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The Gland of the Third Eyelid (Harderian gland) in the Broiler Chickens. II. Histological and Histochemical Peculiarities of the Draining Duct from the Hatching to the 56 Day of the Development

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180 Harderian glands obtained from 90 broiler chickens originating from a commercial stock, from both genders at the age of 1, 7, 14, 21, 28, 35, 42, 49 and 56 days were investigated. After the broiler's narcosis, decapitation and glandular preparation using the classical methods, durable histological specimens were prepared. It was conducted a histochemical staining for polysaccharides – glycogen, mucoproteides, glycoproteides and glycopeptides using a PAS reaction. With the use of a combined staining –MAB followed by a PAS reaction, were simultaneously visualised the acid and the other mucins. The differentiation of the acid sulphated and non sulphated glycoseamminoglycans was conducted with a histochemical staining at pH 1,0 and pH 2,5.

The lightmicroscopy analysis showed that at all the studied ages, the broiler Harderian gland possessed a single draining duct, beginning from its posterior end. In the proximal and distal segment the canal wall possesses lymphocytic agregations. Along all parts of the duct in all of the age groups the epithelium is actively secreting mucosubstances of acid nonsulphated and some neutral mucopolysacharides.

Key words: Harderian gland, broiler chickens, draining duct, histology, histochemistry.

Introduction

In the intraorbital glandular complex in birds, in contrast to the mammalian animal species, the dominating in size is the gland of the third eyelid, most commonly called Harderian, in honour of its discoverer. Long ago it was stated, that the Harderian gland in the birds is a place and a source of a lymphoid tissue and immune producing cells [3, 10]. Nowadays there are evidences, that the gland is a "center" for migrated through the blood flow, or a source of immune bodies [6, 11, 5, 13, 2, 7]. This scientific data makes the gland of the third eyelid in the birds a desired research biological object.

According to the nowadays accepted among the researchers of the Harderian gland classification, in the "birds kingdom" account in of over 1200 bird species, there exists

four types of Harderian glands. The domestic fowl and the galinacea bird species possess a complex tubuloacinous gland of the third eyelid.

In contrast to the well studied basic secretory structural elements of the Harderian gland – acins and different classes of secretory tubules, the draining duct of the gland is rarely mentioned in the specialized literature. Making an anatomical description of the gland in the domestic fowl, some authors mention some scarce morphological peculiarities of its draining duct [3, 16, 9]. In two consecutive experimental scientific studies the authors ligatures and drains the draining duct of the Harderian gland in fowls, but gives in their publications only a short description of the condition of the duct after the experiment conduction [14, 15].In the literature there is a lack of data about the microstructure of the duct. There is also a lack of information about the structural peculiarities of the draining duct of the gland in birds of the broiler type during their development.

In the present study we aimed, using the method of the light microscopy analysis, to determine the microstructural condition and some histochemical peculiarities of the draining duct of the broiler chicken's Harderian gland – from the hatching till the 56th day of the postnatal development.

Materials and Methods

Harderian glands of 90 stock broiler chickens – 1, 7, 14, 21, 28, 35, 42, 49 and 56 days old were used for the conduction of the present study. Every age group consisted of 5 male and 5 female birds. The glands of the third eyelid were taken from previously anaesthetized broiler chickens after decapitation followed by orbitotomy and a careful separation from the surrounding structures and tissues [1]. The obtained 180 Harderian glands were fixated in 10% water solution of neutral formaldehid and in the fixating mixtures of Bouen and Carnoa. After embedding in paraffin with the use of a microtome (Reichert Jung, Austria) were prepared a considerable number of longitodinal and transversal sections with a maximal thickness of 6 μm. From the formalin sectios after staining with Ehrlich' s hematoxylin and eosin and a polychrome Heidenhein's Azan, durable histological specimens were made [12]. Over a part of the sections fixated in the fixating mixture of Karnua we conducted a histochemical (PAS) – reaction for demonstrating the presence of polysaccharides, glycogen, mucoproteides, glycoproteides and glycolipids [12]. Over sections fixated in the fixating mixture of Carnoa was conducted a combined staining method using Mowris's alcian blue, followed immediately by PAS reaction, for the simultaneous staining of the acid and other mucins at pH 2.5 [8]. A selective differentiating staining with Alcian blue at pH 1.0 was conducted for determinating of the sulphated glucoseaminoglycans [12], also the staining with Alcian blue at pH2,5 for the differentiation of the acid nonsulphated glucoseaminoglycans [12].

