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HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF HARDERIAN GLAND, LACRIMAL GLAND AND BURSA OF FABRICIUS IN MULARD DUCKS (*ANAS STERILIS*) WITH CHLAMYDIAL INFECTION

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Summary

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Specimens from the Harderian gland, the lacrimal gland and bursa of Fabricius in ducks with chlamydiosis were processed by the classical histological methods and permanent preparations were obtained. On single and serial histological cross-sections from material fixed in non-aqueous fixator, histochemical tests for PAS reactivity, staining with alcian blue at pH 1.0 and 2.5 as well as staining with Mowry's alcian blue followed by PAS reaction were performed. The light microscopic observation did not reveal considerable changes in the lacrimal gland. Structural and functional changes with various character and degree in the central lobular parts of the Harderian gland, the epithelium, the propria and thymus-like lobules of the bursa of Fabricius were observed. The histochemical analyses showed that the secretion of intraorbital glands in Mulard ducks was with a heteropolysaccharide nature, similarly to that in other waterfowl.

Key words: bursa of Fabricius, chlamydiosis, Harderian gland, histochemistry, histological structure, lacrimal gland, Mulard duck

INTRODUCTION

Chlamydiosis is an infectious disease on domestic and wild birds that is also transmitted to man. According to Obreshkov *et al.* (1978), the disease was discovered in Bulgaria by various authors in parrots, in hens, ducks and the personnel of a poultry slaughterhouse and in pigeons. By now, it is accepted that the chlamydial infection is a natural borne disease and that its classical clinical signs are manifested under the influence of favorable factors. Various avian species could be vectors of the disease.

After the finding that the Harderian gland in birds produces or participates in the transmission of three classes of immunoglobulins (Albini *et al*., 1974; Gallego *et al*., 1992a; Gallego *et al*., 1992b; Baba *et al*., 1996; Ohshima & Hiramatsu, 2003), many authors declared it as responsible for the local immunity performance in the eye orbit. At the same time, it is accepted that the gland is a peripheral lymphoepithelial organ that together with the spleen, the bursa of Fabricius and the caecal tonsils form a system of avian organs that determines both the general and the local immunity (Mueller *et al*., 1971; Vrakin & Sidorova, 1984; Payne, 1994; Shirama *et al*., 1996; Korbel *et al*., 1997;

Fix & Arp, 1998). In the specialized literature there are no reports about the structural or functional status of the bursa of Fabricius and the intraorbital glands in birds with chlamydial infections. Also, data about the structural features and the type of secretions of the lacrimal and Harderian glands of Mulard ducks are lacking.

This motivated us to use biological material from a duck farm in the region of Stara Zagora with a spontaneous outbreak of chlamydiosis aiming to determine the type of secretions released by the lacrimal and Harderian glands via histochemical tests and light microscopy and to check out whether there were chlamydiae-induced structural and functional changes in intraorbital glands and the bursa of Fabricius in Mulard ducks.

MATERIALS AND METHODS

The present study was performed on 18 Mulard ducks with chlamydiosis, confirmed by clinical, pathoanatomical, haematological and microbiological examinations. Material was obtained from 10 dead Mulard ducks and from 8 live birds with most evident clinical symptoms. The Harderian and lacrimal glands were carefully separated from surrounding structures and tissues according to the method of Aitken and Survashe (1976). The intraorbital glands and the samples from the bursae of Fabricius were fixed in 10% neutral formalin, in Bouin's and Carnoy's fixation liquids. The fixed material was embedded in paraffine using classical histological techniques. Single and serial cross sections (5 μm) were prepared. One part of sections were stained with haematoxylin (Erlich)-eosin and protected with cover glasses for making permanent histological preparations. The other histological sections were submitted to histochemical reactions for detection and differentiation of polysaccharide content. The PAS reaction, staining with alcian blue at pH 1.0 and 2.5 (Everson Pearse, 1962), as well as with Mowry's alcian blue followed by PAS were used (Lilie,1965).

