



### Original Contribution

## ENCAPSULATED NERVE ENDINGS IN THE LACHRYMAL GLANDS OF BROILER CHICKENS – A LIGHT MICROSCOPY STUDY

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### ABSTRACT

The study was performed on 180 lachrymal glands, obtained from 90 stock broiler chickens from both genders at the age of 1, 7, 14, 21, 28, 35, 42, 49 and 56 days. Following anaesthesia, decapitation and dissection of the glands using the classical methods, durable histological specimens were prepared. The light microscopy revealed encapsulated nerve endings in the lachrymal glands of three chickens. Histologically, we determined those structures as capsulate lamellar sense endings and one of them - as a compound sense body. Micrometrical studies on nerve corpuscles were also performed and the results were statistically processed. The dimensions of corpuscles were 67.22  $\mu$ m/78.06  $\mu$ m; 72.18  $\mu$ m/105.22  $\mu$ m and 95.2  $\mu$ m/138.26  $\mu$ m. We consider that this is the first report giving an evidence for the presence of encapsulated nerve endings in the lachrymal glands of broiler chickens.

**Key words:** Broiler chickens, Encapsulated nerve corpuscles, Lachrymal gland

### INTRODUCTION

Almost all vertebrates have a lachrymal gland (1). In birds, *Glandula membranae nictitans* (Harderian gland) is dominating by its dimensions while the lachrymal gland is the less developed (2, 3). According to Burns (4) who presented the first histological description of the lachrymal gland in domestic fowl and duck, it is covered by a thin connective tissue capsule entering the parenchyma as a interlobular tissue, forming lobules with a different shape and size within. Burns (4) as well as other investigators have determined the lachrymal glands in *gallinaceous* birds as a compound tubular acinose gland. The references about the presence of free nerve endings in the lachrymal gland are predominating. Only three research groups have reported the presence of capsulate nerve endings in the region of the eye, however, these reports concerned structures, adjacent to the lachrymal gland. Nestorov and Ivanov (5) described Krause's end bulbs and Golgi-Mazzoni's corpuscles in the eyelid conjunctiva in birds. Halata and Munger (6)

described three types of nerve endings, one of them being the Ruffini's corpuscles in the eyelids of primates. Lawrenson and Ruskell (7) observed the structure of corpuscular nerve endings in the end (limbic) conjunctiva of human eye. However, there are no reports for the presence of encapsulated nerve endings in the lachrymal gland of any animal species.

Taking into account the fact that the structure of the lachrymal gland in broiler chickens is insufficiently investigated, as well as the lack of evidence for the presence of encapsulated nerve endings within, we aimed to establish their presence or absence in the lachrymal gland of stock broiler chickens by light microscopy, to describe their location, structure and to make an attempt for their determination.

### MATERIALS AND METHODS

Lachrymal glands from stock broiler chickens of both genders at the age of 1, 7, 14, 21, 28, 35, 42, 49 and 56 days were used. The donor birds for each age group were 5 male and 5 female. The glands were obtained after halothane anaesthesia and decapitation of the birds followed by dissection of the glene, careful removal of glands from the surrounding structures and tissues according to the method of Aitken and Survache (8).

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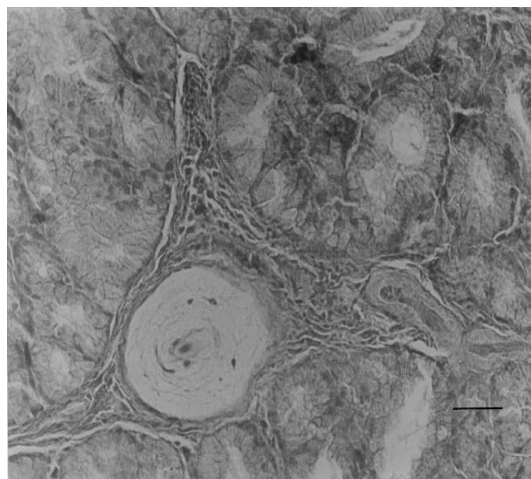
The experiments with animals were conformed according to the Act and Code of Practice for the Housing and Care of Animals used in Scientific Procedures (1986; 1989). The specimens were fixed in 10% neutral formaldehyde, formal-saline, Bouin's and Carnoy's liquids. After embedding in paraffine, a minimum of 5 serial and single (5  $\mu\text{m}$ ) sections were prepared from the left and right lachrymal glands of each bird in each age group. Following staining with haematoxylin (Erlisch)-eosin, azan polychromic staining (according to Heidenhain) and impregnation with silver nitrate, durable histological specimens were prepared (9). The micrometric parameters of the encapsulated sense bodies were determined by a light microscope (Ergaval) and a micrometer eye-piece (Zeiss) by the method of Avtandilov (10). The data from all histological sections were statistically processed (11). The light microscopy of lachrymal glands and the microphotography of encapsulated receptors were performed with a universal microscope NU-2 (Carl Zeiss, Jena).

