Journal of Animal and Veterinary Advances 9 (13): 1875-1879, 2010
ISSN: 1680-5593

Histological and Histochemical Studies on the Harderian Gland of the Osprey (*Pandion haliaetus*)

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Abstract: The aim of the present study was to demonstrate the histological and histochemical structure of the Harderian gland of the osprey. The Harderian gland of the osprey (*Pandion haliaetus*) is a tubuloalveolar gland. The gland lumen was lined by columnar epithelial cells of varying height which were filled with secretory vesicles and by dark cells found among these cells. It was observed that the secretion was produced in the corpus glandulae and was extracted by primary and secondary ducts. In addition, plasma cells were present in interlobular trabeculae. Histochemical stainings which were performed to determine the character of the glandular secretion revealed that the majority of the secretory cells contained only acidic mucins whilst the remaining secretory cells contained both neutral and acidic mucins. Furthermore, it was observed that some cells of the primary and secondary ducts epithelium had secretory vesicles containing only neutral mucin. The presence of reticulum fibres was demonstrated in the interlobular trabeculae.

Key words: Osprey, harderian gland, histology, histochemistry, reticulum fibers, Turkey

INTRODUCTION

The Harderian gland is an orbital gland which occupies a position medioventral to the eyeball in many terrestrial vertebrates (Djaridane *et al*., 1999; Payne, 1994; Sakai, 1981; Shirama *et al*., 1982). The gland was first described by the Swiss physician Johann Jacob Harder in 1694, in red and fallow deer. However, it was only after the 1970s that detailed research was conducted on the structure and functions of the Harderian gland.

The Harderian gland has been studied in many vertebrate species mainly in mammals and it has been reported that the gland serves important functions which may vary with species (Djaridane *et al*., 1999; Payne, 1994; Sakai, 1981). The known functions of this gland include: lubrication of the eye and nictitating membrane, a source of pheromones, a source of growth factors, a site of immune response particularly in birds, a source of salt in some chelonians, osmoregulation in some rodents, photoreception in rodents and thermoregulation in some rodents (Chieffi *et al*., 1993). In recent years, a great many histological and ultrastructural studies have been carried out on the Harderian gland of many avian species (Maxwell *et al*., 1986). However, to the knowledge, the structure of the Harderian gland of the osprey (*Pandion haliaetus*) has not been studied previously. The present study was aimed at the demonstration of the histological and histochemical structure of the Harderian gland of the osprey (*Pandion haliaetus*).

MATERIALS AND METHODS

Animals: Ospreys do not live in Turkey but are sometimes found along their migratory routes. The encounter of the adult female osprey examined in this study in the Province Antakya was coincidental. The osprey which had a gun-shot wound was referred to the faculty clinics but died before being able to be treated. Clinical examination revealed that there was no systemic damage to the gland that would affect the tissue. Furthermore, the gland was examined by pathologists and no pathological finding was observed.

Histological and histochemical stainings: The Harderian gland was excised 15 min after the death of the osprey. Part of the tissues was fixed in formol-alcohol for 12 h

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while some other tissue samples were fixed in formaldehyde for 24 h. Following routine histological processing for light microscopy, tissue was embedded in paraffin. About 6 micron-thick serial sections were cut and stained with Mallory's modified triple stain (Crossmon, 1937) for the demonstration of the general structure.

The Periodic Acid-Schiff (PAS) and combined periodic acid-Schiff/alcian blue (PAS/AB) pH 2.5 staining methods were applied to determine histochemical characteristics and to analyze the presence and distribution of neutral and acidic mucins. In addition, the methyl green-pyronin method (Bock, 1989) was used for the demonstration of plasma cells and the silver staining method of Gordon and Sweets was used for reticulum fibres (Bancroft and Cook, 1984).

RESULTS AND DISCUSSION

Macroscopically, it was observed that the Harderian gland of the osprey resembled a droplet in shape and was pink in colour. The gland was observed to be of tubuloalveolar type and had a lobulated structure enclosed by a capsule of connective tissue. It was determined that the secretion produced in the corpus glandulae was transported via primary and secondary excretory ducts. The gland lumen was lined by columnar epithelial cells of varying height which were filled with secretory vesicles and displayed a holocrine mode of secretion and by dark cells found among these cells (Fig. 1 and 2). Plasma cells were present in the interlobular trabeculae (Fig. 3).

