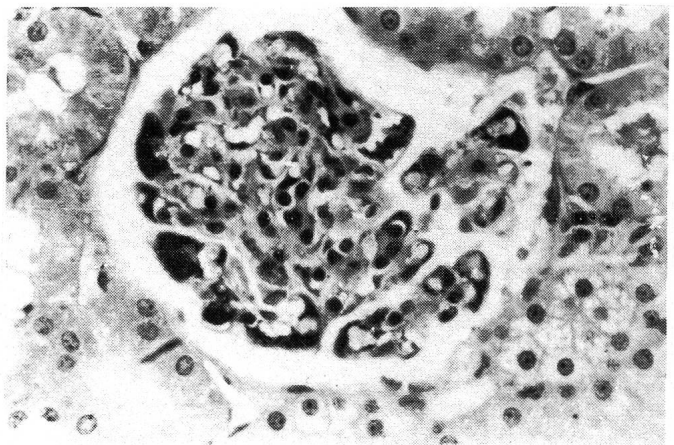


Fig. 2: MPGN: Heavy deposits of IgM. PAP Method. 25x.