RESULTS

The histological examination of the *lacrimal gland* in Mulard ducks with chlamydial infection revealed relatively few deviations from the microstructural model of this organ, determined as a complicated tubuloacinous gland, presented for the first time by Burns (1976). Its connective tissue skeleton (capsule, interlobular and interstitial tissues) did not exhibit any particularities. In several birds however, interlobularly or within the interstitium of the lobular connective tissue, normally diffuse and less commonly, microlymphocytic clusters were observed. The glandular epithelial cells of acini were with clear, sometime vacuolized cytoplasm. Within, single basophil granules most commonly located near the free end of cells could be often observed, and in some of them their apical surface was covered with a thin basophil layer. The epithelium, lining the complex system of tertiary, secondary and primary tubules, varying from prysmatic to cubical, differed from the epithelium of acini. Its cells were rarely and in a lower degree vacuolized and their cytoplasm was eosinophilously stained. The secretory activity of the epithelium of the entire system of acini and tubules was preserved and very often, the lumen of studied structures was fill with secretion. Sometimes, the secretion was mixed with small amounts of desquamated material but most commonly, it was found out inde-

Table 1. Histochemical reactivity of mucoid substances in intraorbital glands and the bursa of Fabricius of ducks with chlamydial infection

Method	Acini	Channels			Connective tissue	Glandular crypts	Follicles
		Tertiary	Secondary	Primary			
Lacrimal gland							
PAS	+	+	±	±	±	0	0
MAB/PAS	++	++	+++	+++	±	0	0
AB pH 1.0	–	–	±	±	–	0	0
AB pH 2.5	++	++	+++	+++	±	0	0
Harderian gland							
PAS	0	–	–	±	±	0	0
MAB/PAS	0	–	±	+++	±	0	0
AB pH 1.0	0	–	±	++	–	0	0
AB pH 2.5	0	–	±	+++	±	0	0
Bursa of Fabricius							
PAS	0	0	0	0	±	–	–
MAB/PAS	0	0	0	0	±	+±+	±
AB pH 1.0	0	0	0	0	–	±	–
AB pH 2.5	0	0	0	0	±	+±+	±

PAS = periodic acid – Schif; MAB = Mowry's alcian blue; AB = alcian blue; (–) = lack of effect; (+) = weak reactivity; (++)= medium reactivity; (+++) = good reactivity.

pendently into the lumen and then, it had a foamy appearance.

The performed histochemical studies (Table 1) showed a weak PAS reactivity. A very weak positive PAS reaction was exhibited only by single cells, localized mainly adjacently to the diffuse microlymphocytic clusters (observed in the interlobular or the interstitial connective tissue) or entering rarely into the composition of these cell clusters. The same degree of reactivity was observed among segments of lumen contents of acini and tertiary tubules whereas in secondary and primary tubules it was found out only in some birds. The other histochemical stainings showed a weak to moderate differentiated reactivity of both epithelial cells and lumen content in acini and in the different types of tubules. The tests showed that the composition of lacrimal gland secretion in Mulard ducks was mixed with predominantly sialomucin content (Fig. 1).

The connective tissue capsule covering the *Harderian gland* in studied Mulard ducks was usually with unchanged histological structure. In most glands, the interlobular tissue surrounding the lobules with different size and with polygonal shape was with preserved structure. Only in some birds (those with the most clear clinical manifestation of the disease), diffuse or local lymphoid cell clusters were

Fig. 1. Lacrimal gland. Glandular lobes without histostructural changes with well expressed secretory activity. MAB/PAS staining; bar =10 μm.

Fig 2. Harderian gland. Area of a glandular lobule with well visible alcianophilia in the central zone; peripherally, the secondary and tertiary tubules into the lobule do not show a staining reactivity. MAB/PAS staining; bar =5 μm.