Histochemical stainings applied to determine the character of the glandular secretion demonstrated that the majority of secretory cells contained only acidic mucins whilst the remaining secretory cells contained both neutral and acidic mucins (Fig. 5-7).

Furthermore, it was observed that duct epithelium contained secretory vesicles with only neutral mucins. The presence of reticulum fibres in the interlobular trabeculae was demonstrated by silver staining (Fig. 8). Chieffi *et al.* (1993) stated that the localization of the Harderian gland in the orbit varied among species while Olcese and Wesche (1989) reported that this gland was

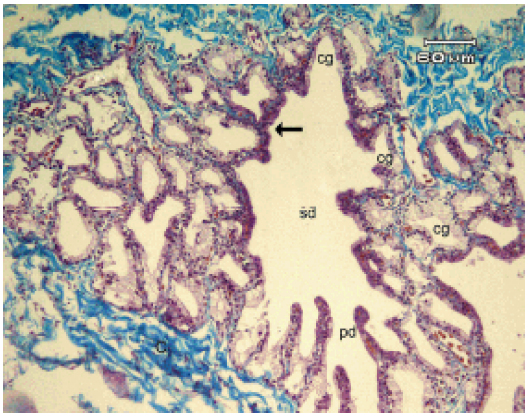

Fig. 1: A histological appearance of the Harderian gland in the osprey. cg; corpus glandula, pd; primary duct, sd; secondary duct, arrow; dark cell and C; capsule. Triple

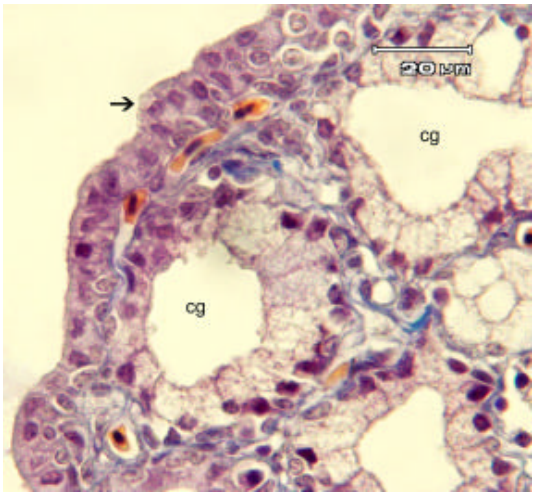

Fig. 2: Dark cells which were observed among the columnar epithelial cells lining the lumen in the Harderian gland of the osprey, arrow; duct epithelial cells, cg; Corpus Glandulae. Triple

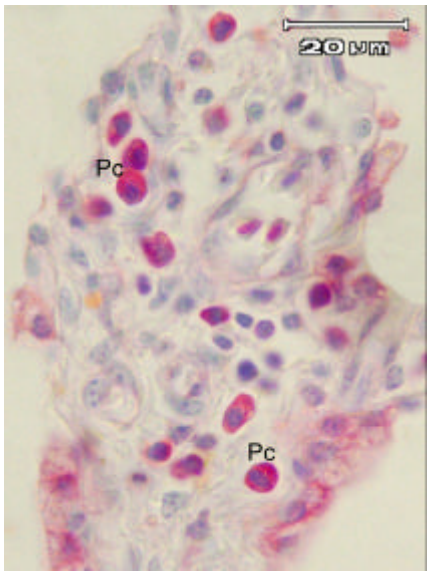

Fig. 3: Plasma cells were observed in the interlobular trabeculae Pc; Plasma Cells. Methyl green-Pyronin