Results and Discussion

According to the scarse literature data [16, 9], in the galinaceous bird species only one non pair canal leaves from the posterior end every Harderian gland (left and right), after which reaches the base of the third eyelid where drains the products generated in the gland. The examined from us large number of histological sections determined that the draining duct of the gland of the third eyelid in the stock broiler chickens leaves the gland also through its posterior pole. It begins in a different way in every bird. We came across findings, in which the duct began spontaneously among some of the last glandu-

lar lobules in the organ. In other cases some of the terminal lobules in the posterior end of the gland was "transformed" into a beginning of the draining duct.

Independently of this in what way the initial segment of the Harderian gland draining duct begins, in every age group of older chickens, its wall shows a similar structure throughaut the whole length of the duct. The duct was surrounded by a comparatively thin layer of connective tissue. The connective tissue coating was wrapped in a different thickness of adipose pillow throuhout the whale length ol the duct consistina of white adipose tissue. Although the precision of the conducted study in all age groups of birds was not determined the presence of a connective tissue capsule. With the help of the light microscopy analysis was determined in the duct tissue covering a presence of connective and white adipose tissue, well blood circulation and inervation, which can be treated as an indirect evidence for the duct functional load. Another common structural element, for which however we determined an age related dependency, were the diffuse or local concentrations of lymphocytes and lymphoid cells agregations. They were observed in the draining duct of the Harderian gland of the 4-week old broilers (28th day), and were present in almost every histo section of birds belonging to the last four age groups – 35th, 42nd, 49th and 56th day. If the draining duct of the broiler Harderian gland is conditionally divide along its length into a proximal (initial), medial (middle) and distal (terminal) parts beginning from the posterior glandular end to the conjunctiva, the lymphatic aggregations are mostly determined in the initial and terminal parts of the duct. Along with the local lymphatic aggregations along the whole length of the draining duct, were also diffuse intercellular lymphocytic infiltrations among the ducts epithelium.

Light microscopically was determined, that even in the old broiler chickens, the draining duct of the Harderian gland is with formed and activity functioning covering epithelium (Fig. 1). The epithelial cells covering the inner surface of the broiler Harderian gland draining duct varied in their shape along the ducts length – from cubic

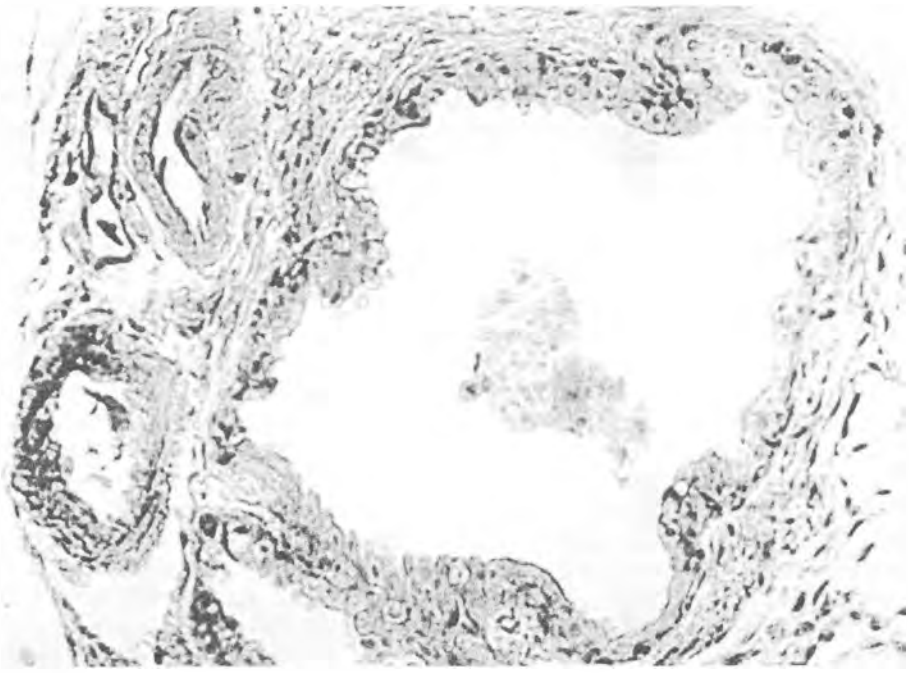

Fig. 1. Broiler chicken – first day. Transversal section from the opening segment of the draining duct, which is already covered with an actively functioning epithelium. Heidenhein's Azan, × 250