Fig. 3. Harderian gland. Central lobular zone with total lymphoid cellular and pleiomorphocellular infiltration. Rarely encountered small alcianophilic secretory islets are also visible. MAB/PAS staining; bar=10 μm.

discovered. In the thin streaks of interstitial connective tissue staying among the compactly situated tubules of the compound tubulous gland, changes were commonly not present. Only in cases with interlobular lymphoid cell clusters, similar groups were present among the interstitial interlobular connective tissue as well. The single-layer prysmatic epithelium lining the complex system of tertiary, secondary and primary tubules as well as the highly branched furcations of the end parts of primary tubules prominating towards the lumen of the central channel, was in a different structural and functional state. The glandular epithelial cells of tertiary tubules, located in lobule's periphery and those of most secondary tubules were with similar intensity of staining. Most frequently, their big oval nucleus was located near to cellular base and their cytoplasm was clear. It was stained slightly eosinophilously and in some cells it contained small amounts of diffusely located basophilic granules. The margins of the apical cutting surface were free of secretory material and were clearly differentiated under a light microscope. Unlike them, the eosinophilic staining of glandular epithelial cells located centrally in the lobule (covering the primary tubules and their furcations in the central lobular channel) was more intense. The free cell margins in those areas were covered by a basophilously stained layer with different thickness. The secretory activity of glandular epithelial cells was preserved but in a different degree according to their localization in the glandular lobule. Into the peripheral parts of the lobules there was a very poor or no secretion in the tertiary and some of secondary tubules, whereas in primary ones and their furcations prominating towards the central channel, it was very well expressed (Fig. 2). In

areas with slight secretion, the apical surface of some epithelial cells was covered by a thin, barely visible basophilic layer. At sites where the intensely basophilously stained secretion was located independently, it had a foamy appearance. Very frequently, the secretion in the lumen of the central lobular channel was mixed with desquamated eosinophilously stained cell fragments. In Harderian glands with described interlobular and interstitial lymphoid cell clusters (from birds with most clearly manifested clinical signs), the secretory activity was almost lacking. A similar morphological picture and lack of secretory activity was rarely observed in Harderian glands from Mulard ducks dead from chlamydiosis. In them, the secretory activity of the gland was rather preserved. In areas with active glandular secretion, threre were affected lobules with an almost complete infiltration of their central part with pleiomorphic and lymphoid cell clusters mixed with desquamation material. At sites where the epithelium of lobules was not yet desquamated, the cells were with necrobiotic changes. Into the wall and the lumen of the central channel of these lobules, alcianophilic secretory material was pictured (Fig. 3) as small hardly visualized islets (visible only after histochemical staining).

The histochemical tests on Harderian gland cross sections in Mulard ducks with chlamydial infection showed a PAS positive reaction of single cells among the cell clusters observed within the connective tissue glandular structures and the furcations prominating inside the central lobular duct. The histochemical reactions showed a high degree of alcianophilia in glandular epithelial cells from the central glandular parts whereas in the peripheral ones, only traces or no acid mucosubstances were present (Fig. 4). In all histochemical tests, the secretion from the central lobular ducts was mixed and consisted of sialomucins and sulfomucins (Table 1).

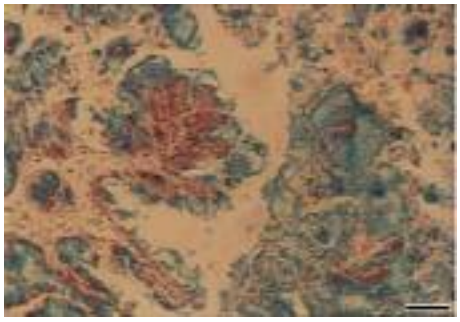

Fig. 4. Harderian gland. A well distinguished secretory activity in a lobular centre. Part of the desquamated cellular material contained in the secretion and some cells in the propria of the mucosal fold are slightly PAS positive. MAB/PAS staining; bar=10 μm.