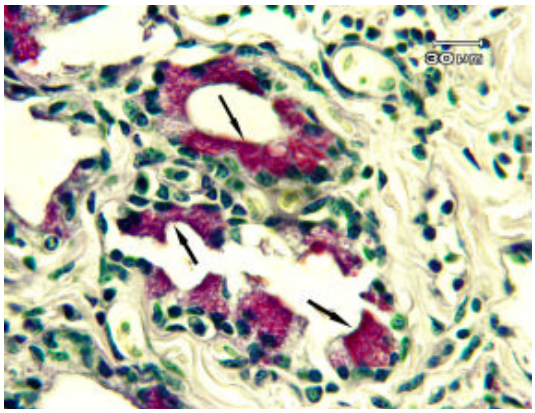

Fig. 4: PAS (+) secretory epithelial cells in the Harderian gland of the osprey, arrow; PAS (+) secretory epithelial cells. Periodic Acid-Schiff (PAS)

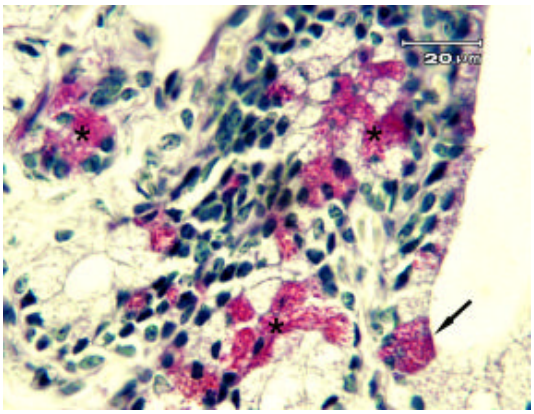

Fig. 5: PAS (+) duct epithelial cells and secretory epithelial cells in the Harderian gland of the osprey. arrow; PAS (+) duct epithelial cells. Periodic acid-Schiff (PAS)

generally located medially to the eyeball in all species and that the gland showed a tubular or tubuloalveolar structure.

Meanwhile, Burns (1975) stated that in birds, the Harderian gland was generally located ventromedially to the eyeball. In the present study, it was observed that the Harderian gland of the osprey occupied a position ventromedial to the eyeball as in other avian species and that it had a tubuloalveolar structure as in many other vertebrates. In the Harderian gland of the osprey, the luminal lining epithelium was observed to be composed of columnar cells of varying height. This was in agreement with the reports of Rothwell *et al*. (1972) for domestic chicken, Maxwell *et al*. (1986) for Turkeys and Altunay and Kozlu (2004) for ostriches.

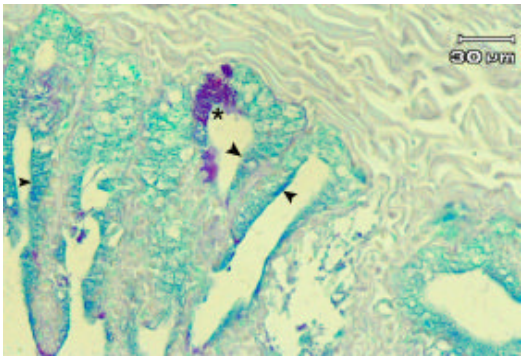

Fig. 6: Asterisk; PAS/AB (+) secretory epithelial cells arrow; AB (+) secretory epithelial cells

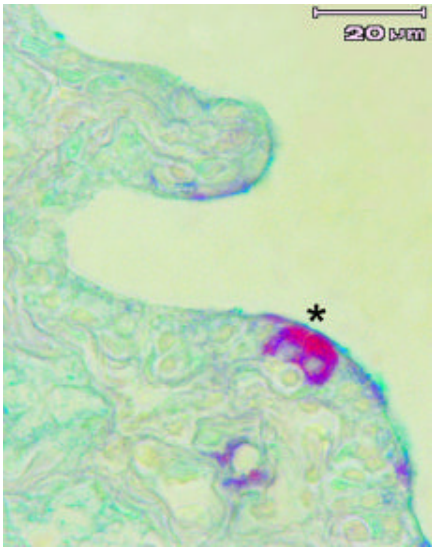

Fig. 7: Asterisk; PAS/AB (+) duct epithelial cells

In general, the interlobular trabeculae which contain blood vessels, nerve fibres and fibroblasts in many avian species were observed to line the corpus glandulae as stated by Rothwell *et al*. (1972).