## RESULTS

In our study, encapsulated nerve endings (ENE) were observed only in the lachrymal glands of three birds from different age groups. In the first case, adjacently to the outer surface of the left lachrymal gland, we fell upon a transversally cut ENE near a small arteriole in the interlobular tissue of a 42-day old bird (Figure 1).

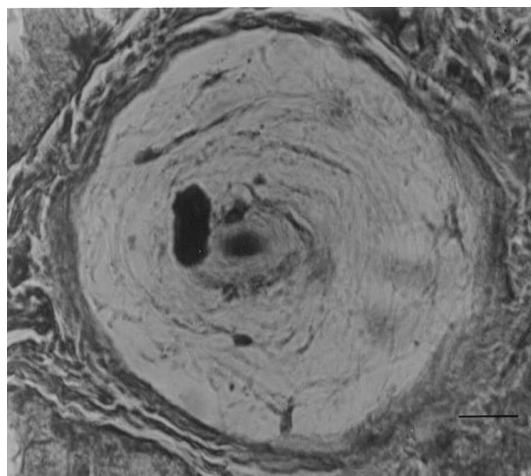
The measurement of the corpuscle in all histological sections using the micrometer eye-piece, yielded average dimensions of 78.06/67.22  $\mu\text{m}$ . The outer surface of the body was covered by a thin connective tissue capsule (containing 6-8 layers in the different cross-sections). In the central area, stained in red (or black after impregnation), we observed the cross-section of a central sensory nerve fibre. Around, there was a hardly visible, gray-bluish, extremely thin homogenous layer resembling an inner gliose bulb in encapsulated sense bodies (ESB). Directly above it, without a sharp delineation, a layer composed by concentrically situated structural elements was visible, resembling a configuration of a lamellar type. It contained a small number of nuclei (2-3) with an oval shape and stained in red, resembling those of leucocytes, while its outer cross-section was

lined by elongated dark-stained fibrocyte-like nuclei.



**Figure 1.** Lachrymal gland of a 42-day-old broiler chicken. A transversally cut encapsulated receptor endings among the interlobular connective tissue. Bar = 10  $\mu\text{m}$ . Azan (according to Heidenhain).

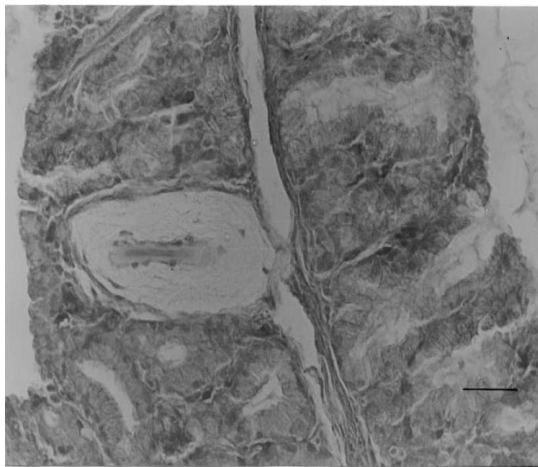
The interspace between the described centrally located structures and the covering connective tissue capsule was filled with concentrically layered thin lamellar structures, cut in a different manner. Among them, upon the cutting surface, the dark-colored nuclei of fibrocyte-like cells with elongated shape and single oval red-colored nuclei of neurogliocytes were visible. The magnifications over  $650\times$  showed thin nerve branches resembling red (or black after impregnation) threads found in almost all regions of the body (Figure 2).



**Figure 2.** A transversally cut corpuscle in the lachrymal gland of a 42-day-old broiler chicken. Next to the central nerve fiber, an identically stained section (possibly through the end top part of the fiber). Bar = 20  $\mu\text{m}$ . Azan (according to Heidenhain).

In the second case, an almost longitudinally cut corpuscle was found out in the left lachrymal gland of a 49-day-old broiler.

It was localized among the acini and tubules of a glandular lobule in such a manner that its first pole reached the main channel nearly subepithelially, while the other pole was in contact with the elements of the interlobular connective tissue (Figure 3).



**Figure 3.** Lachrymal gland of a 49-day-old broiler chicken. A longitudinally cut body among the tubules and acini of a glandular lobule. Bar = 10  $\mu\text{m}$ . Azan (according to Heidenhain).