The microscopical analysis of the *bursa of Fabricius* showed changes mainly in the mucosal layer whereas the histostructure of the musculature and the lining was normal. The changes affected mostly the epithelial and the own sublayers of *Tunica mucosa*. The changes were the best manifested in both big mucosal folds filling almost entirely the inner surface of the organ whereas in the lower and less numerous secondary leaflets of the mucosa, they were less commonly observed. The architectonics of the epithelial leaflet of the mucosa (composed of epithelial cells with various height in the different regions of the mucosa) was altered almost all over the organ. Most commonly, on the convexe areas of mucosal folds, the epithelium was desquamated at a different degree or was found out in the lumen of the organ under the form of clusters with a varying size. Rarely, only in areas of epithelial bends inward the propria (glandular crypts) the epithelium

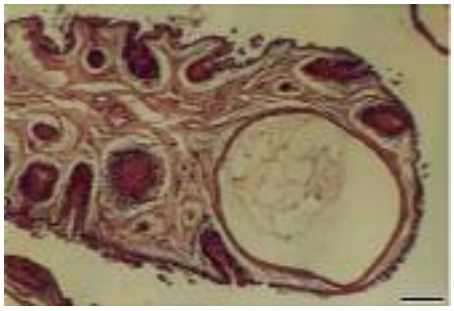

Fig. 5. Bursa of Fabricius. Subepithelial cystic formation with foamy content in the propria of a mucosal leaflet. H/E staining; bar = 5 μm.

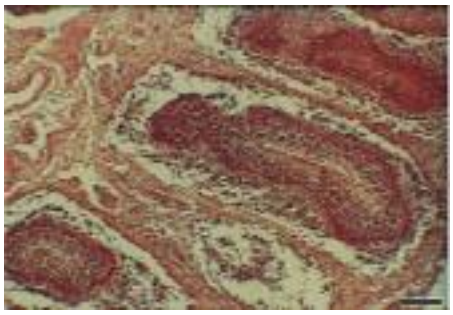

Fig. 6. Bursa of Fabricius. Thymus-like lobules with various degree of cellular reduction in the cortical part. H/E staining; bar=10 μm.

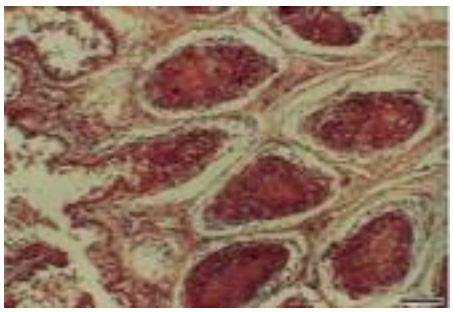

Fig. 7. Bursa of Fabricius. Lobules, whose core is necrotically changed, surrounded by an aureole of the missing cortex. H/E staining; bar=10 μm.

was hyperplastically changed and as a rule, with preserved secretory activity.

The performed histochemical tests revealed that the secretion in such area contained mainly acid nonsulfated and very few neutral mucopolysaccharides (Table 1). Capillaries, small arteries and veins (in a state of hyperaemia or with microperivasal haemorrhagic foci) were encountered among the loose connective tissue of the mucosal propria. Nearby, in tissue cleft-like spaces, diffuse pleiomorphic clusters were most commonly situated. On the background of the pale stained surrounding connective tissue (slight reactivity obtained from the histochemical tests for polysaccharides), single cells within the clusters were PAS positive. Into the propria of both big mucosal folds, some cystic formations with a different size were subepithelially located (Fig. 5). In all of them, the wall was well constructed, multilayer and with a connective tissue composition. In most cases the findings were with a luminal content with a foamy appearance and rarely, with a homogenous look. The content of the cystic formations was with a heteropolysaccharide nature similar to that of the glandular crypt secretion. The structure of the thymus-like lobules located into the propria were changed at a various extent. In most of them, the two zones of the lobule – cortex and medulla could be still distinguished. In lobules, where the cutting surface (delimiting both lobular parts) revealed the epithelium entering into the composition of the core zone, a weak secretory activity of epithelium, similar to that observed in glandular crypts was histochemically shown (Table 1). The cortex of these lobules was with strongly reduced cellular composition of small and medium lymphocytes thus determining a demarcation of a cell-free space between the cortical and the periglandular connective tissue (Fig. 6). A part of lobules protruded on

the observation field as embraced by the aureole of empty fields of the lacking cortical zone. The core of such lobules usually contained few cell elements, hardly typifiable and most frequently appearing as a dense, necrotically changed mass (Fig. 7).