In their study on the Harderian gland of domestic chickens, Rothwell *et al*. (1972) found that the secretory epithelium was of four different types. These were type II cells with developed golgi body, type III cells characterized by multiple rough endoplasmic reticulum, type IV cells with tightly packed secretory vesicles in the cytoplasm and type I cells with typical columnar cell structure, lacking the above characteristics. Despite the mention of uniform cells in a study of Maxwell *et al*. (1986) in Turkeys in a study by Altunay and Kozlu (2004) in ostriches, the epithelium of the Harderian gland was found to be composed of two different types of cells. The first were type I cells with a basally located nucleus and

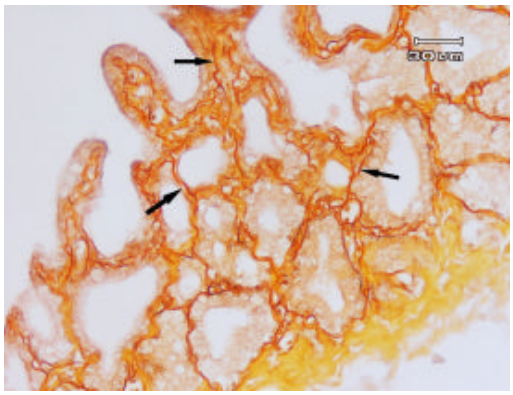

Fig. 8: Reticular fibres were observed in the interlobular trabeculae of the Harderian gland of the osprey arrow; reticular fibres, Gordon and Sweet's silver staining method

light coloured secretory vesicles and the second were type II cells with a basally located nucleus and dark coloured secretory vesicles. In the present study, the epithelial cells lining the lumen were found to be uniform but among these there were dark cells with darkly stained cytoplasms and nuclei.

Olah *et al.* (1992) obtained similar findings in chicken and reported that dark cells were localized close to plasma cells. Rothwell *et al.* (1972) mentioned that these cells were observed in the subepithelial region with myoepithelial cells and Olah *et al.* (1992) reported the presence of these cells among secretory cells. The findings obtained in the present study on the localization of dark cells were in agreement with those reported by Olah *et al.* (1992).

Another type of cell localized in the subepithelial region of the gland is plasma cells. These cells exist in high numbers in many avian species. For this reason, the avian Harderian gland has been studied extensively for its immunological functions. Wight *et al.* (1971) and Rothwell *et al.* (1972) have also reported the presence of plasma cells in the Harderian gland of chickens. Furthermore, Maxwell and Burns (1979) determined that plasma cells and lymphocytes existed in the ducts of the Harderian gland of Turkeys, ducks and chickens. In the present study, it was observed that although in small numbers, these cells were present in the intertrabecular areas. In their histochemical study on the Harderian gland of domestic fowl, Wight *et al.* (1971) observed PAS-positive reactions of varying intensity particularly in duct epithelial cells. While secretory cells composing the corpus glandulae showed strong reaction, the number of cells displaying positive reaction decreased significantly in the secondary ducts. It was reported by Ohshima and Hiramatsu (2002) for young chickens and by Dimitrov and Nikiforov (2005) for ducks that PAS-positive reactions increased in some infections. PAS staining results obtained for the Harderian gland of the osprey in the present study were in agreement with those reported by Wight *et al.* (1971) mentioned that following PAS/AB staining, most of the granules in the cytoplasm of acinar cells stained dark blue whilst a greater number of granules stained red. In the present study, it was observed that the granules of secretory cells usually stained a light blue colour and to a less extent a distinct red.

CONCLUSION

Participation of plasma cells in the mechanism of local immune system especially with regard to the course of the immunoresponse after antigen application or eye inflammation has been well documented in birds. Attempts to demonstrate a similar immunological role for the Harderian gland of avian have been successful. The suggestion by Burns (1975) and Aitken and Survashe that habitat may determine the gland types and immune system is an important clue to the eventual understanding of Harderian gland function. Having gone through literature, we did not come across with any histological study previously conducted in osprey. Further observations will be needed to reach definite conclusions. We do believe that the information that we have obtained will guide researchers who will conduct studies in this area in the future.

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