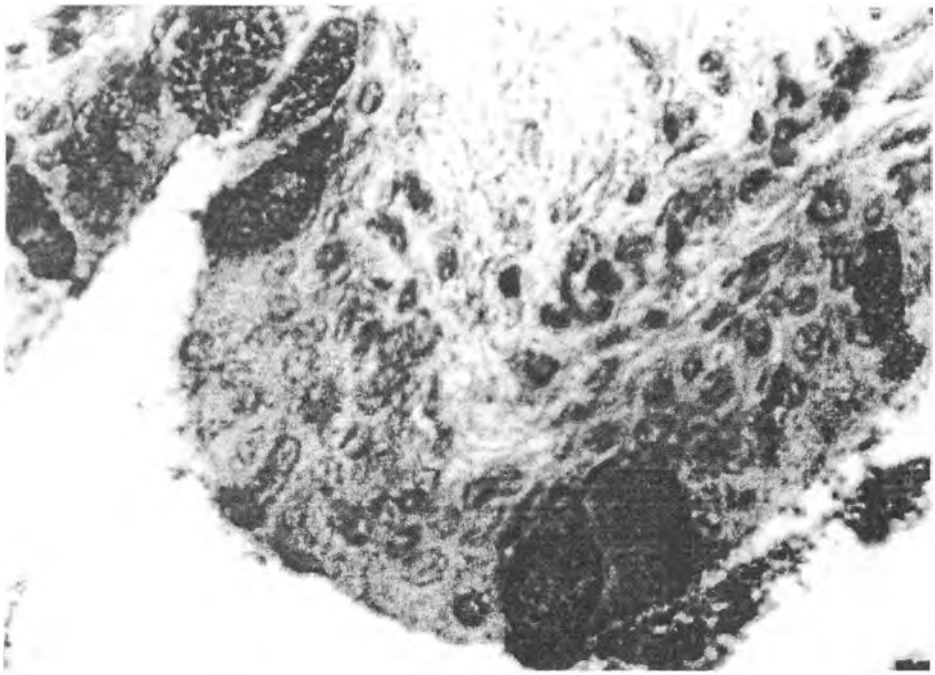

Fig. 2. Broiler chicken – 49th day. Goblet cells from the distal duct segment in different phases of secretion an secretion formation, demonstrating alcianophilie properties. MAB/PAS, × 650

to prismatic. The epithelial cells covering the inner surface of the broiler Harderian gland draining duct varied in their shape along the ducts length – from cubic to prismatic. The tipisation of the epithelium was impeded especially in the proximal and distal parts of the duct where its wall was sepeatedly folded. In the areas with folds and the spaces between them, the epithelium looked like it consisted of layers and was tipisated like a stratified. The segments of the duct with non folded, flat autline of the section surface, were covered with simple cuboidal, or simple columnar epithelium, demonstrating a secretory function. Simple cuboidal epithelium was also covering the lymphocytic aggregations, dividing them from the duct lumen. Among the epithelial cells lining the inner surface of the duct there where also goblet cells, whose quantisy was big in the proximal and distal parts, and very little in the middle segment of the duct. All the observed goblet cells were with high secretory activity.

In our study using the larger magnifications of the microscope we determinided that in all the parts of the duct, the epithelium is situated over a basal membrane in one layer of flat, mononuclear cells, whose nucleus is globe-shaped, big and light stained. The fact, that the previously described epithelium in all parts of the duct does not perform only covering functions was proved by the shown reactively with the different staining methodes. The conducted histochemical tests proved, that along the whole length of the duct surface, the epithelial cells are PAS – negative, which is an argument to treat them as mucus – secreting cells. When stained with Mowris alcian blue with pH1.0, the epithelium reacted extremlly weak, and in most areas was not detected reactivity at all. When the staining medium (Alcian blue) is with pH2.5 – the shown reactivity was mainly medium expressed. The best pronounced reactivity was observed after a combined staining with a MAB, followed immediately by a PAS reaction (Fig. 2).

These results gives us a reason to think, that the mucosubstances secreted in the draining duct of the stock broiler chickens Harderian gland possesses a mixed composition – mainly acid non sulphated and a little quantity of neutral glucoseaminnoglycans.

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