The micrometrical study determined average dimensions of the corpuscle 105.22  $\mu\text{m}$ /72.18  $\mu\text{m}$ . Its structure was built of the same elements as in the first described ENE but here, the central nerve fiber and the inner gliose bulb with bigger dimensions (that was in a close proximity), were better visible at the cutting surface. The fine network of thin red-colored nerve branches (or black after impregnation) involving almost all regions of the corpuscle, was well-developed.

The third ESB was found out in the right lachrymal gland of a 56-days old bird. Its localization was similar to that in the second case. It was situated among the structural elements of the glandular tissue. The one pole of the body reached beneath the epithelium, covering the main channel of the lobule, while the other pole touched the interlobular space (Figure 4).

The micrometry gave average dimensions of 138.26  $\mu\text{m}$ /95.25  $\mu\text{m}$ . The structural analysis showed that the receptor nerve ending in our third case possessed structural elements giving grounds to determine it as an encapsulated compound sense body of a lamellar type. On the cutting surfaces of all histological cross-sections, a common thin (up to 6 layers) connective tissue capsule covering two almost transversally cut corpuscles was clearly outlined. They were of a different size (46.08  $\mu\text{m}$ /31.75  $\mu\text{m}$  for the smaller and 69.13  $\mu\text{m}$ /47.62  $\mu\text{m}$  for the bigger ones) and their location, described in the first

transversally cut corpuscle was excellently presented in each of the structural elements of the complex capsulated lamellar corpuscle.



**Figure 4.** A compound encapsulated lamellar sense ending in the lachrymal gland of a 56-day-old broiler chicken. Both parts of the compound body are almost transversally cut. Bar = 10  $\mu\text{m}$ . Azan (according to Heidenhain).

## DISCUSSION

The reference data that could be used for comparison with our results, are numerous reports upon the incidence, structure or functions of the capsulated nerve endings in different animal species. The present scientific concepts are that the typical representatives of the lamellar type of capsulated nerve receptors are the Vater-Pacinian and Herbst's corpuscles. Furthermore, in birds, apart from Herbst's corpuscles, those of Grandri's are accepted as specific as well. The Vater-Pacinian corpuscles could be with various dimensions - the biggest ones, reaching several millimeters, could be observed with a naked eye. For the first time, Herbst's corpuscles of various size were described by Halata (12) in aquatic birds. Later, Herbst's corpuscles in the joint capsule of the pigeon (13) and the beak of the Japanese quail (14) were categorized into small-sized (5  $\mu\text{m}$ /100  $\mu\text{m}$ ), medium-sized (80  $\mu\text{m}$ /200  $\mu\text{m}$ ) and large-sized (100  $\mu\text{m}$ /600  $\mu\text{m}$ ). Herbst's corpuscles were studied in various biological species by other authors (15, 16) that also classify them depending on their size. Accepting those views, we could place the corpuscles observed by us in an intermediate group between small- and medium-sized encapsulated lamellar receptors. We also consider that stock broiler chickens at the age of 8 weeks although being appropriate from a commercial point of view, are still actively developing biosystems that have not reached a structural maturity and full

morphogenesis of their organs and systems. For the first time in Bulgaria, Hadjiolov (17) reported that *corpuscula Herbsti* in the avian beak, tongue and skin were built like the Vater-Pacinian corpuscles. He determined the smaller dimensions of Herbst's corpuscles, the lesser number of lamellae of their capsule and the lack of nuclei in the inner lamellae, arranged around the inner nervous branch as structural differences. A similar opinion for the similarity of corpuscular structure have been expressed by Moskov (18) in domestic animals, Stefanov (19) in some biological species and Popivanov et al. (1) in higher vertebrates. Studying the ultrastructural particularities of Schwann's cells in Herbst's corpuscles in the pigeon's beak, Malinovsky and Pac (15) have suggested a structural resemblance with Vater-Pacinian corpuscles. Saxod (20) have also commented upon the exceptional structural similarities between Herbst's and Pacinian lamellar corpuscles following out the ontogenesis in mammalian Merkel's cells in cutaneous sensory organs. Taking into account the structural particularities in our study and the opinions of fore-mentioned authors, we determined our three findings as encapsulated receptor endings of lamellar type. We suppose that our findings could be small or medium-sized Herbst's corpuscles because of the impossibility to present an adequate explanation for the presence of these mechanical receptors among the glandular components of the lachrymal gland.

Our results suggested that the lachrymal gland in broiler chickens possessed encapsulated nerve endings of a lamellar type, the histostructure of which up to the 56<sup>th</sup> day of their ontogenesis was similar to that of adult birds living in volieres. We also suggest that this is the first report giving evidence for the existence of encapsulated sense bodies in the lachrymal gland of broiler chickens.

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