DISCUSSION

The light microscopic study of Harderian and lacrimal glands, performed by us showed that in Mulard ducks with chlamydial infection the principal histological structure of those organs, described by several authors in various breeds and lines of ducks as well as other waterfowl (MacLeod., 1880; Ballantyne & Fourman, 1967; Brobby, 1972; Kühnel & Beier, 1973; Burns, 1976; Vrakin & Sidorova, 1984), was preserved. The histochemical tests confirmed that the secreting mode and the type of discharge of both intraorbital glands in ducks were identical with most findings in waterfowl, described by Brobby (1972), Kühnel & Beier (1973), Wight & Mackenzie (1974), Burns (1976) etc.

The analysis of the lacrimal gland in Mulard ducks revealed that chlamydial infection did not change significantly its histological structure. This is evidenced by the observed insignificant alterations in the organ as well as by the conserved secretory activity of all structural untis of the gland. The observed and described local or diffuse microlymphocytic clusters into the organ are not to be considered as pathognomic and therefore, consequent to the disease. This opinion of ours is supported by their sporadic appearance (only in some of studied birds), their microscopical size and their appearance only in the interlobular and interstitial connective glandular tissue, as reported by Burns (1976). The latter described for the first time the histological structure of the lacrimal gland in ducks and accepted the presence of similar cell clusters in the connective tissue skeleton of the organ as normal in this avian species.

The similar interlobular and interstitial lymphoid cell clusters observed by us in the other intraorbital (Harderian) gland and the described structural and functional changes in the central zone of lobules are in our opinion a resutls of the infection. This hypothesis is supported by the facts that necrobiotic changes, partial or total desquamation of the glandular epithelium, diffuse pleiomorphic infiltration and total lack of secretory activity were found out in the central lobular parts of Harderian glands in Mulard ducks with the most evident manifestation of the disease. Those structural and morphological changes were very rarely present in Harderian glands obtained from dead Mulard ducks. We assume that the different structural and functional reactivity of lacrimal and Harderian glands in Mulard ducks with chlamydial infections could be explained to a great extent by their different vascularization. Authors having studied avian intraorbital glands (Burns, 1976; Ballantyne & Fourman, 1967; Payne, 1994; Shirama *et al*., 1996 etc), report that the Harderian gland has a better developed and branched blood supply that could be a favorable factor for the spreading of the infectuous agents in the organ. Furthermore, Obreshkov *et al.* (1978) speculate that the infectious agent penetrates into the cells of the reticuloendothelial system. There, it reproduces, releases a toxin and causes haemorrhagies and necrotic foci in the spleen, liver, pericardium, the respiratory airways and conjuctives.

The similar structural changes that were present in our investigation of bursa of Fabricius in studied Mulard ducks are supporting and adding further to the thoughts of forementioned authors. Apart the perivasal microhaemorrhagic foci, the local and diffuse pleiomorphocellular clusters in the propria, the Mulard ducks with most evident clinical signs manifested cystic formations as well. In same birds, part of thymus-like lobules in mucosal propria were necrobiotically to necrotically changed. Accounting for the beliefs of authors that have studied the structure of the bursa of Fabricius both normally and following various treatments (Edwards *et al*., 1985; Ackerman, & Knoff, 1989; Quesada & Agulleiro, 1994; Strelnikov, 1996; Olah *et al*., 2001), we assume that altered lobules are consequent to processes affecting intially their cortex and then, the medullary zone. The discovery of necrobiotically changed cells in the deep cortical zones (the boundary between cortex and medulla is marked by a dense capillary network forming a fine membrane) was indicative for onset of pathogenesis into the lobule. Then followed reduction of cells in the cortex and only afterwards, necrotic processes in lobular core did occur.

On the basis of this study and our comments it could be concluded that in Mulard ducks with chlamydial infection, the structure of the function of lacrimal glands did not change considerably. Both the Harderian gland and the bursa of Fabricius changed structurally and functionally, the latter being more significantly altered.

The present study introduced for the first time histological and histochemical data about the intraorbital glands in Mulard ducks with chlamydial infection.

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